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METHYL HALIDE PRODUCTION IN FUNGI

BY

Gail D. Dailey

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Natural Resources

September, 2007

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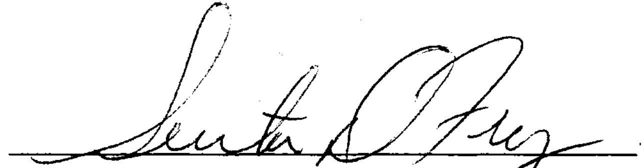

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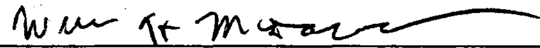

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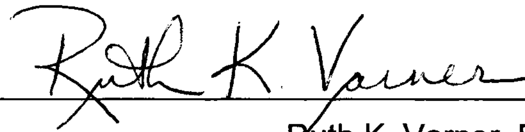

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ACKNOWLEDGEMENTS

I would like to thank the following individuals for their contribution to this research:

My thesis committee, Dr. Ruth Varner, Dr. Serita Frey, and Dr. William McDowell, for contributing their time and critique of this work. I would especially like to thank Dr. Varner for her efforts in obtaining funding for this project, her belief in my ability, and many hours teaching me about atmospheric chemistry;

Thank you to Dr. Andrew B. Cooper for providing statistical analysis consulting;

Sallie Whitlow, of the CCRC Ion Chromatography Laboratory for your interest in my research and for providing ion chromatography analysis of the soil samples;

Dr. Michael Palace for collecting numerous mushrooms used in the study;

Dr. Robert O. Blanchard and Dr. Therese M. Thompson for mycology training, advice, and use of the mycology laboratory;

Funding assistance from the National Science Foundation was greatly appreciated.

Thank you to the following people for their help in the field and the laboratory:

Jordan Goodrich, Kate Petzack, Sarah Hines, Christine San Antonio, Maria Hunter, Jennifer Richards, Nick Robertson, Danielle Ouimette, and Lisa Brunie

Finally, thank you to Laurier St. Onge, for his love, support, and patience, which have allowed me to pursue this degree.

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PREFACE

This document reports the levels of methyl chloride, methyl bromide, and methyl iodide that were produced by fungi in laboratory culture. Included are descriptions of the experimental procedures used to culture fungi, obtain pure isolates, and assay the fungi for methyl halide production. Table 1 lists the common terms used within this document.

Table 1: Common Terminology Used in this Document

Term	Definition
halocarbon	a chemical compound containing carbon and a halogen
halomethane	a methane molecule (CH_4) minus a hydrogen atom plus a halogen atom
halogens	the chemical elements, fluorine (F), chlorine (Cl), bromine (Br), iodine (I), and astatine (At)
halide	a halogen anion; halogen atom with a negative charge (e.g. Fl^-, Cl^-, Br^- or I^-)
CH_3Cl	methyl chloride, also known as chloromethane
CH_3Br	methyl bromide, also known as bromomethane
CH_3I	methyl iodide, also known as iodomethane
CH_3X	X=Cl, Br, or I
Gg	10^9 grams
Tg	10^{12} grams
Pg	10^{15} grams
T, t	tonne or metric ton = 1000 kg
1 pptv	1 part per trillion by volume or 1 molecule gas/10^{12} molecules air
1 ppt	1 picomol mol^{-1} or 1×10^{-12} mol^{-1}
flux	the exchange of a gas between the soil or ocean and the atmosphere; calculated as the amount of gas /area /unit of time
sink	a mechanism or reaction that removes a gas from the atmosphere; consumption of a gas
source	a mechanism or reaction that adds a gas to the atmosphere; production of a gas
budget	an accounting of additions to and subtractions from the atmosphere for a gas

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ABSTRACT

METHYL HALIDE PRODUCTION IN FUNGI

by

Gail D. Dailey

University of New Hampshire, September, 2007

Methyl halide gases are a source of halogen radicals that can react with and destroy stratospheric ozone. The sources of methyl halide gases are both anthropogenic and biogenic, that is, they are human induced and they occur naturally. This research focused on the emission of methyl halides from fungi in the phylum Basidiomycota, which are one of the known biogenic sources.

Previous studies have measured methyl halide production and consumption in soils using field chambers. The objective of this study was to compare production from individual fungi in laboratory cultures to the field measured fluxes to examine whether fungi are a significant source of methyl halide emissions.

This study included fungi from four different ecosystems: an agricultural field, a temperate forest, a fresh water wetland, and coastal salt marshes in southern New Hampshire, USA. Fungal samples were collected from each site and cultured in the laboratory using tissue culture, wood bait, and direct soil

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plating methods. Once pure isolates were obtained, the fungi were assayed for methyl halide production using cryotrapping-gas chromatography. Samples of headspace gas were extracted from flasks containing fungi grown in liquid media or soil substrate and measured on a gas chromatograph/electron capture detector (GC/ECD). By sampling individual fungi from different ecosystems and assaying them in media and soil substrate the halide ratio from fungi could be examined.

Two types of statistical analyses were used to determine the methyl halide fluxes: linear least squares fit of the methyl halide concentration vs. time, and a Bayesian model with Markov Chain Monte Carlo (MCMC) sampling. Both statistical methods calculated a slope of the flux for each flask. The fluxes were averaged by isolate, and then normalized to mass by dividing by fungal biomass. The Bayesian model provided a rigorous analysis of the data that could be compared to data derived from the traditional linear fit method in order to determine whether a linear regression fit causes an over-estimation of the production rate.

Methyl halide production was observed in fungi collected from all of the sites. Specifically, the highest levels of methyl halides were produced in all species of fungi isolated from salt marshes; the highest levels of methyl bromide were produced by basidiomycetes from all sites and zygomycete

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isolates from the freshwater fen; the non-basidiomycete fungi isolated from the freshwater fen and from the salt marshes produced the highest levels of methyl iodide.

Although the primary focus was methyl halide production in basidiomycetes, the results clearly show that the non-basidiomycete fungi are a potential source of methyl halide emissions that may represent a greater environmental significance than expected from basidiomycetes.

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CHAPTER 1

1.0 BACKGROUND ON ATMOSPHERIC METHYL HALIDES

The composition of the earth's atmosphere contains a small percentage (0.04%) of trace gases that include organic halogen compounds (Schimel and Holland, 2003). Within this group of compounds are three methyl halides, methyl chloride (CH_3Cl), methyl bromide (CH_3Br) and methyl iodide (CH_3I), which are the focus of this study.

When chemical reactions involving methyl halides occur in the stratosphere, halogen radicals, chloride (Cl^-), bromide (Br^-), or iodide (I^-) can be released. Here, they may react with and destroy ozone. The ozone layer in the stratosphere protects the earth from incoming radiation.

The sources of methyl halides to the atmosphere are both biogenic (natural processes) and anthropogenic (caused by human activities). While the anthropogenic sources of methyl halides can be controlled through regulations, such as the Montreal Protocol and the US Clean Air Act, a better understanding of the biogenic sources of methyl halides is required in order to accurately quantify the global atmospheric budget of these compounds and, therefore, the effect of anthropogenic emissions.

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1.1 Global Methyl Halide Budget

In order to calculate a budget for methyl halides on a global scale, we need to know the magnitude and distribution of the sources and sinks. To date, not all of the methyl halide sources and sinks have been identified. Estimates indicate that the global budget for methyl chloride and methyl bromide is out of balance because the known sinks are greater than known sources (Yvon-Lewis and Butler, 1997; Reeves, 2003; Saltzman et al., 2004). The missing source (or sources) of methyl bromide has been estimated at 60–70 Gg yr^{-1} [Gg=10^9 g] (Yvon-Lewis and Butler, 1997; Reeves, 2003), while "the combined emissions [of methyl chloride] from known sources account for about half of the modeled sink" (Keene, et al., 1999).

According to the latest assessment panel of the U.N. Montreal Protocol on Substances that Deplete the Ozone Layer, the annual mean concentration for methyl chloride in the troposphere is about 550 pptv (pptv=parts per trillion by volume) (Clerbaux and Cunnold, 2006). The estimated global concentration for methyl bromide is about 10 pptv (Lobert et al., 1995; Yvon-Lewis and Butler, 1997; Reeves, 2003). The tropospheric levels of methyl iodide are the lowest of the three methyl halides and concentrations have been estimated at 0.2–5 pptv (Dimmer et al., 2001) and <0.005–5 pptv (Redeker et al., 2000).

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1.2 Historical Record

One approach that is often used to determine methyl halide levels is to examine the historical atmospheric record that exists inside polar firn and ice core air. Firn is defined as "the porous layer of compacted snow overlying an ice sheet" (Trudinger et al., 2004). Using the polar firn and ice core air data, researchers build models that can estimate past concentration levels and trends.

Halocarbons that were trapped inside polar firn air in Antarctica showed that the rate of increase in atmospheric methyl bromide grew from 0.01 pmol mol^{-1} yr^{-1} to 0.05–0.06 (+/- 0.01) pmol mol^{-1} yr^{-1} from the early 1900's to the 1970's and 1980's (Butler et al., 1999). The methyl chloride concentrations "were significant in the deepest samples and increased gradually toward the surface" (Butler et al., 1999). In another study, ice core measurements of methyl bromide from West Antarctica were consistent with modeled estimates of the preindustrial atmospheric budget (Saltzman et al., 2004).

In a similar model analysis using Antarctic firn data, Reeves (2003) concluded after finding the same imbalance in the methyl bromide budget that calculations of the known sinks must be overestimated, the sources must be underestimated, or there is an unknown biogenic source. This uncertainty demonstrates that the natural cycling of these compounds is not well understood.

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1.3 Anthropogenic Sources of Methyl Halides

The historical record supports the view that methyl halide levels have increased as a result of human influence, particularly following industrialization. The anthropogenic (human activities) sources that cause methyl halide emissions include biomass burning, automobile engine exhaust, and fumigation for agriculture.

Biomass burning is considered one of the major anthropogenic sources of ozone-depleting gases. When forests are burned to clear land for agriculture and development or when plant material is burned for fuel, the combustion of organic matter releases carbon-based gases including methyl chloride and methyl bromide into the atmosphere (Levine, 2002). Because chlorine is a component of all biomass (Graedel and Keene, 1995) biomass burning is a major source of methyl chloride in the atmosphere (Lobert et al., 1999). Worldwide biomass burning releases significant amounts of methyl chloride (1100–1510 Gg yr^{-1}) and methyl bromide (19–24 Gg yr^{-1}); however, methyl iodide emissions from biomass burning (3.4–8.5 Gg yr^{-1}) are not as large (Andreae et al., 1996). It should be noted that while the cause of most biomass burning is anthropogenic, it can also be the result of natural combustion from lightning.

Automobile exhaust is another combustion source of methyl halides. Methyl bromide emissions caused by burning leaded gasoline have varied annual global estimates published by the World Meteorological Organization (WMO) of

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0.5–1.5 kT yr^{-1} and 9–22 kT yr^{-1} (Penkett et al., 1995), 1.5–3.0 kT CH_3Br yr^{-1} (Baker et al., 1998), and 0–10 Gg yr^{-1} (Warwick et al., 2006). [Note: kT=Gg.]

For many years, methyl bromide was used as an agricultural and domestic fumigant to kill pests. As a result of the Montreal Protocol agreement and the US Clean Air Act, a reduction in the commercial use of methyl bromide was necessary and a phase-out was implemented as of January, 2005 (with critical use exceptions in the US). The global methyl chloride and methyl bromide budget estimates continue to be revised as more research becomes available. Levels must be monitored since regulations restricting anthropogenic emissions are now in effect.

1.4 Natural Sources

The natural sources of methyl halide emissions include abiotic reactions and production in biological organisms (biogenic sources). The following sections describe some of these natural sources.

1.4.1 Abiotic Production of Methyl Halides

There is evidence that methyl halides are produced abiotically, through natural geochemical processes. Examples of abiotic sources include weathering of rock (Gribble, 2003) and volcanic gas (Frische et al., 2006). Methyl iodide is formed in oceans by photochemical reactions with dissolved organic carbon (Bell

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et al., 2002). Seawater experiments conducted by Moore (2006) showed that variable amounts of methyl iodide and methyl bromide were produced by an unknown mechanism when seawater was stored in polyethylene containers. Similarly, methyl chloride, methyl bromide, and methyl iodide were produced when seawater was stored in polyvinyl fluoride containers and exposed to sunlight.

Keppler et al. (2000) found that methyl halides were produced during oxidation of organic matter in soils without the aid of microbes. Their study showed that methyl halide production depended on the organic matter content and the presence of halide ions and ferrihydrite (Fe^{+3}) in the soil. Production increased when halide ions and amounts of ferrihydrite increased; whereas, when they were absent, methyl halides were not produced.

Abiotic processes that remove methyl halides (known as "sinks") include reactions with hydroxyl (OH) radicals, which are considered a major sink for methyl chloride and methyl bromide (Graedel and Keene, 1995; Redeker et al., 2000; Harper, 2000). Abiotic removal processes for methyl bromide and methyl iodide also include chemical reactions with chloride ion (Cl^-) in the ocean (Bell et al., 2002; Moore, 2006).

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1.4.2 Biogenic Sources of Methyl Halides

As the potential sources of ozone-destroying compounds were being identified in the 1980's, the need to understand the mechanisms involved in methyl halide biosynthesis took on environmental significance.

Methyl chloride and methyl bromide production have been identified in *Fomes* cultures by Hutchinson (1971) and Cowan et al. (1973). In 1982, White investigated the biosynthesis of methyl chloride in a wood-decay fungus, *Phellinus pomaceus* (Pers.) Maire. *(Phellinus pomaceus* is also known by the synonym *Fomes pomaceus.)* Harper (1985) then reported methyl halide production in *P. pomaceus* grown in flask cultures that were supplemented with halides. Subsequent studies of fungal metabolism indicated that production of methyl chloride probably occurred in more species of fungi than *Phellinus* and also in higher plants (Harper et al., 1989; Watling and Harper, 1998). Researchers continued to investigate the production of methyl chloride in fungi, as well as other methyl halides and in other living organisms.

1.4.3 Methylation

A methyl chloride transferase enzyme was identified as the biosynthetic mechanism involved in the biogenic production of methyl chloride (Wuosmaa

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and Hager, 1990). White (1982) suggested that S-adenosylmethionine (SAM) was the methyl donor in the biosynthesis of methyl chloride in fungal cultures. Several researchers agree that during the methylation process, S-adenosyl-L-methionine (SAM) serves as a methyl (CH_3) donor and the chloride, bromide, or iodide anions are methylated (added to the methyl group) by a catalyst, methyl chloride transferase (MCT) (Attieh et al., 1995; Ni and Hager, 1998; Saxena et al., 1998).

However, in 2003, Hamilton et al. proposed that methyl chloride production in senescent and dead plant leaves is an abiotic, rather than an enzymatic process that involves plant pectin and heat. Under this scheme, chloride methylation occurs when pectin (a component of plant cell walls) serves as the methyl donor. They estimated a global annual production rate of 0.03–0.64 Tg CH_3Cl yr^{-1} between latitudes 30°N and 30°S.

1.4.4 Genetic Mechanism

The genetic mechanism responsible for methyl halide production was recently discovered in *Arabidopsis thaliana* plants (Rhew et al., 2003). The gene, known as the "harmless to ozone layer" (HOL) gene, encodes the methyltransferase enzyme that catalyzes the methylation of halide ions in plants. The gene is believed to be common in vascular plants (Rhew et al., 2003). Similar gene sequences have been found in *Prochlorococcus* phytoplankton and

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are being investigated since methyl iodide production in laboratory cultures of this species has been confirmed (Smythe-Wright et al., 2006).

1.4.5 Why Do Organisms Produce Methyl Halides?

The reason for biogenic methyl halide production is unclear. Methyl halide emissions may be a mechanism for plants to detoxify their system of halides; however, Manley (2002) found that the levels of chloride in salt marsh plant tissue remained high despite methyl chloride production. Likewise, Rhew et al. (2002) noted that based on the methyl halide fluxes they observed in California salt marshes, only small amounts of Cl^- and Br^- would be removed from the plants.

On the other hand, the production of methyl halides by plants may serve no function, and may simply be a metabolic by-product (Manley, 2002). Experiments in simulated rice paddies showed that rice plants volatilized less than 1% of chlorine or bromine as methyl chloride or methyl bromide, while over 90% of iodide that was stored in the plant was volatilized as methyl iodide (Redeker et al., 2004). This suggested to the researchers that rice plants are able to maintain constant levels of chloride and bromide in their tissues.

Another reason for methyl halide production in plants may be to provide a chemical defense mechanism (Harper, 2000). Seaweeds (marine algae) may produce methyl halides as a form of natural pesticide to prevent predation

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(Gschwend et al., 1985; Attieh et al., 1995; Gribble, 2003). There is more evidence to support the view that methyl halide production may be related to the decay of plant matter. Most plants contain halogens in different amounts; in fact, chlorine is required by plants for photosynthesis (Kabata-Pendias and Pendias, 1992), and it is found in high amounts. (See Table 1-1: Halogen Concentrations in Soils and Plants.)

Soil microorganisms and fungi are known to accumulate large amounts of iodine, and soil bacteria release iodine when breaking down organic matter (Kabata-Pendias and Pendias, 1992). Lee-Taylor and Holland (2000) proposed litter decomposition as a source of methyl bromide emissions. Using a model based on a range of bromine content in litter (0.065–16 mg Br- kg^{-1} dry matter) and three litter fractions (woody, aboveground fine, and belowground), they calculated methyl bromide emissions in the range 2.5–37 kT CH_3Br yr^{-1} from decaying vegetation.

In addition, an examination of the chemical forms of chlorine by Myneni (2002) showed that senescent and humified plant material (the mineralized form of organic matter) contained organo-Cl compounds. Because the chlorine-containing organic molecules in humics are biologically stable and recalcitrant, it is possible that halogenation helps stabilize and store carbon (Myneni, 2002). As noted in Keppler et al. (2000), methyl chloride production during abiotic oxidation of organic matter depended on the presence of Fe-oxyhydroxides in the soil.

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If decaying vegetation is a source of methyl halides, then the microbes involved in decomposition are implicated. Lignin molecules, which provide structure and strength to plants, are an example of a recalcitrant plant material. Several white-rot fungi and some bacteria are able to degrade lignin (Palmer and Evans, 1983). Methyl halides have been found to serve as methyl donors in fungal metabolism (Harper et al., 1989) and methyl halide production is possibly part of the lignin degradation process (Harper et al., 1989; Redeker et al., 2004).

Studies have shown that microbial abundance, in particular fungal biomass, is greatest at the soil surface, probably due to the availability of resources (Fierer et al., 2003). Although the study did not examine methyl chloride specifically, Myneni (2002) found that "halogenation of organic molecules was pronounced" in soil samples collected from the surface, which suggested that "soil fauna and flora influence halogenation reactions" (Myneni 2002). Yet, studies of methyl halide emissions in rice paddies found "no significant correlation between available organic matter and methyl chloride production" (Redeker et al., 2000).

1.4.6 Methyl Halides from Plants

The biogeochemistry of chlorine, bromine, and iodine is similar. Each of the halogens is soluble, easily transported by water and leached from soils; all three can be taken up by plants. Halophytes and marine plants, in particular, can

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accumulate large amounts of halogens in their plant tissue, especially the leaves. Table 1-1 lists the concentrations of halogens found in soils and plants.

Table 1-1: Halogen Concentrations in Soils and Plants. (Source: Kabata-Pendias & Pendias, 1992 unless otherwise noted); dw=dry weight; ppm=parts per million.

Halogen	Soils (ppm dw)	Plants (ppm dw)
Chlorine	35 – 20,000	200 – 10,000 mg Cl^- kg^{-1} dw (Harper, 1985); 700 – 23,000 mg Cl^- kg^{-1} dw (Hamilton et al., 2003); 1,300 – 5,500 ppm dw potato tubers
Bromine	5 - 40	<40 ppm dw plants; 0.065 mg kg^{-1} dw temperate deciduous forest litter; 2 – 36 ppm dw mushrooms
Iodine	<0.1 – 10	0.4 – 16 μM in plant tissue (Harper, 2000); 2.8 – 10.4 ppm dw US garden vegetables; 5.2 – 9.5 ppm dw mushrooms

As indicated in Table 1-1, the chloride concentration in plants is highly variable. In their review of wood-rotting fungi, Watling and Harper (1998) noted that the mean chloride content in wood ranged from 8–2,535 mg Cl^- kg^{-1} dw (dry weight) in temperate species and 9–5,100 mg Cl^- kg^{-1} dw for tropical wood species. Factors such as genetics, soil conditions, and climate contribute to this variability.

In 1995, Attieh et al. purified and characterized a methyltransferase enzyme in *Brassica oleracea*. These plants had been shown to produce high levels of methyl halides (Attieh et al., 1995; Saini et al., 1995). Subsequently, Gan et al. (1998) observed that plants from the *Brassicaceae* family (rapeseed, broccoli, cabbage, and wild mustard) emitted methyl bromide gas. Plant emission rates were linearly correlated to plant bromide content. The results

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indicated that “the aboveground part of the plants” produced and released methyl bromide (Gan et al., 1998). Similarly, Rhew et al. (2002) proposed that the aboveground plant material was the likely source of emissions that were observed in California salt marshes.

On a larger scale, Moore et al. (2005) made tower measurements above the forest canopy (36 m) and at ground level in the Brazilian rainforest. Methyl chloride concentrations of 785 ppt at 36 meters high and 673 ppt at ground level suggested vegetation or a vegetation-related source.

Rhew et al. (2001) measured methyl chloride and methyl bromide emissions from three California shrubland sites. Their estimate of net global uptake from shrublands was <1 Gg (0.7 +/- 0.2) CH_3Br $yr^{-1}$and <20 Gg (15 +/- 6) CH_3Cl yr^{-1}. The sites appeared to have competing consumption and production processes. Methyl chloride and methyl bromide emissions were associated with living and decayed plant biomass, but consumption occurred in soils and showed some relationship to soil moisture content (Rhew et al., 2001).

Methyl halide emissions from tropical plants were detected in the atmosphere inside a tropical rainforest glasshouse at the Tsukuba Botanical Gardens in Japan (Yokouchi et al., 2002). Flux chambers on the soil and Teflon bag enclosures on plants were used to measure methyl chloride. The researchers measured uptake by the soil in the range of 0.2–0.6 $\mu g\ m^{-2}\ h^{-1}$, and

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average emissions of 0.4 μg CH_3Cl g dry leaf^{-1} h^{-1} from nine plants. They also made flux measurements at two forest sites and found 0.15-3.7 μg CH_3Cl g dry leaf^{-1} h^{-1}, which supported their glasshouse measurements. From this study, a global annual methyl chloride emission rate of 0.82 Tg yr^{-1} from tropical forests was estimated.

The diurnal cycles observed in methyl halide emissions from saltmarsh environments (Rhew et al., 2002; Drewer et al., 2006) and peatlands (Dimmer et al., 2001) have also indicated that plants may be a source for methyl halides. Methyl chloride and methyl iodide emissions were also measured in potted tropical ferns (Saito and Yokouchi, 2006). Surprisingly, the ferns showed diurnal cycles in methyl halide emissions but the cycles were different for two plants of the same genus. In addition, the diurnal variations in methyl chloride and methyl bromide were correlated with each other, suggesting a similar production mechanism (Saito and Yokouchi, 2006).

1.4.7 Methyl Halides from Oceans, Algae, and Phytoplankton

The ocean is both a source and sink for methyl halides (Moore, 2006). Biogenic sources that have been found in the marine environment include Antarctic macroalgae (Laturnus et al., 1998), marine red algae *Endocladia muricata* (Wuosmaa and Hager, 1990), and plankton (Oram and Penkett, 1994; Scarratt and Moore, 1996; Smythe-Wright et al., 2006).

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Global emissions from the oceans are estimated at 460 Gg Cl yr^{-1} for methyl chloride (Khalil et al., 1999) and 56 Gg CH_3Br yr^{-1} (Yvon-Lewis and Butler, 1997; Yvon-Lewis et al., 2002). Smythe-Wright et al. (2006) estimated global methyl iodide emissions of 4.3 x 10^9 mol yr^{-1} [4.3 Gg CH_3I yr^{-1}] from the North Atlantic Ocean at latitudes below 40° based on their measurements of methyl iodide production in the picoplankton species, *Prochlorococcus.*

Cohan et al. (2003) used a three-layer model to calculate a flux of methyl iodide in the Southern Ocean. Average mixing ratios in the marine boundary layer were adjusted for seasonal variability (0–1.0 km above the ocean for spring and autumn; 0.9 km in summer, and 1.1 km in winter). The free troposphere layer was set at 1.1–10 km above the ocean. The resulting calculations showed a global annual oceanic flux of methyl iodide between the marine boundary layer and the free troposphere ranging from 1.9 x 10^{-6} g m^{-2} d^{-1} (winter) to 5.2 x 10^{-6} g m^{-2} d^{-1} (summer). The researchers did not extrapolate a global atmospheric flux from these data due to seasonal and latitudinal variability and uncertain losses due to photolysis.

In another study, methyl chloride and methyl bromide production was measured in three phytoplankton species, *Phaeodactylum tricornutum*, *Phaeocystis* sp., and *Thalassiosira weissflogii* (Scarratt and Moore, 1996). The production rates observed in their laboratory cultures are shown in Table 1-2.

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Table 1-2: Methyl Chloride and Methyl Bromide Production Rates in Marine Phytoplankton Grown in Laboratory Cultures. Rates have been normalized to biomass using average chlorophyll *a* or utilized nitrate (Scarratt & Moore, 1996).

Normalized Method	mol CH_3Cl y^{-1}	mol CH_3Br y^{-1}
chlorophyll *a*	~$1.3 \times 10^8 - 4 \times 10^9$	~$1.3 \times 10^7 - 5.5 \times 10^8$
Nitrogen	$2.0 \times 10^8 - 1.3 \times 10^9$	$1.6 \times 10^7 - 9.5 \times 10^7$

The processes in the ocean that remove methyl halides from the atmosphere (sinks) are abiotic chemical reactions (Moore, 2006) and consumption of methyl bromide by bacteria (King and Saltzman, 1997). For example, the marine bacterium, *Leisingera methylohalidivorans,* grows on methyl bromide and uses it for a carbon source (Schaefer et al., 2002).

1.4.8 Methyl Halides from Freshwater Wetlands

Environments with saturated soils have been identified as sources and sinks of methyl halides. Studies have measured methyl halide fluxes in peatlands (Dimmer et al., 2001), fens and bogs (Varner et al., 1999; White et al., 2005), and rice paddies (Muramatsu and Yoshida 1995; Redeker et al., 2000; Redeker et al., 2004).

Methyl chloride and methyl bromide mixing ratios in two freshwater wetland sites in southern New Hampshire were measured by Varner et al. (1999). From these measurements they estimated global fluxes of 48 Gg yr^{-1} for methyl chloride and 4.6 Gg yr^{-1} for methyl bromide. Additionally, a

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relationship between temperature (at a 10-cm depth in the peat) and methyl halide flux was observed. Previous studies at the same site identified a similar relationship between temperature/methane and DMS (dimethyl sulfide) flux, which was considered to be microbially mediated (Kiene and Hines, 1995; Varner et al., 1999).

The results of further flux chamber measurements and a vegetation removal experiment at this fen indicated that the methyl bromide flux is highly variable and appears to depend on environmental conditions, such as vegetation, microbial community composition, and peat moisture (White et al., 2005).

Based on measurements made in seven peatland sites in Co. Galway, Ireland, Dimmer et al. (2001) calculated global annual fluxes of 5.5, 0.9, and 1.4 Gg yr^{-1} for methyl chloride, methyl bromide, and methyl iodide, respectively. The annual daytime methyl iodide flux (123×10^{-4} g m^{-2} yr^{-1}) from their coastal marsh site was higher than the other halocarbons measured. These researchers also observed that the fluxes occurred in a diurnal cycle and were greater during the growing season.

Rice paddies are another source of methyl halides (Muramatsu and Yoshida, 1995; Redeker et al., 2000) that has been investigated. Estimated global emissions of 2.4–4.9 Gg CH_3Cl yr^{-1}, 0.5–0.9 Gg CH_3Br yr^{-1}, and 16–29 Gg CH_3I yr^{-1} from rice paddies were made by Lee-Taylor and Redeker (2005).

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1.4.9 Methyl Halides from Salt Marshes

Methyl chloride and methyl bromide emissions in two southern California salt marshes have been estimated at 170 Gg CH_3Cl and 14 Gg CH_3Br yr^{-1} (Rhew et al., 2000). Studies showed that methyl chloride and methyl bromide emissions had strong seasonal and spatial variability. In addition, temperature, sunlight, vegetation types were also important factors (Rhew et al., 2002). In another study of California salt marshes, Manley et al., (2006) estimated a methyl iodide rate of 1.2×10^4 g CH_3I yr^{-1} from salt marsh emissions.

The methyl halide production in salt marshes may explain the higher emissions reported in coastal waters. The ocean has been considered a net sink for methyl bromide because of undersaturation in the open ocean and supersaturation in coastal waters (Sæmundsdóttir and Matrai, 1998; Sturrock et al., 2003). Similarly, concentrations of methyl iodide were higher in coastal waters in the North Atlantic than those in the deeper ocean (Ballschmiter, 2003).

The seasonal and diurnal cycles observed in methyl halide emissions from salt marshes suggests that plants (or plant-mediated processes) are the source in these environments (Rhew et al., 2002; Drewer et al., 2006). Drewer et al. (2006) measured methyl bromide fluxes in a salt marsh in Scotland using static chambers and calculated global methyl bromide emissions of 0.5–3 Gg yr^{-1}. They found that net emissions of methyl bromide

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had high spatial and diurnal variability; emissions were strongly associated with sunlight and vegetation. There was no correlation with emissions and water table and only slight positive correlation with temperature.

It has been demonstrated that the salt marsh plant known as smooth cordgrass (*Spartina alterniflora* Loisel.) oxidizes hydrogen sulfide gas (H_2S) to less toxic sulfur species (Lee et al., 1999; Rhew et al., 2002). *Spartina alterniflora* is a facultative halophyte; it can tolerate conditions of high salinity and flooding. *Spartina* plants have adapted to the salt marsh environment by developing aerenchyma, spongy tissue in the plant stems (Seago et al., 2005). The aerenchyma allows chemical gases, such as methane, oxygen, and carbon dioxide to pass between the leaves and the roots (Teal and Kanwisher, 1965) and be transported from the soil to the atmosphere (Le Mer and Roger, 2001). Thus, methyl halide emissions in the salt marsh may be transported from the sediments by way of the plant aerenchyma (Rhew et al., 2002).

The halocarbon emissions from Irish peatland sites also showed a diurnal cycle and indicated distinct differences in production between plant types. Not only did emissions vary among the methyl halides but also between the plant species. In contrast to the salt marsh plants, higher emissions of methyl chloride in peatlands came from plants without lacunae (air spaces that are formed in plants with aerenchyma tissue) rather than plants with lacunae (Dimmer et al., 2001).

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Although methane fluxes showed strong correlations with temperature and water table level in a temperate fen (Treat et al., 2006) and a relationship between temperature and methyl halide flux was also observed, there are conflicting reports of correlations between methane flux and methyl halide flux. Rhew (2002) found no correlation between methyl halide emissions and methane emissions in California salt marshes, yet reported a positive correlation between methyl chloride and methyl bromide fluxes and methane emissions in a northern Alaskan coastal tundra (Rhew et al., 2007) where these gases were consumed.

Methane had no effect on the uptake of methyl bromide in soil incubation experiments by Hines et al. (1998). Likewise, Redeker et al. (2000) found differences between the methane emission profile and methyl halide emissions, which suggest that methane and methyl halides are products of different biological processes.

In a recent study by Manley et al. (2007), methyl halide emissions were measured in greenhouse-grown mangrove plants. Mangroves are halophytes that grow in tropical coastal areas. From the results of this study, global emissions of 12 Gg CH_3Cl yr^{-1}, 1.3 Gg CH_3Br yr^{-1} and 11 Gg CH_3I yr^{-1} from mangroves were calculated.

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1.4.10 Methyl Halides from Bacteria and Soil

Research has shown that soils are a sink for methyl bromide (Shorter et al., 1995; Serça et al., 1998; Varner et al., 1999; Varner, 2000) and methyl chloride (Moore et al., 2005). Based on incubation experiments of soils from various locations, a global methyl bromide sink estimate of 42 ± 3 Gg yr^{-1} was made by Shorter et al. (1995). A study of methyl chloride fluxes from the rainforest in Brazil indicated that the forest floor acted as a sink for methyl chloride. Using static flux chambers, Moore et al. (2005) measured average CH_3Cl uptake in the range of 18–161 pmol m^{-2} min^{-1}).

The mechanism of methyl bromide consumption in soils has been attributed to bacteria (Oremland et al., 1994; Hines et al., 1998; Varner et al., 1999; Goodwin et al., 2001). Varner (2000) observed that uptake of methyl bromide was greatest in the 0–5 cm soil layer, and activity was affected by temperature and moisture. In addition, after sterilizing the soils with antibiotics and autoclaving, methyl bromide uptake was inhibited (Hines et al., 1998).

Researchers have identified several strains of bacteria that use methyl chloride (Coulter et al., 1999) or methyl bromide (Miller et al., 1997; Connell Hancock, et al., 1998) as a sole carbon source. Goodwin et al. (2001) observed methyl bromide consumption at tropospheric levels by methylotrophic and methanotrophic bacteria in culture. Harper et al. (2000) found a strain of soil bacteria that converts methyl bromide to methyl chloride

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in a process called transhalogenation, which may represent another significant methyl bromide sink.

In 2003, Varner et al. reported net production of methyl bromide in temperate forest soils and indicated that fungi may be the responsible source. Measurements were made in College Woods, Durham, NH using field enclosures, and soil samples were collected and incubated in the laboratory. The methyl bromide flux measurements made by these investigators ranged from −3.0 to +4.0 μg CH_3Br m^{-2} d^{-1} (Varner, 2000; Varner et al., 2003).

The net flux measurements of methyl halides are a result of competing production and consumption processes by different mechanisms and organisms within the soil or sediment ecosystem (Rhew et al., 2001; Varner et al, 2003; White et al., 2005). Field studies using chamber measurements have shown high spatial and temporal variability in methyl halide emission rates. The emission rates were associated with various environmental conditions including light level (Dimmer et al., 2001), vegetation cover (White et al., 2005; Rhew et al., 2002) and plant species (Saini et al., 1995; Rhew, 2000), temperature (Varner et al., 1999), season (Rhew, 2000; Varner et al., 1999), and ground water level (White et al., 2005).

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1.4.11 Methyl Halides from Fungi

Fungi have been investigated as a source for methyl halide emissions (Harper, 1985; Watling and Harper, 1998; Harper, 2000). Lee-Taylor and Holland (2000) calculated a rate of 0.5–5.2 Gg yr^{-1} (1.7 Gg yr^{-1} geometric mean) as the potential methyl bromide flux from the decay of aboveground woody litter by fungi. Watling and Harper (1998) estimated global methyl chloride emissions of 160 Gg yr^{-1} by fungi in all forest types.

Table 1-3 lists the estimates of global methyl halide emissions from fungi that have been calculated by several authors.

Table 1-3: Global Methyl Halide Emissions from Fungi. A global emission estimate from ectomycorrhizal fungi was not calculated by Redeker et al. due to the variability of CH_3Cl, CH_3Br, and CH_3I conversion in species tested.

Source	Methyl Chloride	Methyl Bromide	Methyl Iodide	Reference
Temperate forests (excluding Australia)	38.7 Gg CH_3Cl yr^{-1}	-	-	Watling & Harper (1998)
All forest types	162.7 GgCH_3Cl yr^{-1}	-	-	
Terrestrial wood-decay fungi	100 Gg Cl yr^{-1}	-	-	Khalil et al. (1999)
Above-ground woody litter decomposition by wood-rot fungi	-	0.5-5.2 Gg CH_3Br yr^{-1}	-	Lee-Taylor & Holland (2000)
Ectomycorrhizal fungi	0.003-65 μg CH_3Cl g^{-1} dw fungi day^{-1}	0.001-3 μg CH_3Br g^{-1} dw fungi day^{-1}	0.02-12 μg CH_3I g^{-1} dw fungi day^{-1}	Redeker et al. (2004)

Moore et al. (2005) measured a methyl chloride concentration of 843 ppt (1.5 times ambient levels) directly near a polypore growing on a tree in Brazil. The

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majority of fungi species investigated for production of methyl halides have been polypores. However, over 1200 known polypore species remain untested for methyl halide emissions (Lee-Taylor and Holland, 2000).

1.5 Overview of Fungi

Fungi comprise a separate biological kingdom because of their unique characteristics. Although some fungi may look like plants, they do not carry out photosynthesis. They are heterotrophs; that is, they cannot produce their own food but obtain it from an external source. Fungi perform a critical role as decomposers in the environment, functioning wherever there is an opportunity and under a variety of conditions. These opportunists exist in nearly every habitat on earth, including many harsh environments. Their presence can be detrimental, causing human and plant diseases, or they can be beneficial, producing important drugs like penicillin and cyclosporine (Hodge and Palmer, 2006).

The most readily recognized fungi are the basidiomycetes; these are the typical capped mushrooms that are associated with the forest. The remaining fungal phyla contain species that are more inconspicuous and difficult to see without microscopic magnification. Although these fungi do not form large fruiting structures, they are very common in nature.

There are thousands of known species of fungi; in fact, many fungi remain unidentified (Hawksworth, 2001; Frey, 2002), and the current estimate of 1.5

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million species continues to be discussed. Gams (2007) estimated "3,300 currently known species of soil fungi". Researchers can now use molecular techniques to assess species diversity and classify fungi. O'Brien et al., (2005) estimated global species richness of soil fungi in the range of 3.5 to 5.1 million after extracting DNA from soil and litter samples in a temperate forest.

Terrestrial ecosystems can contain a large amount of fungal biomass. For example, in a northern hardwood forest in New Hampshire, Taylor et al. (1999) calculated active fungal biomass in the range of 41–795 mg m^{-2} forest floor organic matter. Clearly, the prevalence of fungi, the potential number of species, and the amount of fungal biomass has a significant impact on the earth's environment.

1.5.1 Fungi in Salt Marshes

Many species of fungi inhabit salt marshes. However, the larger, more visible fruiting bodies that are typical of basidiomycetes are uncommon in salt marsh environments. For example, the marine basidiomycete *Nia* spp., which have been isolated from submerged horsehair baits (Rossello et al., 1993) and the salt marsh plant *Spartina maritima* (Curtis) Fernald (Barata et al., 1997), have fruiting bodies under 5 mm in diameter. The *Puccinia* species, also found on salt marsh plants, *Spartina alterniflora*, (Gessner and Kohlmeyer, 1976) is a rust fungus. Its fruiting bodies are smaller than 2.5 mm. The most common types of fungi associated with decomposing salt marsh grasses, such as *Spartina* spp.,

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are ascomycetes (Gessner and Kohlmeyer, 1976; Castro and Freitas, 2000; Newell et al., 2000).

Because the dead leaves of *Spartina* are not abscised but remain attached to the plant, fungal production occurs on the standing decaying plant (Newell et al., 2000). Castro and Freitas (2000) studied leaf decomposition of *Spartina maritima,* and found that fungal activity was greater before leaf fall. They suggested that bacteria take over the decay process once the leaves fall to the ground. However, this may not be the case, according to Newell (2003). Instead, a shift in fungal community structure may be taking place on the sediment (Newell, 2003). Or, there may be an ecological interaction between the bacterial and fungal decomposers of *Spartina* (Buchan et al., 2003).

1.5.2 Mycorrhizal Associations in Marine Fungi

The success of plants living in harsh environments may be due to mycorrhizal associations, which are plant-fungi symbioses that can provide the plant with nutrients. Thus, it is not unusual that similar plant-fungi interactions are found in salt marshes. Mason (1928) first described the presence of mycorrhizal fungi in the roots of salt marsh plants. The mycorrhizae may enhance oxygen uptake and reduce salt stress in the plants (Hyde et al., 1998). Arbuscular mycorrhizae form associations with plants located in the higher zones of the marsh where species, such as *Spartina patens* and *Distichlis spicata* occur (Burke et al.,

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2003). Muhsin and Booth (1986) documented the presence of several species of fungi on inland salt marsh halophytes (salt tolerant plants) in Manitoba, Canada. The association that mycorrhizal fungi form with salt marsh plants suggests that the fungus has developed a tolerance to flooding. However, salinity has been shown to have a negative affect on the growth of mycorrhizae and its ability to infect halophytes, such the sea aster plant, *Aster tripolium* L. (Carvalho et al., 2003).

1.6 Investigation of Fungi as Potential Source of Methyl Halides

Since 1994, research on the cycling of methyl chloride and methyl bromide has been conducted in upland soils, temperate forests, agricultural fields, wetlands, and coastal salt marshes located in southeastern New Hampshire (see previously published works by Shorter et al., 1995; Hines et al., 1998; Varner et al., 1999a; Varner et al., 1999b; Varner 2000; Varner et al., 2003; and White et al., 2005). These studies have provided more insight into the controlling processes in these environments, and have led to the present investigation of fungi as a potential source of methyl halide emissions.

In this investigation, fungi were collected from fruiting bodies, plant and root tissue, and soils from various locations in southeastern New Hampshire. The fungi from these sources were cultured in the laboratory and assayed for methyl halide production in an effort to answer the following questions:

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1. **Do fungi isolated from these sites produce methyl halides?**

Fungi were cultured in media and the methyl chloride, methyl bromide, and methyl iodide emission rates were determined.

2. **Are the rates significant enough to be responsible for observed fluxes in these and other environments?**

Methyl halide production rates by fungi in the laboratory soil incubations were compared to field measured fluxes.

3. **Do the halide ratios observed in culture reflect those observed in field measurements?**

Emission rates were quantified to identify a signature ratio. This ratio was also compared to the halide ion content in soils from the field sites.

4. **Does the linear least squares fit model yield an overestimation of fungal emissions?**

Two types of data analyses were used to determine whether the linear regression fit method, which is traditionally used to measure methyl halide production, can potentially over-estimate production rates.

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The following chapters in this document describe the research procedures that were used and the results of the investigation. An analysis and discussion of the data are also provided.

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CHAPTER 2

2.0 COLLECTION OF FUNGI AND LABORATORY PROCEDURES

This chapter describes the procedures that were used to collect and culture fungal samples, obtain pure isolates in laboratory culture, and assay the fungi for methyl halide production using gas chromatography.

2.1 Overview

The goal of this study was to expand the current knowledge of methyl halide emissions from basidiomycete fungi. To accomplish this goal, several fungi samples that were putatively identified as basidiomycetes were collected in the field and isolated in laboratory culture. The individual fungal cultures were grown in flasks and the headspace gas was analyzed to measure levels of methyl halides. These emission measurements were then compared to rates of other fungi that have been reported in the literature.

2.2 Fungi Samples

Fungi samples were collected during the spring, summer and fall months between June 2004 and October 2005.

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The samples were collected from the following different sources:

- fruiting bodies (mushrooms)
- wood baits
- soils
- plant tissue

The samples were returned to the laboratory and refrigerated. Generally, the fungi were cultured within two days of receiving them from the field; beyond that time, the samples deteriorated too rapidly and could not be used.

The procedures that were used to culture each sample type are described in Section 2.4 Fungal Isolation Protocol. The entire sample inventory is listed in Appendix A.

2.3 Experimental Sites

The experimental sites from which samples were obtained included four different ecosystem types:

- agricultural fields
- a fresh water fen
- salt marshes
- a temperate forest

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2.3.1 Agricultural Fields

Soils and fruiting body samples were collected from two fields at Kingman Farm, (43° 10′ 08″N, 70° 56′ 05″W), an agricultural research farm maintained by the University of New Hampshire near the Durham, NH campus. The soils are drained glacial till spodosols. Methyl bromide flux measurements were made at this site in 1994 and 1999 by Varner (Varner et al., 1999; Varner 2000). Field measurements showed both consumption and production of methyl bromide in the soil, with a net uptake of 0.0007 mg m^{-2} d^{-1} of methyl bromide consumed by the cornfield soil (Varner, 2000).

Kingman Farm 1 (Site KF1). Site KF1 is a fallow field, that was last used in 2003 to grow cucurbits; it contained some weeds predominantly, clover (*Trifolium* spp.) and chickweed (*Cerastium* sp.). Several "LBM" (Little Brown Mushrooms, possibly *Mycena* spp.) small, light brown gilled mushrooms were observed at different times at this location and two samples were collected, cultured, and assayed for methyl halide production. During the 2006 season, the field was planted with cucurbits.

Kingman Farm 2 (Site KF2). Site KF2 is a cultivated cornfield that has been used to grow corn every year; an application of dairy manure was applied in the spring. Soil samples were collected for soil incubations in August 2005, November 2005, February 2006, and September 2006. Soil was collected and

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aggregated in the field from rows and between post-harvested corn stalks that had been chopped but remained enrooted.

2.3.2 Freshwater Fen

Sallie's Fen is a nutrient poor fen located in Barrington, New Hampshire (43° 12.5'N, 71° 03.5'W). The 1.9 x 10^4 m^2 wetland is dominated by *Sphagnum* spp., *Carex* spp., and ericacious plants (Varner, 2000). Ongoing monitoring at this research site has provided a wealth of meteorological and trace gas exchange data (Crill et al., 1994; Frolking and Crill, 1994; Melloh and Crill, 1996; Carroll and Crill, 1997; Bubier et al., 2002; Bubier et al., 2003; Treat et al., 2007), including methyl bromide and methyl chloride cycling (Varner et al., 1999; Varner, 2000; White et al., 2005).

Fruiting bodies that were growing directly on the *Sphagnum* plants, as well as in the sediment underneath marsh shrubs were collected at this site. Fungi were also isolated from wood baits buried at this site.

2.3.3 Salt Marshes

Plant samples were collected from the following four salt marsh locations along the southern New Hampshire and Maine coastline:

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- Odiorne Point, Rye, New Hampshire
- Chappy's Landing, Stratham, New Hampshire
- Brave Boat Harbor, Kittery, Maine
- Mill Pond, York Harbor, Maine

The salt marsh sites at Odiorne Point, Brave Boat Harbor and Mill Pond were in tidal river marshes located within one mile of the Atlantic Ocean. The Chappy's Landing site is an inland tidal marsh along the Squamscott River, approximately 10 miles from the open ocean. All sites are dominated by salt marsh grass, *Spartina* spp. plants.

2.3.4 Temperate Forest

The College Woods site is a 28-hectare mixed deciduous-conifer temperate forest. Soils are drained upland inceptisols (Varner et al., 1999; Varner, 2000). Methyl bromide fluxes were measured at this site in 1994 and 1999 by Varner (2000).

Fruiting body samples were collected from various locations within College Woods (43° 08′N, 71° 57′ W) in Durham, New Hampshire. Fungi were also isolated from wood baits buried at this site.

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2.4 Fungal Isolation Protocol

Different procedures were used to isolate fungi from field samples in order to successfully obtain a variety of basidiomycete species. When available, small segments of fungal tissue from fruiting bodies were used; otherwise, direct plating of soil, plant tissue, or a piece of wood substrate was used. The isolation of fungi for assaying was performed using standard microbiological methods with aseptic technique (Parkinson, 1994). Sterile growth medium was prepared in sterile petri dishes in advance, covered, and refrigerated until needed (Bills and Foster, 2004). (See Section 2.14 at the end of this chapter for the media recipes.)

2.4.1 Fruiting Body Tissue Culture

Small segments (<1 cm) of fruiting body were cut from the sample and soaked in a sterilizing solution (20% household bleach, 20% ethanol, 60% distilled H_20) to remove any surface contaminants (R. Blanchard, unpublished data, 2004). The segments of tissue were then blotted dry and placed on MYBDA selective growth medium. The MYBDA medium contains fungicides and antibiotics (benomyl, chlortetracycline-HCl, streptomycin sulfate, and penicillin G); MYBDA was developed to isolate basidiomycete species and to inhibit the growth of bacteria, as well as “weedy” fungi species (Thompson, 1998).

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The covered petri dishes were set aside in an incubation room (24°C) for several days. When growth characteristics could be visually differentiated a small piece of mycelium and attached media was removed and placed on a new plate of media to grow separately. This procedure was repeated as many times as needed until a single fungal isolate was obtained. In most cases, mycelia covered a full plate (8 cm diameter) in one to three weeks. After a single isolate was well established on the plate, it was prepared for storage in cryovials. (See Section 2.6.)

2.4.2 Direct Soil Culture

Soil cultures were prepared by sprinkling a small amount (approximately 0.05 g) of soil collected from the field directly onto prepared selective growth medium. The soil was not rinsed with sterilizing solution. Soil cultures were incubated and re-plated following the procedure in Section 2.4.1, until a pure isolate was obtained.

2.4.3 Plant Tissue Culture

The plant tissue was cultured to obtain fungi that inhabit salt marshes. Segments of salt marsh grass, *Spartina* tall- and short-form species, were collected from different salt marshes along the southern New Hampshire and Maine coastline. Upon return to the laboratory, the sediment was rinsed from the roots using cool tap water followed by a mild soap solution (0.1 g dish soap L^{-1}

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water) and a final rinse with dH_2O. Small segments of root were cut from the plant and surface sterilized, as described in Section 2.4.1. The green plant leaves and dead culms (hollow plant stems) were surface sterilized then plated onto MYBDA medium. The upper parts of the plant were not rinsed in water or soap solution.

2.4.4 Wood Bait Culture

To obtain fungi located below the soil surface, wood baits were prepared and buried in 2 x 2 m field plots. The plots were set up in duplicate at College Woods, Sallie's Fen, and Chappy's Landing. Due to time constraints, wood baits were not planted in the agricultural fields at Kingman Farm. Two plots containing ten baits each of pine (n=20), maple (n=20), and oak (n=20) were used.

Small pieces of wood were pre-cut to roughly 3 cm x 2.5 cm x 2.5 cm chunks. A nylon string approximately 12 inches long was tied around each bait, and a plastic identification label was attached to the opposite end of the string. Figure 2-1 shows an example of a wood bait.

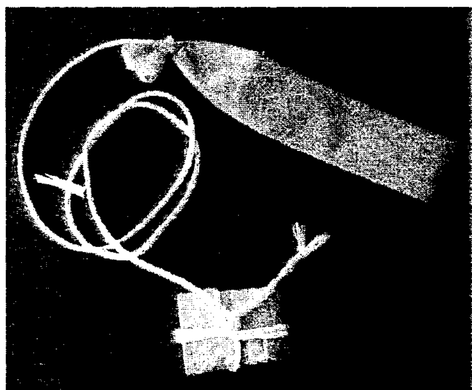

Figure 2-1: Wood Bait

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The bait, string, and label were wrapped in aluminum foil and autoclaved for 20 minutes at 123°C before burying at the field sites. The sterilized bait was unwrapped in the field and buried approximately 10 cm deep using a sterile trowel or metal spatula. The string was unwound as the soil/peat was back filled so the identification label remained on the ground surface. To prevent contamination, the trowel was cleaned with sterilizing solution and wiped with sterile paper towel between burying each bait.

The wood baits were left in the ground for 6–12 months and then retrieved. Upon return to the laboratory, a small sliver of wood and any attached soil or roots were removed from the bait using a sterile scalpel and placed on a plate of sterile MYBDA medium. Wood bait cultures were incubated and re-plated until a pure isolate was obtained.

2.5 Species Identification

Microscope slides were prepared using a small piece of fungal hyphae that was stained with Phloxine and Cotton Blue stains. Spores were viewed under 40x magnification for identification of ascomycetes and zygomycetes; the presence of clamp connections (microscopic reproductive structures unique to many species of basidiomycetes) was used to confirm basidiomycete samples (Thorn et al., 1996). Identification was made using Barnett and Barry (1998) and Wang and Zabel (1990).

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2.6 Storing Fungal Isolates

Once a fungal isolate was obtained in culture and was well established on the growth medium, it was prepared for long-term storage. A small piece of mycelium was removed from the medium and re-plated onto sterile CMEA medium, which provides a mild, nutrient-rich substrate. After growing on the plate for approximately two weeks, cultures were stored in sterile water in cryogenic vials (1.2 ml Nalgene or 1.5 ml Nunc CryoTube), as described by Burdsall and Dorworth (1994). Figure 2-2 is an example of a plated fungal isolate and a cryovial that has been prepared for storage.

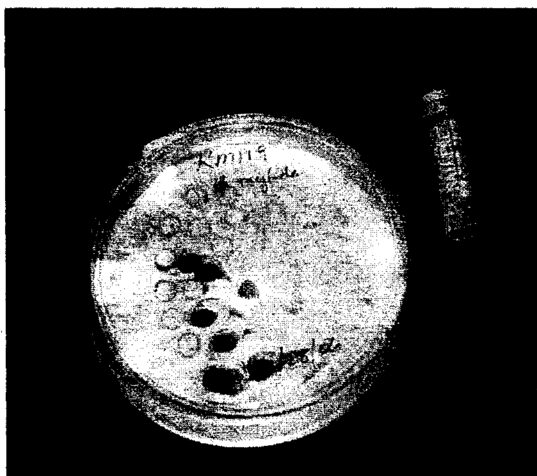

Figure 2-2: Sample Plate and Cryovial

2.7 Fungal Incubation Assays

To prepare a fungal isolate for methyl halide assays, a 6-mm plug was removed from the stored cryovial, placed on a new plate of sterile growth MYA medium (see 2.14 for media recipes) and set aside at room temperature to grow

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for approximately 1–3 weeks. The length of time required for the fungi to cover the plate (8 mm diameter) was monitored and recorded so that the same amount of time could be allotted to that isolate when it was grown in flask cultures.

Two types of substrate were used for the incubation assays: liquid media or soil (or both). The substrate chosen was based on the original location from which the fungal sample was collected and whether the fungus was a ground-inhabiting species.

2.7.1 Preparing Liquid Media Incubations

Standard growth medium from malt and yeast extract was prepared for liquid media incubations. Agar was omitted so that the media would remain liquid and allow later filtering of fungal biomass. The liquid media was supplemented with halide ions using potassium chloride (KCl) and pre-made potassium bromide (KBr) and potassium iodide (KI) solutions. (See Section 2.14 for the media recipes.)

A 100-ml portion of medium was poured into individual 250-ml Erlenmeyer flasks. The flask was sealed with a cotton plug and silicone stoppers were autoclaved separately. (In some instances, stoppers were set loosely onto the flasks, wrapped in foil, and autoclaved together).

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To prevent photolytic production of methyl iodide, the flasks were entirely covered in aluminum foil. The flasks were labeled and autoclaved for 20 minutes at 123°C. After the flasks were removed from the autoclave and cooled to room temperature, three flasks were inoculated with fungi. A 6-mm plug of fungal mat was punched out of the MYA medium and dropped into each flask. Three replicate flasks without fungi provided the controls. The flasks were re-sealed with a sterile silicone stopper. The inoculations were performed under low light conditions inside a laminar flow hood using aseptic technique. The flasks remained wrapped with aluminum foil for the entire incubation and assay period.

2.7.2 Preparing Soil Incubations

The soil incubation assays were prepared to measure methyl halide emissions from soil-inhabiting fungi. Soil incubation assays contained soil that was collected from College Woods and Kingman Farm. The soils were inoculated with fungi that had been originally collected and isolated from the same location.

Soil weights were pre-measured to determine flask headspace volume. A College Woods soil sample of 35 grams and a Kingman Farm soil sample of 60 grams filled the flask to approximately 100 ml. The weights differed between sites due to the differing soil texture.

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Because the soil surface contains the greatest microbial abundance (Fierer et al., 2003), soils were collected from the surface layer (~ 0 –10 cm) at both sites. To maintain consistency between samples, the soils were collected randomly from the site and aggregated in the field.

Two sets of soil incubations were prepared. In the first set, the soils were not sieved. However, any twigs, whole leaves, and stones greater than 1 cm were removed by hand upon return to the laboratory.. Fine roots, small stones, and some coarse debris were left in the sample in order to retain as much of the field characteristics as possible. This set of soil was immediately autoclaved at 123˚C for 20 minutes and stored in closed plastic bins. Later, when the flasks were prepared for assaying, the soil was consecutively autoclaved two more times to ensure destruction of any bacteria or fungi. To test whether autoclaving destroyed pre-existing microorganisms, the dried soil from a soil incubation (CWM62-B) was plated onto growth medium after assaying the sample for methyl halides. Fungal hyphae containing clamp connections re-emerged on the plates of the soil samples while no growth was observed after one week in plates containing the soil controls (soil incubations that were not inoculated with fungi after autoclaving).

The second set of soil incubations was used for comparison to the autoclaved soils. In these incubations, approximately 50 grams of soil was placed directly into sterilized 250-ml flasks and immediately capped with sterile

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silicone stoppers in the field. The amount of soil added to each flask in the field was approximated because the empty flasks were covered with aluminum foil and autoclaved before field collection.

The second set of soil incubations was inoculated with fungi the same day it was collected from the field; it was not sieved, hand picked, or autoclaved. Three replicates were prepared for each fungal sample. Three flasks containing soil were each inoculated with a single 6-mm plug of fungi from the MYA medium, and three replicates without fungi provided the controls. One milliliter of sterile dH_20 was added to each flask to moisten the soil; no halide solutions were added. The flask was gently shaken for approximately three seconds and a sterile silicone stopper was used to seal each flask.

After incubating the flask cultures for 1–3 weeks at room temperature, flasks were set up on the Zero Air Flushing system to assay for methyl halide emissions.

To prevent photolytic production of methyl iodide, the soil incubations were prepared under low light conditions and flasks were covered with aluminum foil for the entire collection, incubation, and assaying period.

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2.8 Zero Air Flushing System

The zero air flushing system is constructed from a series of 10 brass T-joint fittings installed approximately 2.25 inches apart along a 24-inch length of copper pipe. Each T-joint fitting is controlled by a toggle valve at the top and has approximately 12 inches of plastic tubing attached at the bottom. The opposite end of the plastic tubing is fitted with a stopcock valve onto which a hypodermic needle is attached. A carbon filter (Hydrocarbon Trap, Alltech Assoc., Inc.,) containing approximately 115.6 g activated carbon (cocoanut charcoal, Fisher Scientific) was installed inline between the output valve from the ultra high-purity (UHP) zero air cylinder (2400 psi) and the input end of the zero air flushing system pipe. The filter was added to trap any methyl halide gas contained in the zero air gas cylinder. The end of the zero air flushing system pipe is capped. The entire system is mounted on the wall approximately 17 inches above the laboratory bench top.

Following the incubation period for the fungal samples, the flasks were set up on the zero air flushing system. Two sterile 20 gauge, 1½ inch hypodermic needles were inserted in the stopper of each flask. One needle was connected to the plastic tube supplying air from the zero air flushing system, and the other needle served as a vent from the flask.

At the beginning of each run, the flask headspace was flushed with ultra high-purity (UHP) zero air (19.5%–23.5% oxygen, nitrogen balance; total

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hydrocarbons <1.0 ppm), which was obtained from a commercial gas distributor. The UHP zero air was set at a pressure of 20 psi and flushed the flasks for approximately one hour. After one hour had elapsed, the air was shut off, the flasks were disconnected, and the hypodermic needles removed from the stoppers. This sealed the flasks.

The gas chromatography/electron capture detector (GC/ECD) cryotrapping system was started by first initializing the HP ChemStation (B.02.04) software application. At this time, the GC/ECD cryotrapping system cleared the sample loop by heating to 180° C. (The system is described in more detail in Section 2.9 GC/ECD Cryotrapping System.)

Meanwhile, a 60-ml glass syringe was “cleaned” by withdrawing and expelling a full syringe of UHP zero air four consecutive times. Then, to maintain pressure within the flask, a 10-ml sample of zero air was injected into the flask through the stopper. The headspace was mixed by withdrawing and injecting the air back into the flask using the syringe without removing the needle. This was repeated four times. A final 10-ml headspace sample was extracted from the flask and the syringe locked to prevent loss or contamination of the sample. After three minutes from when the system was initialized had elapsed, the 10-ml sample was injected into the GC/ECD cryotrapping system injection port. After approximately 23 minutes, the three methyl halide gases in the sample were detected at predicted response times and the levels displayed in a

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chromatogram. A series of gas samples were extracted from each flask over a period of 0–120 hours and run on the GC/ECD cryotrapping system. The glass syringe was cleaned with UHP zero air between every injection sample to prevent contamination.

2.9 GC/ECD Cryotrapping System

The GC/ECD cryotrapping system used in this study is based on the design by Kerwin et al. (1996) that was developed for detecting low-level mixing ratios of methyl bromide. The system is also described in detail in Varner (2000). The gas chromatograph (Shimadzu GC-8A) uses non-reactive, ultra high-purity nitrogen (N_2) as the carrier gas flowing at a rate of 5ml/min to sweep the sample into the electron capture detector.

In the electron capture detector, the radioactive source, ^{63}Ni, emits beta particles that create a high electrical signal. When the gas sample moves into the detector, the halogens in the gas absorb electrons from the radioactive source and reduce the electrical signal. The reduction in current is directly proportional to the mass of the constituent halogen. The reduced signal is then converted to a positive signal and is displayed in a chromatogram. The peak height of the signal in the chromatogram is compared to the peak height and response time of the signal in a standard gas that contains known concentrations of the halogen. Aliquots of a standard cylinder containing mixing ratios of 131

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ppbv CH_3Cl, 120 ppbv CH_3I and 126 ppbv CH_3Br (uncertainty is +/-5%) were also run (Cylinder #CC169180, Apel/Reimer, Miami, Florida).

The methyl chloride, methyl bromide or methyl iodide constituents in the gas sample can be separated by their adsorption-desorption properties. As the gas sample moves into the cryotrapping system (Cry-O-Trap Model 951, Scientific Instruments, Inc.), rapid cooling (-78°C) and subsequent rapid heating (180°C) converts the sample between its gas and solid phases. A 15 m x 0.53 mm (ID) GSQ capillary column (J&W Scientific, Folsom, CA) that is located inside the GC/ECD system is packed with a material that selects for the target adsorption/desorption properties of the specific constituents. These properties help to identify the constituent gas.

2.10 Standard Curve

Methyl halide concentrations in the sample assays were determined by comparing the peak heights of the sample to a standard curve. Increasing aliquots (0.5 ml, 1.0 ml, and 2.0 ml) of a standard gas containing known concentrations (± 5% uncertainty) of methyl chloride (124 ppb), methyl iodide (99 ppb) and methyl bromide (124 ppb) were run with each sample in order to create the standard curve.

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2.11 Adjustments to the Assaying Procedure

In 2005, a new standard was obtained that included known amounts of the three methyl halides: methyl chloride, methyl bromide, and methyl iodide. Prior to 2005, the samples were only tested for methyl bromide. Also, soil incubation assays were started in 2005. The assays performed in 2003 and 2004 measured only methyl bromide emissions from cultures in liquid media.

2.12 Estimating Methyl Halide Flux from Incubations

Two types of statistical analyses were used to determine the methyl halide fluxes from the fungal samples. One method plotted a linear regression using "least squares fit" of the methyl halide concentrations over time. The second method used a Bayesian model with Markov Chain Monte Carlo (MCMC) sampling. Both methods calculate a slope (the methyl halide concentration vs. time) for each flask.

The flux was then normalized to mass by dividing by the fungal biomass, as follows (CH_3X where X=Cl, Br, or I):

ng CH_3X /hr * flask headspace volume/sample volume * 1/g dry wt fungi (or g dry soil)

The first method (the linear regression least squares method) is typically used in studies such as this one to report fluxes from biogenic sources. A

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limitation of this method is that by not accounting for the uncertainty in the standard, a slightly positive or over-estimated flux can be generated.

The statistical Bayesian model provided a more rigorous analysis of the data by estimating a level of uncertainty and applying that error estimate to the sample observations. In this study, the model could only be applied to a small set of data due to inadequate sample size.

2.12.1 Statistical Model

The statistical model was created for this study by Dr. Andrew Cooper of the UNH Department of Natural Resources using the WinBUGS (Bayesian inference Using Gibbs Sampling for Windows) software program. The WinBUGS software was developed at the Medical Research Council (MRC) Biostatistics Unit, at the University of Cambridge, UK.

The model accounts for a known ± 5% uncertainty in the standard that is reported by the gas manufacturer, and calculates the level of uncertainty in the standard curve. The estimated uncertainty was then incorporated into the estimated methyl halide concentration in the headspace of each sample flask. A slope of concentration over time was calculated for each flask. By averaging the slopes of all flasks for all sampling days by fungal isolate, a resulting mean flux could be reported (ng CH_3X /time). An adjustment for sample volume was made

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by dividing the volume of the flask headspace by the volume of the sample. The flux was normalized to mass (1/grams fungi in soil [or media]) then, adjusted for the control (sample flux − control flux).

2.12.2 Estimating Fungal Biomass

To estimate the emissions from fungi in media assays, the flux rate was divided by the dry weight fungal biomass, except for five samples run in September, 2003 (isolate # CWM5, CWM8, and CWM23) where wet weight was used.

The amount of fungal biomass in the soil incubation assays was not measured. The results of Loss on Ignition analysis show 14% mean organic matter in the temperate forest soils collected from College Woods, and 5% mean organic matter in the agricultural soils from Kingman Farm.

Biomass in Liquid Media. After assaying media samples, fungal growth was rinsed and strained from the medium through 24-cm fluted paper filters using a funnel and evacuation flask. The fungal biomass and filter were then placed in plastic trays and dried at approximately 60°C in a drying oven for several days. The control samples were also filtered and dried. After drying, the fungal biomass dry weight was calculated as the difference between the control average and the fungal sample average.

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Biomass in Soil Incubations. The amount of fungi in soils is highly variable and is affected by many factors, such as soil type, vegetation, moisture, and season. Several methods can be used to assess the amount of fungi in soils (for example, phospholipid fatty acid analysis, chloroform fumigation-extraction, direct count microscopy, selective inhibition and substrate-induced-respiration, and DNA analysis). An assessment of fungal biomass using one or more of these methods was beyond the scope of this project. Therefore, emissions from soil incubations were normalized to the amount of soil (grams) in the flask.

2.13 Soil Properties

Soil properties were analyzed using two additional tests: substrate-induced respiration analysis was conducted to measure the amount of CO_2 respired by microbes in the soils, and organic carbon content was measured by using loss-on-ignition analysis. It is important to note that inhibitors, such as antibiotics and fungicides, were not added to the soil during the analysis; therefore, any observed respiration cannot be attributed exclusively to fungi.

2.13.1 Substrate-Induced Respiration (SIR) Analysis

Before soils were removed from the flasks, substrate-induced respiration analysis was conducted to measure CO_2–C respired by organisms in the soil. First, a baseline measurement was obtained by extracting a 60-ml headspace sample from the flask. The syringe stopcock was locked and the sample was set

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aside for CO_2 analysis on a GC/TCD (Shimadzu GC-8A gas chromatograph) or Li840 (LI-COR, Inc.). Within one hour, each flask received 1 ml of glucose solution (4 g glucose L^{-1} water). The flask was immediately re-capped, gently shaken for approximately three seconds, and set aside at room temperature to incubate. Flasks were opened and the glucose solution was added inside a laminar flow hood to prevent contamination. After four hours had elapsed, another 60-ml headspace sample was extracted from the flask and set aside for CO_2 analysis.

Respiration was determined from CO_2 in the flask headspace samples taken before and after the addition of the glucose substrate. The rate of respiration was calculated from CO_2 concentrations in parts per million by volume (ppmv), using the following equation:

(CO_2 concentration before incubation – CO_2 concentration after incubation) = µg CO_2-C g^{-1} dry weight soil hr^{-1}

2.13.2 Loss-on-Ignition (LOI) Analysis

The Loss-On-Ignition (LOI) method was used to analyze organic carbon content in the soil samples. First, the soil was rinsed from the flask, put in a small plastic tray, and dried in an oven at approximately 60°C for several days.

Then, a 7-g sample of the oven-dried soil was measured into an aluminum tray and placed in a muffle furnace at approximately 475°C for 24 hours. The

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ashed soils were removed from the furnace and re-weighed. The soil organic carbon content was calculated from the difference between the soil weight before and after combustion, using the following equation:

LOI (g kg^{-1}) = [(oven-dried soil wt – soil wt after combustion)/oven-dried soil wt] x 1000

Organic matter in soil will contain varying amounts of fungi, and the loss after combustion does not equate to fungal biomass in the sample.

2.13.3 Ion Chromatography Analysis

Ion chromatography analysis was performed to assess the background levels of halogens, Cl^-, Br^-, and I^-, in the soil samples. Five replicates each of College Woods soil and Kingman Farm soil were analyzed. Controls of water were also analyzed. For comparison, the sterile water used for culturing fungi and a sample of deionized water from the ion chromatography laboratory was tested.

One series of soil samples were control soils that had not been autoclaved before assaying for methyl halides. These soils were collected from College Woods (uCW1–5) and Kingman Farm (uKF1–5) on 3-February-2006, placed directly in sterilized flasks, capped with sterile stoppers, and covered in aluminum foil in the field. The soils were not inoculated with fungi.

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Upon return to the laboratory, the soils were immediately set up on the zero air flushing system. Samples were assayed for methyl halides between 4-February-2006 and 21-February-2006. Substrate induced respiration tests were conducted on 6-March-2006. Soils were dried 28-March-2006, and prepared for ion chromatography on 13-April-2006.

A second series of soil samples were control soils from College Woods (CW1–5) and Kingman Farm (KF1–5) that were autoclaved before assaying for methyl halides. The soils were collected in November, 2005 and autoclaved once that day. The soils were autoclaved an additional two times, consecutively, on 30-January-2006 before assaying for methyl halides. Soils were assayed for methyl halides from 31-January-2006 to 9-February-2006. Substrate induced respiration tests were conducted on 10-Feb-2006. Soils were dried 28-Feb-2006, and prepared for ion chromatography on 9-March-2006.

To prepare the soils for ion chromatography analysis, 10 grams of soil were mixed with 50 ml distilled water in a beaker and placed on a platform stirrer (speed setting 8) for one hour. Then, a 10-ml sample of the soil/water mixture was drawn through a filter (Pall 0.45 µm 10N chromatography acrodisc) using a 60-ml plastic syringe. The filtered water sample was injected into two, sterile 5-ml plastic vials and capped. The syringe was thoroughly rinsed with diH_20 between samples, and a new filter was used for each sample. Vials were

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completely covered to prevent photolytic reaction and were refrigerated until analyzed. Samples were analyzed by another laboratory within two weeks.

Major cations were analyzed using a Dionex CS12, 50 µl sample loop with CSRS suppression and 20 µM MSA element; major anions were analyzed using a Dionex AS11, 50 µl sample loop with ASRS suppression and 6 µM NaOH element. Iodide was analyzed using a Dionex AS16, 500 µl sample loop with ASRS suppression and 35 mM NaOH element. Five mixed-species standards were run prior to sample analysis, and three of five standards were rerun after analysis. Refer to Appendix B for the ion chromatography data.

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2.14 Media Recipes

Table 2-1 lists the media recipes that were used for culturing the fungi.

Table 2-1: Growth Media Recipes. Bacto™ malt extract (0.07% chloride), Bacto™ yeast extract (0.38% chloride), and Bacto™ agar (0.021% chloride) were used.

MYA
5 g malt extract
1 g yeast extract
10 g agar
500 ml dH_2O

Media Incubations (Flasks)
15 g malt extract
3 g yeast extract
15 ml of 20 mM L^{-1} KBr solution
15 ml of 2.0 mM L^{-1} KI solution
2.25 g KCl
1500 ml dH_20

MYBDA
5 g malt extract
1 g yeast extract
10 g agar
5 ml of 60 mg/L chlortetracycline-HCl solution
5 ml of 30 mg/L streptomycin sulfate solution
5 ml of 30 mg/L penicillin G solution
1 ml of 2 mg/L benomyl solution
483 ml dH_2O

CMEA
7.5 g malt extract
10 g agar
500 ml dH_2O

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CHAPTER 3

3.0 RESULTS

Fungal production of methyl halides was observed at varying rates from all field sites: the upland forest, freshwater wetland, salt marsh and agricultural field. The results of sample culturing methods, soil sterilization, and flux estimation methods are also described.

3.1 Sampling Results

A total of 195 samples were collected during the growing seasons between June, 2003 and October, 2005. Out of the 195 samples, 550 cultures were prepared and stored in cryovials. A summary of the fungi collected for this study is shown in Table 3-1. A complete inventory of the fungi is provided in Appendix A.

Forty isolates were putatively identified as basidiomycetes by microscopic identification of clamp connections (Thorn et al., 1996). However, more isolates may be basidiomycetes since they could not be ruled out by the absence of clamp connections. Clamps are only formed during a particular life cycle phase of the fungus, and not all basidiomycetes produce clamps in culture.

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Table 3-1: Summary of Samples Collected

Sample Source	Location	Collected	Cultured	Isolated
Mushrooms	Bartlett Forest	10	17	0
	College Woods	97	311	28
	(Other) Mushroom	2	7	0
	UNH Campus Lawn	3	10	2
	Sallies Fen	18	50	7
	Salt Marsh	0	0	0
	Kingman Farm	3	5	2
	Mushroom Total	**133**	**410**	**39**
Plants	College Woods	0	0	0
	Sallies Fen	11	28	0
	Chappys Landing	0	0	0
	Odiorne Point	2	10	0
	Mill Pond	2	6	0
	Brave Boat Harbor	3	13	1
	Kingman Farm	0	0	0
	Plants Total	**18**	**57**	**1**
Roots	College Woods	2	5	0
	Chappys Landing	2	5	0
	Roots Total	**4**	**10**	**0**
Wood Baits & Soil	Kingman Farm	3	6	0
	College Woods	13	17	0
	Sallies Fen	15	30	0
	Chappys Landing	13	20	0
	Wood & Soil Total	**44**	**73**	**0**
	Total:	**195**	**550**	**40**
Basidiomycetes Isolated by Site				
	Temperate Forest			28
	UNH Campus Lawn			2
	Salt Marsh			1
	Kingman Farm			2
	Sallies Fen			7
	Total:			**40**

Although the primary focus was to measure methyl halide production in basidiomycete fungi, some non-basidiomycetes represented in the phyla, ascomycota and zygomycota were also assayed.

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3.2 Flux Estimation Methods

As described in the methods section, fluxes were estimated two ways: by using a linear least-squares regression of the change in headspace concentration over time, and using a more complex Bayesian approach, which takes into account the uncertainty in the standard gas concentration.

Figure 3-1 is an example of the two fits of the data for the BBH2 fungus (an unidentified basidiomycete from a salt marsh) that was sampled from 9/11/06 to 9/13/06. When the linear least square approach was used, the predicted concentrations of the samples were higher and the slope of the regression fit for the flux was steeper than the Bayesian approach. The flux estimated by using the linear least squares fit was 0.51 ± 0.29 ng g^{-1} d^{-1}. When the Bayesian method was applied, the model returned a flux of 0.0026 ± 0.29 ng g^{-1} d^{-1}, which is approximately 0.5% of the flux estimate using the traditional linear least squares fit method.

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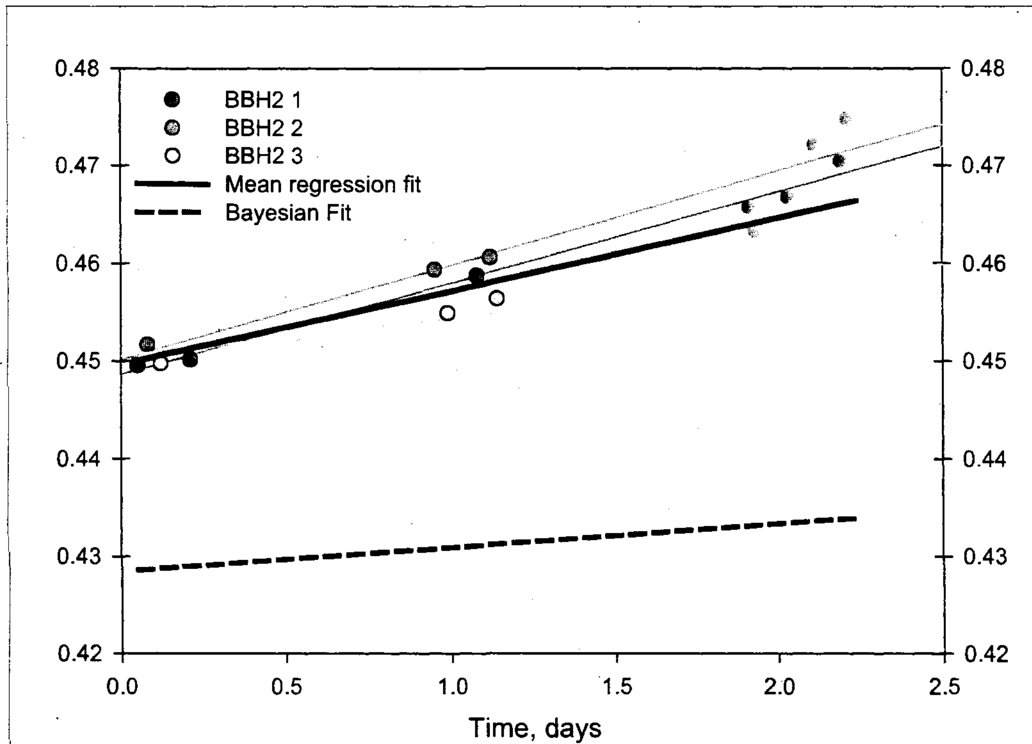

Figure 3-1: Comparison of Fluxes Using Both Estimation Methods. The linear least squares regression of headspace samples from BBH2 triplicate flasks (solid black line) and the Bayesian estimate (dashed line) for the slope of the best fit.

Fluxes were calculated for all fungal species first by using the traditional linear least squares fit of the standards, then using a linear least squares fit of the headspace concentration over time. The Bayesian model approach was applied to a subset of the data that fit the criteria. The results from the BBH2 incubations are representative of those that were observed in a comparison of the two methods for several other species (Table 3-2). In every case except for CH_3I fluxes from *Russala* CWM62B, the fluxes calculated using the Bayesian approach (shaded rows) were lower than those calculated with the least-squares fit. The decrease in flux ranged from 30 to 95% of the flux.

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Table 3-2: Comparison of Linear Fit vs. Bayesian Model Calculations

Genus Isolate# Run Date	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
Unidentified basidiomycete BBH2						
(9/06)	Not available		82.23	30.8	0.51	0.29
(9/06) model	Not available		-1.6	0.81	0.0026	0.34
LBM KM118						
(6/06) Media	Not available		-8.67	8.8	54.0	56.2
(6/06) Media Model	Not available		-0.07	0.72	5.65	3.21
(6/06) Soil, Autoclaved	Not available		0.17	0.03	-2.20	0.44
(6/06) Soil, Autoclaved Model	Not available		0.01	0.02	-0.14	0.04
(9/06) Soil, Not Autoclaved	2.53	2.46	-4.97		-.005	0.0027
(9/06) Soil, Not Autoclaved Model	-0.52	0.11	-0.34	0.16	0.001	0.12
Cantharellus CWM148						
(5/06) Media	Not available		5.06	1.68	Not available	
(5/06) Media Model	Not available		-0.01	0.21	Not available	
(5/06) Soil, Autoclaved	Not available		3.79	7.35	Not available	
(5/06) Soil, Autoclaved Model	Not available		0.02	0.01	Not available	
Russula CWM62B						
(5/06) Media	Not available		1.01	1.88	-4.64	4.22
(5/06) Media Model	Not available		-0.01	0.14	0.08	0.11
(5/06) Soil, Autoclaved	Not available		-0.34	0.06	0.009	1.49
(5/06) Soil, Autoclaved Model	Not available		-0.01	0.02	0.02	0.05
Clavaria CWM35						
(5/06) Soil, Autoclaved	0.14	0.13	-0.31	0.16	Not available	
(5/06) Soil, Autoclaved Model	0.002	0.03	-0.10	0.05	Not available	

3.3 Sampling Method Results

As described in the methods chapter, when the fungal or plant tissue sample was cultured, it was surface sterilized to remove any contaminants and eliminate species that can out-compete basidiomycetes in culture. However, the results show that surface sterilizing the tissue had no effect on the successful isolation of basidiomycetes. Plating the tissue directly onto the growth medium

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produced the highest number of basidiomycete isolates (26 of 76). Additionally, the length of time the tissue was soaked in sterilizing solution had no effect on the successful isolation of basidiomycetes. Soaking the tissue for 1.0 minute produced the next highest number of isolates (21 of 76); whereas, soaking for 0.5 minutes produced the same number of isolates as 2.0 minutes (12 of 76).

One of the methods used to collect fungi samples involved setting out wood baits at field sites. No basidiomycetes were isolated from the baits. Only non-basidiomycetes were isolated from baits that were retrieved from field sites. At the Chappy's Landing site, the baits were lost after being buried in the salt marsh for two years. This was probably due to the tides and ice buildup on the shore in winter. However, some wood samples were collected from a decayed boardwalk that was originally installed at Chappy's Landing in the late 1980's. Similarly, several wood baits could not be located in the freshwater fen site at Sallie's Fen due to overgrown vegetation.

3.3.1 Fluxes from Autoclaved Versus Non-autoclaved Soils

By incubating the upland forest and agricultural fungi samples in both sterilized and non-sterilized soils, the effects of methyl halide production by fungi growing in their substrate of origin could be observed. However, the background levels of methyl halide production from soils not inoculated with fungi first needed to be identified. In addition, because there was some concern that autoclaving

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soils may cause abiotic production of methyl halides, any methyl halides produced abiotically needed to be determined.

A comparison of autoclaved and non-autoclaved soils from Kingman Farm (Figure 3-2) and College Woods (Figure 3-3) shows that methyl chloride and methyl bromide consumption occurs in soils that have not been autoclaved; whereas, production of methyl iodide occurs in both non-autoclaved and autoclaved soils. Note that these soils were not inoculated with fungi.

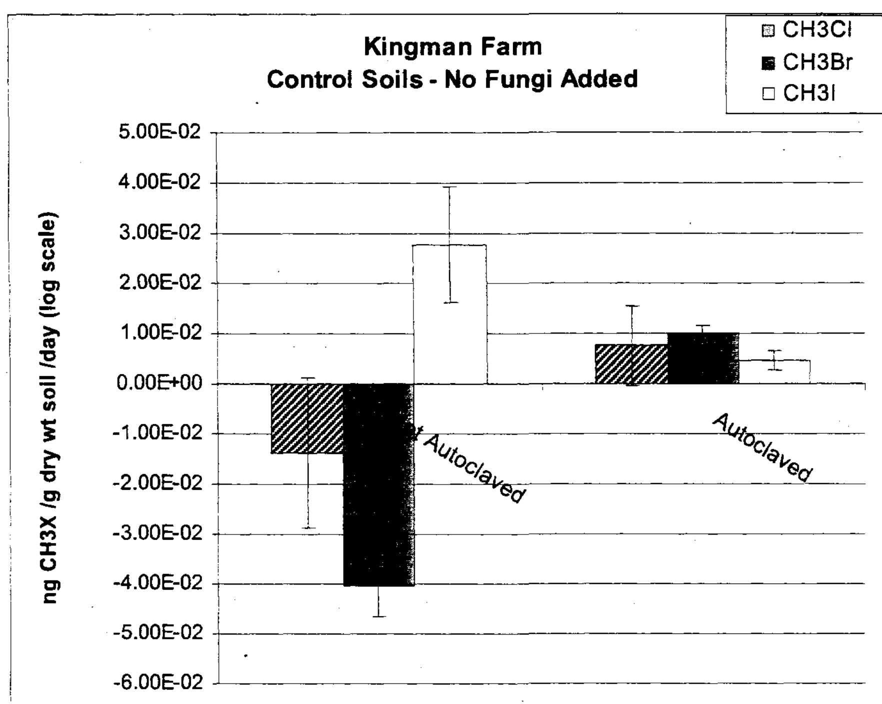

Figure 3-2: Control Soils, Kingman Farm. Methyl chloride and methyl bromide consumption occurs in non-autoclaved soils; methyl iodide production occurs in both autoclaved and non-autoclaved soils. Consumption of methyl halides did not occur in autoclaved soils.

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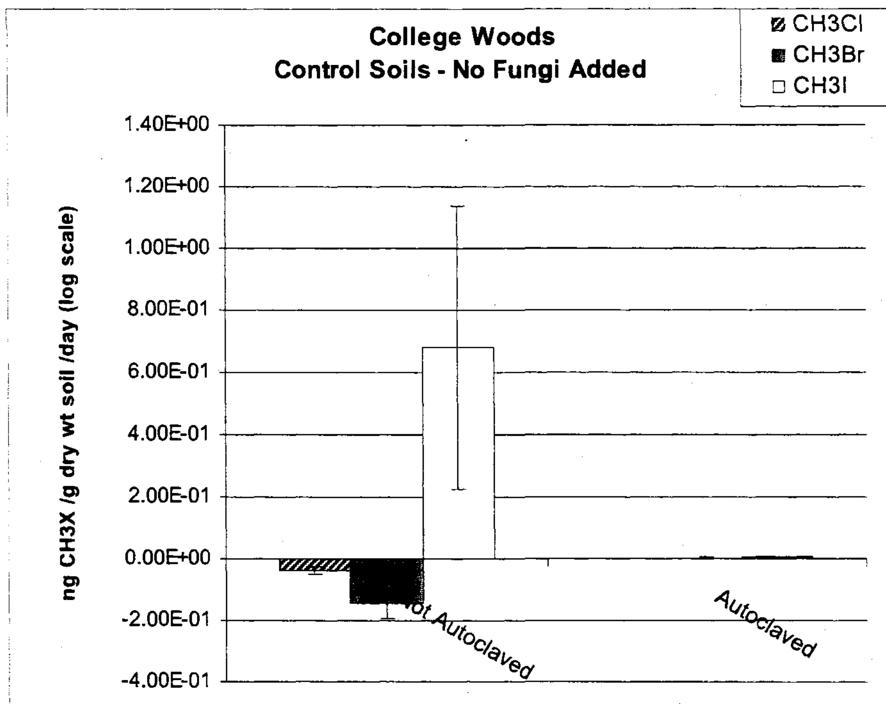

Figure 3-3: Control Soils, College Woods. Similarly, consumption of methyl chloride and methyl bromide occurs in non-autoclaved soils; production of methyl iodide occurs in both autoclaved and non-autoclaved soils. Consumption of methyl halides did not occur in autoclaved soils.

Consumption of methyl halides did not occur in the autoclaved soils. The results show that autoclaving destroyed the biotic mechanism that consumes methyl halides.

In addition, a soil sample (n=2) was plated onto growth medium after the soil had been autoclaved (three times consecutively at 123°C) to ensure that microorganisms were destroyed. After one week, no growth was observed in the sterilized soil sample.

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When the production of methyl halides by fungi is studied in laboratory culture, it is difficult to eliminate any soil microbes that may influence background methyl halide levels without also creating methyl halides from abiotic processes. However, the results indicate that autoclaving caused only a slight increase in methyl halides. This is consistent with Keppler et al. (2000) who found "a similar methyl halide distribution" between heat-dried (105°C) and freeze-dried soils, which had been treated to kill biological material, and their untreated soils.

Although methyl iodide production occurs in both autoclaved and non-autoclaved soils, it is greatly reduced after autoclaving. The difference in the response of methyl chloride and methyl bromide to autoclaving compared to the methyl iodide response indicates a separate mechanism for the production and consumption of these gases. The reduced rates of methyl halide production after autoclaving supports the theory of a biogenic source of methyl halides in soils based on the higher amount of organic matter in the College Woods soils. To summarize, it appears that consumption of methyl halides by bacteria and production of methyl halides by fungi are competing processes that occur in the soil at these sites.

3.4 Fluxes from Fungi

The remaining sections of this chapter describe the methyl halide fluxes from fungi in soil and media incubations. If the rates were calculated using both the traditional linear fit and the Bayesian model they are both listed. In some cases, a rate could not be calculated due to insufficient data. These are marked

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"not available." When the emission rate was below the detection level of the instrument, "bd" is indicated. Note that assays performed in 2003 measured only methyl bromide.

3.4.1 Freshwater Fen Fungi

Methyl chloride, methyl bromide, and methyl iodide production was observed in fungi from the freshwater fen (Table 3-3). Three Zygomycetes were isolated; two were isolated from *Galerina* fruiting bodies (SFM129, SFM130), and the other was isolated from a maple wood bait (SF162M12040). Four fungi identified as basidiomycetes (SFM44, SFM46B, SFM53, SFM54) were also isolated from the fen. All assays were in media incubations.

Table 3-3: Freshwater Fen Fungi Rates (ng CH_3X g dry wt fungi^{-1} d^{-1}; bd=below limit of detection)

***Genus* Isolate# (Run date)**	**CH_3Cl**		**CH_3Br**		**CH_3I**	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Wood bait SF162M1204						
(7/05)	76		24.2		70.5	
(7/06)	0.82	1.34	19.8	12.2	2.24	2.96
Galerina SFM129 (7/05)	85.4	25	3.98	6.15	16.9	13.9
Galerina SFM130 (7/05)	bd		269.75		223.02	
Hygrophorus SFM44						
(11/03)			1.64	0.02		
(7/06)	0.43	0.40	137.58	78.3	1.83	0.87
Hygrophorus SFM46B 0 (11/03)			0.2	0.13		
Hygrophorus SFM46B 1.5 (11/03)			1.98	0.35		
Marasmius SFM53 (7/06)	Not available		2.80	2.1	Not available	
Alnicola SFM54 1.5 (11/03)			0.32	0.07		
Alnicola SFM54 0 (12/03)			3.15	0.98		
SFM54 0 (7/06)	0.55	0.05	4.39	3.7	44.4	51.6

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The rates varied between and within genera; methyl bromide emissions from samples at this site included the highest levels measured in this study.

3.4.2 Salt Marsh Fungi

Methyl halide production was observed in all the fungi collected from the salt marsh sites (Table 3-4). One basidiomycete (BBH2) and four ascomycetes (OD2 a, OD2 b, BBH1, CLR) were assayed in media incubations.

Table 3-4: Salt Marsh Fungi Rates (ng CH_3X g dry wt fungi^{-1} d^{-1}; bd=below limit of detection)

Genus Isolate# (Run date)	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
Unidentified basidiomycete BBH2						
(7/05)	108.01		439.40		89.54	
(9/06)	bd		82.23	30.8	0.51	0.29
(9/06) model	bd		-1.6	0.81	0.0026	0.34
Alternaria OD2 b (9/06)	103		3.7		out of range	
Fusarium OD2 a (7/05)	187.91	176	142.28	85.8	98.66	90
Fusarium BBHI (7/05)	33.05	1.7	31.71	6.31	76.12	12
Fusarium CLR 3 a						
(7/05)	387.26	516	20.01	22.4	915.65	291
(7/06)	25.71	24.7	97.38	107	out of range	

The basidiomycete isolate (BBH2) was obtained from a piece of dead *Spartina* plant tissue collected from the Brave Boat Harbor salt marsh. This was the only basidiomycete that was isolated that had not been cultured from an identifiable basidiocarp. The isolate was incubated in media cultures and assayed in July 2005 and September 2006. This isolate produced the highest levels of methyl bromide of all the basidiomycetes tested.

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Three isolates from salt marsh sites were putatively identified as *Fusarium* spp. based on microscopic spore characteristics. The *Fusarium* genus includes many species that are commonly found in soils and plants. One *Fusarium* isolate (CLR) was obtained from root tissue of a *Spartina* plant collected from the Chappy's Landing salt marsh site. The second isolate (OD2 a) was obtained from the above-ground tissue of a *Spartina* plant collected from the Odiorne Point salt marsh. The third *Fusarium* isolate (BBH1) was also obtained from a piece of above-ground plant tissue of *Spartina* collected from the Brave Boat Harbor salt marsh site.

The *Fusarium* isolates produced high levels of methyl halides in media culture. In particular, the isolates produced the highest levels of methyl iodide; in some cases, methyl iodide levels exceeded the detection limit of the instrument.

Finally, another ascomycete isolate was obtained from the above-ground tissue of a *Spartina* plant from the Odiorne Point salt marsh site. This isolate (OD2 b) was identified in the genus *Alternaria,* which is another common fungus found on plants.

3.4.3 Agricultural Field Fungi

Methyl chloride, methyl bromide, and methyl iodide production was observed in fungi collected from the agricultural site at Kingman Farm (Table 3-5). Two basidiomycete isolates (KM118 and KM119) were obtained from what appeared to be the same species of unidentified "Little Brown Mushroom" fruiting

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bodies collected from a fallow field. Assays were performed on fungi growing in liquid media and soil incubations.

Table 3-5: Fluxes from Agricultural Field Fungi (ng CH_3X g dry wt fungi^{-1} d^{-1}; bd=below limit of detection)

Isolate# (Run date)	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
LBM KM118						
(7/05) Media	10.9		69.7		4640	
(6/06) Media	Not available		-8.67	8.8	54.0	56.2
(6/06) Media Model	Not available		-0.07	0.72	5.65	3.21
(6/06) Soil, autoclaved	Not available		0.17	0.03	-2.20	0.44
(6/06) Soil, autoclaved Model	Not available		0.01	0.02	-0.14	0.04
(9/06) Media	Not available		bd		Not available	
(9/06) Media Model	Not available		Not available		Not available	
(9/06) Soil, not autoclaved	2.53	2.46	-4.97	3.1	bd	
(9/06) Soil, not autoclaved Model	-0.52	0.11	-0.34	0.16	0.001	0.12
LBM KM119						
(7/05) Media	48.8		3.76		417	
(9/06) Media	57.4	78	76.4	49	18.6	65
(10/06) Soil, not autoclaved	0.78	0.11	-0.76	1.8	bd	

3.4.4 Upland Forest Fungi

Methyl halide production was observed in basidiomycetes collected from the upland forest site at College Woods (Table 3-6). Assays were performed on fungal isolates growing in media and in soil incubations.

Table 3-6: Fluxes from Temperate Forest Fungi (ng CH_3X g dry wt soil^{-1} d^{-1}; bd=below limit of detection)

Genus Isolate# Run Date	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
Crepidotus CWM3 (11/03) Media			9.04	0.07		
Trametes CWM4						
(10/03) Media			0.073	0.04		
(11/05) Soil	0.0004		0.005		0.007	
Tyromyces CWM8						

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Genus Isolate# Run Date	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
(9/03) Media			2.06			
(7/06) Media	3.60	0.31	30.68	100	22.8	
Tyromyces CWM23 (9/03) Media			0.033	0.01		
Russula CWM29						
(11/03) Media			1.8	0.20		
(11/05) Soil	bd		0.0004		0.0002	
No ID CWM30						
(11/03) Media			0.627	0.50		
(11/05) Soil	bd		0.0002		0.002	
No ID CWM34 1.0 (10/03) Media			1.76	0.83		
Clavaria CWM35						
(12/03) Media			8.14	0.12		
(5/06) Media	-0.26	0.37	0.40	0.28	-0.57	1.39
(5/06) Soil, Autoclaved	0.14	0.13	-0.31	0.16	-0.55	1.89
(5/06) Soil, Autoclaved Model	0.002	0.03	-0.10	0.05	Not available	
No ID CWM40 (11/03) Media			0.82	0.75		
Russula CWM62B						
(12/03) Media						
(5/06) Media	Not available		1.01	1.88	-4.64	4.22
(5/06) Media Model	Not available		-0.01	0.14	0.08	0.11
(5/06) Soil, Autoclaved	Not available		-0.34	0.06	0.009	1.49
(5/06) Soil, Autoclaved Model	Not available		-0.01	0.02	0.02	0.05
Stereum CWM98 (11/05) Soil	bd		bd		0.0002	
Gymnopilus CWM140 (11/05) Soil	0.00001		0.00001		0.0001	
Trametes CWM142						
(9/06) Media	Not available		-82.14	43.3	1.16	0.37
Cantharellus CWM148						
(5/06) Media	Not available		5.06	1.68	11.9	5.84
(5/06) Media Model	Not available		-0.01	0.21	na	
(5/06) Soil, Autoclaved	Not available		3.79	7.35	0.26	0.66
(5/06) Soil, Autoclaved Model	Not available		0.02	0.01	na	
Russula CWM159 (11/05) Soil	bd		bd		0.0002	

In addition to the basidiomycetes isolated from College Woods, an ascomycete was isolated from the tissue of a cup fungus putatively identified as *Peziza badioconfusa*. Four isolates were obtained from this individual sample (CWM5). Media incubations were assayed in 2003 for methyl bromide

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production only; soil incubations were assayed in 2005. The emissions from this fungus are shown in Table 3-7.

Table 3-7: Methyl Halides from *Peziza* (media=ng CH_3X g wet wt fungi^{-1} d^{-1}; soil=ng CH_3X g dry wt soil^{-1} d^{-1}; bd=below limit of detection)

CH_3Cl			CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
Media (9/03)			0.09 ± 0.02 – 1.23 ± 0.17			
Soil (11/05)	bd		2.23E-05		3.15E-08	

3.4.5 Mycorrhizal Fungi

Some of the samples that were assayed are fungi that are known to form mycorrhizae (Table 3-8). The highest fluxes observed in this group were from the unidentified LBM samples collected at the agricultural site.

Table 3-8: Fluxes from Mycorrhizal Fungi (media=ng CH_3X g dry wt fungi^{-1} d^{-1}; soil=ng CH_3X g dry wt soil^{-1} d^{-1}; bd=below limit of detection)

Genus Isolate# Run Date	CH_3Cl		CH_3Br		CH_3I	
	Mean	sd	Mean	sd	Mean	sd
Russula CWM29						
(10/03) Media			1.8	0.20		
(11/05) Soil	bd		0.0001		0.0002	
Russula CWM62B						
(12/03) Media			19.80			
(5/06) Media	Not available		1.01	1.88	-4.64	4.22
(5/06) Media Model	Not available		-0.01	0.14	0.08	0.11
(5/06) Soil, Autoclaved	Not available		-0.34	0.06	0.009	1.49
(5/06) Soil, Autoclaved Model	Not available		-0.01	0.02	0.02	0.05
Russula CWM159 (11/05) Soil	bd		bd		0.0002	
Gymnopilus CWM140 (11/05) Soil	bd		bd		0.0001	
Cantharellus CWM148						
(5/06) Media	Not available		5.06	1.68	11.9	5.84
(5/06) Media Model	Not available		-0.01	0.21	Not available	
(5/06) Soil, Autoclaved	Not available		3.79	7.35	0.26	0.66
(5/06) Soil, Autoclaved Model	Not available		0.02	0.01	Not available	
Marasmius SFM53 (7/06) Media	0.14	0.15	0.6	2.08	Not available	

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Genus **Isolate# Run Date**	CH_3Cl		CH_3Br		CH_3I	
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**
Alnicola SFM54 1.5 (11/03)			0.32	0.07		
Alnicola SFM54 0 (12/03)			3.15	0.98		
0 (7/06)	0.55	0.05	4.39	3.7	44.4	51.6
LBM KM118						
(7/05) Media	10.9		69.7		4640	
(6/06) Media	Not available		-8.67	8.8	54.0	56.2
(6/06) Media Model	Not available		-0.07	0.72	5.65	3.21
(6/06) Soil, Autoclaved	Not available		0.17	0.03	-2.20	0.44
(6/06) Soil, Autoclaved Model	Not available		0.01	0.02	-0.14	0.04
(9/06) Media	Not available		bd		Not available	
(9/06) Media Model	Not available		Not available		Not available	
(9/06) Soil, Not Autoclaved	2.53	2.46	-4.97		bd	
(9/06) Soil, Not Autoclaved Model	-0.52	0.11	-0.34	0.16	0.001	0.12
LBM KM119						
(7/05) Media	48.8		3.76		417	
(9/06) Media	57.4	78.0	76.4	49.1	18.6	65
(10/06) Soil, Not Autoclaved	0.78	0.11	-0.76	1.8	bd	

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CHAPTER 4

4.0 DISCUSSION

This study resulted in the isolation of fungi from four ecosystems. Fungi have been previously identified as net producers of methyl bromide (Harper, 1985; Watling and Harper, 1998; Harper, 2000), indicating that they are a potential mechanism for production in these environments. The fungi that were isolated in this study produced methyl halides at rates similar to those observed for fungi cultured from other ecosystems (Redeker et al., 2004). The implications of flux estimation methods and extrapolations to real fluxes from emission measurements are discussed in this chapter.

4.1 Effect of Flux Estimation Methods

The Bayesian approach that was used to estimate a flux from the data resulted in lower fluxes. This was because the error of the standard was used to estimate concentration. In flux measurement studies, the error in the standard concentration is generally ignored either because it is small when compared to measured concentrations (for example, 0.05% for methane and <1% for CO_2) or because there is not an easy way to propagate this error to the flux estimate. This has implications for previously published work of methyl halide fluxes and the magnitude of their extrapolations. For example, the model results for the

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fluxes of all three methyl halides from ectomycorrhizal fungi tested in this study (Table 4-4) are significantly lower than those previously published by Redeker et al. (2004). In their work, the authors do not propagate the 3, 3% and 10% precision of their gas chromatography method for analysis of methyl chloride, methyl bromide and methyl iodide, respectively (Redeker et al., 2004). The analysis performed in this study implies that the fluxes estimated using the linear least squares method are likely an overestimation of the emission from these fungi by 30% to 95%.

In other studies, field measured fluxes of methyl halides have been calculated using similar methods. Dimmer et al. (2001) report fluxes of methyl halides from peatlands. The precision of their instrumentation is reported as varying between 0.3% and 4%. Depending on where the analyses occurred within this precision range, the fluxes could be significantly overestimated. Rhew et al. (2000, 2001, 2007) have made extensive flux measurements ranging from scrubland and coastal salt marsh ecosystems in California to Alaskan tundra ecosystems. In every case, the precision of the instrumentation is reported as 3% for methyl chloride and methyl bromide and 12% for methyl iodide. Previous work by Varner et al. (1999, 2003) report methyl bromide fluxes with an analytical error of 2% to 3%, which is unaccounted for in their flux estimates of methyl bromide from temperate soils and wetlands.

A second source of error that is often ignored in flux measurements is related to the number of samples drawn from the fungal headspace. In this

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study, a minimum of three samples was used to calculate a flux. Redeker et al. (2004) reported that only two samples were drawn (0 and 4 hr) for emission estimates. There is no discussion of the error associated with fitting a linear regression to two points. Similarly, two sampling points were used to estimate methyl halide fluxes in field sampling by Rhew et al. (2000, 2001, 2007). Thus, the fluxes reported by both researchers are suspect.

When calculating fluxes, ignoring the error in variables associated with estimating the concentration of samples has serious implications. These flux measurements are often used to determine a global flux of methyl halides for an ecosystem or organism. The global flux is then used by atmospheric chemists and modelers to interpret atmospheric concentrations of these trace gases and, in turn, determine the distribution of the sinks and sources.

4.2 Emissions from Fungi in Four Ecosystems

To identify whether production from fungi represents a significant source of methyl halides at the ecosystems studied, the rates from fungi in soil incubations were extrapolated to surface area and compared to field-measured fluxes from these and other field sites.

Table 4-1 compares methyl halide emissions from fungi at different sites in this study to fluxes that were measured using enclosure methods in the field in other studies.

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Table 4-1: Comparison of Fluxes from Fungi in Soil to Chamber Fluxes

Methyl Halide ($ng\ CH_3X\ m^{-2}\ d^{-1}$)			Study
CH_3Cl	CH_3Br	CH_3I	
			This study:
			Temperate forest
-0.18 – 2.41	-49.47 – 18.70	-3.02 – 13.88	Media
0 – 0.08	-0.21 – 2.41	-0.33 – 0.18	Soil
			Agricultural fields
0 – 18.78	-2.97– 25.05	1.98 – 1516	Media
-0.16 – 0.99	-1.65 – 0.07	-0.66– 0	Soil
7200	4560	6000	Coastal wetland, Cape Grim, Tasmania; Cox et al. (2004)
-31303.8	-768.52	-	N. Alaskan coastal tundra (drained); Rhew et al. (2007)
-706.86	104.43	-	Flooded tundra; Rhew et al. (2007)
-	-	567.76	Average depended on time of year and hydrologic regime; Rhew et al. (2007)
60000	5000	55000	S. California coastal salt marshes; *Spartina* plants; Manley et al. (2006)
14520.55	2465.75	3835.62	Irish peatlands; median daytime annual flux; Dimmer et al. (2001)
92602.74	4657.53	5205.48	Conifer forest; Dimmer et al. (2001)
18082.19	2739.73	3835.62	Wetlands; Dimmer et al. (2001)
-	664.98	-	Temperate peatland; mean flux 1999 ; White et al. (2005)
-	379.76	-	Mean flux 2000; White et al. (2005)
-	-4000 – 3300	-	Temperate forest soils; Varner et al. (2003)

The emissions observed in this study are consistent with Dimmer et al. (2001) who reported a “very high CH_3I flux” from a freshwater coastal marsh in Ireland. Comparisons can also be made with studies of methyl halide emissions from salt marshes in southern California. For example, Manley et al. (2006)

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found that plants, including *Spartina* spp., in the mid and upper marsh produced 41% of the observed methyl iodide emissions, while 59% was from unvegetated areas of coastal California salt marshes. The methyl halide emissions were correlated to seasonality, biomass, and flowering. There were apparent differences in methyl halide emissions between plant species. The plant roots, associated microbes, or mycorrhizae were identified as possible sources of the strong methyl iodide emissions from soils/muds at the California salt marshes.

Using flux chambers in various southern California shrubland sites, Rhew et al. (2001) measured net production of methyl chloride and methyl bromide. Emissions were very high during the dry season from plants adapted to dry conditions. Overall, they found that methyl chloride and methyl bromide production and consumption were competing processes in shrublands. This contrasted with the strong production of methyl chloride and methyl bromide that was observed in southern California salt marshes in an earlier study. They believe that vegetation, rather than soils and mudflats, were the primary source of methyl chloride and methyl bromide emissions in the salt marshes, but they did not rule out the possibility that methyl chloride emissions may be caused by either fungi or abiotic processes associated with plant decomposition (Rhew et al., 2002). Likewise, Varner et al. (2003) concluded from combined field and laboratory measurements that an abiotic mechanism or fungi may be responsible for net production of methyl bromide from temperate forest soils.

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Both negative and positive fluxes were observed from fungi growing in the soil incubations. The fluxes suggest that consumption, as well as production of methyl halides occurs in the upland and agricultural soils. These results are consistent with similar reports of methyl bromide production and consumption in temperate forest soils (Varner et al. 2003), temperate peatlands (White et al. 2005), and shrublands (Rhew et al., 2001).

As Table 4-1 shows, fluxes from fungi in agricultural soil were consistent with observations by Varner (2000), who suggested that fungi from agricultural fields could be responsible for observed fluxes (1 – 200 ng m^{-2} d^{-1}). However, the fluxes from forest soil fungi are not significant when compared to efflux measurements by Varner et al. (2003) and Varner (2000).

4.3 How Much of Methyl Halide Production is Due to Fungi?

To identify whether the methyl halide production that was observed in field measurements can be attributed to fungi, the molar ratio of emissions from fungi can be compared with field emissions (Table 4-2). The mean methyl bromide and methyl iodide production rates (nmol CH_3X g $fungi^{-1}$ day^{-1}) were normalized to the methyl chloride rate.

Table 4-2: Methyl Halide Ratios of Production Rates by Fungi in Media Incubations (nmol CH_3X g $fungi^{-1}$ day^{-1})

Methyl Halide Ratio $CH_3Cl : CH_3Br : CH_3I$	Ecosystem Type
1 : 3 : 1	Temperate forest
1 : 0.5 : 12	Agricultural fields
1 : 0.6 : 0.7	Freshwater fen
1 : 0.5 : 0.6	Salt marshes

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When the ratios of production rates by fungi in culture in this study are compared to other studies, they reflect a similar "signature" for methyl halide emissions observed in salt marsh (Cox et al., 2004; Manley et al., 2006) and Irish peatland (Dimmer et al., 2001) ecosystems (Table 4-3).

Table 4-3: Methyl Halide Ratios in Field Emissions (n.a.= not available, not measured)

Ratios	Site/Organism	Ion	Measurement	Reference
1 : 4 : 19	*P. pomaceous*	Equimolar	Fungal incubation (basidiomycete)	Harper et al., 1985
1 : 0.005 : 0.03 1 : 0.035 : 0.0006 1 : 30 : 2,553	*I. immacula* *L. laccata*	0.20 mM 0.20 mM 20 mM	Fungal incubations (ectomycorrhizae)	Redeker et al., 2004
1 : 0.05 : n.a.	Temperate wetland	n.a.	Clear chamber flux	Varner et al., 1999
1 : 0.021 : 0.006	Tundra	n.a.	Dark chamber flux	Rhew et al., 2007
1:0.13-0.20:0.0-0.6	Salt marsh plants and controls	(plants)	Clear chamber flux	Manley et al., 2006
1 : 0.20 : n.a.	Salt marsh	n.a.	Dark chamber flux	Rhew et al., 2000
1 : 0.10 : n.a.	Boreal soils	n.a.	Incubations	Rhew et al., 2003
1 : 0.17 : 0.26 1 : 0.05 : 0.06	Irish peatland Conifer forest	n.a.	Dark chamber flux	Dimmer et al., (2001)
1 : 0.26 : 0.26 1 : 0.006 : 0.001 1 : 0.02 : 0.003	Coastal salt-marsh Eucalyptus forest Soil leaf litter	n.a.	Chamber flux (type not described)	Cox et al., 2004

The results show that methyl halide ratios from fungi were not equal to those observed in the field (Rhew et al., 2007; Cox et al., 2004; and Dimmer et al., 2001), but fungi are likely to be part of the source. Methyl halide ratios observed in the field were likely a net flux, which is the result of combined consumption and production mechanisms. This would cause the ratio to be lower, as reflected in the results 1:0.2:<0.3 vs. 1:0.6:0.5 (from fungi).

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4.4 Mycorrhizal Fungi

The methyl halide emissions from mycorrhizal species of fungi tested in the present study are compared in Table 4-4 to emissions from ectomycorrhizal fungi grown in culture, as reported by Redeker et al. (2004).

Table 4-4: Comparison to Emissions from Ectomycorrhizal Fungi in Culture

Methyl Halide Ranges (media=ng CH_3X g dw fungi^{-1} d^{-1}; soil=ng CH_3X g dw soil^{-1} d^{-1})			Study
CH_3Cl	CH_3Br	CH_3I	
			This study; mycorrhizal species of fungi; media incubations=0.02, 20, & 2 mM halide concentrations for Cl^-, Br^-, and I^-, respectively.
			Temperate Forest
0	-0.01– 12.07	-3.02 – 7.24	Media
0	-0.21 – 2.41	0 – 0.16	Soil
			Agricultural Fields
0 – 18.8	-2.97 – 25.05	1.98 – 1516	Media
-0.16 – 0.99	-1.65 – 0.07	-0.66 – 0	Soil
1.9 – 130,000	21 – 320,000	90 – 18,000	Ectomycorrhizae; range of emissions from fungal isolates in media cultures supplemented with 20 mM halide concentration; Redeker et al. (2004)

Although the methyl halide emissions from the present study are lower than the emissions in the Redeker et al. study, they are within the range observed. It is important to note that Redeker et al. (2004) found significant variation in emissions between the nine ectomycorrhizal isolates they measured. Furthermore, the species of fungi tested in the two studies are not the same.

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4.5 Unexpected Production in Non-basdiomycetes

The high levels of methyl halides produced by non-basidiomycete fungi were not expected. The non-basidiomycetes isolated from the freshwater fen and salt marshes produced the highest levels of methyl iodide. In particular, the isolates identified as *Fusarium* spp. produced the highest levels of methyl iodide in media culture, in some cases exceeding the detection level of the instrument.

The results show that ascomycetes and zygomycetes are a potential source of methyl halide emissions that may represent a greater environmental significance than expected from basidiomycetes.

4.6 Summary

The goal in this study was to eliminate environmental factors, such as temperature, moisture, and light that can influence field measurements of methyl halide emissions. Competing biogenic sources of methyl halides were removed by isolating and assaying a single species of fungi in sterile laboratory cultures.

Targeting a single organism within an ecosystem, isolating it from the environment, measuring its production or consumption, and quantifying its biomass can be difficult, especially when dealing with microorganisms. Many factors influence fungal biomass in the soil, such as moisture, temperature, soil structure and resource availability (Sulzman and Frey, 1999). These factors can all contribute to the high variability in methyl halide fluxes that were observed.

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CHAPTER 5

5.0 RECOMMENDATIONS FOR FUTURE STUDIES

While the data presented here are highly variable, clearly, the non-basidiomycete fungi are a potential source of methyl halide emissions, and further investigation is indicated.

More soil incubation assays of ascomycetes, such as *Fusarium* spp. and *Alternaria* spp. fungi, which are common in soil and on plants, should be performed in order to better establish the net methyl halide fluxes from these fungi. Ironically, they are easy to isolate and culture, grow quickly in the laboratory, and are more easily identified by their microscopic characteristics than basidiomycetes.

In a recent survey of fungal biodiversity, many more species of heat- and salt-tolerant fungi have been identified (Gams, 2007); many more species that can adapt to stressful environments, such as the ascomycetes, have yet to be identified (Zak and Wildman, (2004).

The *Fusarium* spp. and *Alternaria* spp. are common fungi that occur on grasses. The presence of these and other unidentified species of fungi on plants and in soils of salt marshes are likely. Their potential for methyl halide emissions

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may represent a greater environmental significance than expected from basidiomycetes.

It is also recommended that fungi be isolated and cultured from tissue samples taken from plants inside static chambers after field measurements to identify whether fungi on the plants are responsible for the methyl halide emissions.

Further, to reduce the compounding effects of error factors, the sample size of isolates should be increased, more assays per isolate should be performed, and the daily assays of standard aliquots should be increased.

Finally, when estimating a flux, consideration should be given to the type of curve-fitting technique that is used if the error of the standard concentration is high.

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APPENDIX A

A.0 FUNGI INVENTORY

Table A-1: Inventory of All Fungi Cultured and Stored in Cryovials. CW=College Woods; Sall=SF, Sallies Fen; Chap=Chappys Landing; KF=Kingman Farm; Bar=Bartlett Forest; Hvd=Harvard Forest; ME=Maine; cl=clamps identified.

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Bar	Mush	B1 0 mybda		*Clavaria inequalis*	8/21/2003	9/8/2003
Bar	Mush	B1 .5 mybda mybda		*or Clauvinopsis fusiformis*	8/21/2003	9/16/2003
Bar	Mush	B1 2.0 mybda2			8/21/2003	9/16/2003
Bar	Mush	B2		*Amanita sp*	8/21/2003	lost
Bar	Mush	B3 0 mybda 1		*Amanita caesarea*	8/21/2003	9/8/2003
Bar	Mush	B3 .5 mybda			8/21/2003	9/8/2003
Bar	Mush	B4 1.5 mybda		*Ramaria*	8/21/2003	9/16/2003
Bar	Mush	B4 2.0 mybda			8/21/2003	10/7/2003
Bar	Mush	B5 .5 mybda2		*Kuehneromyces mutabilis*	8/21/2003	9/16/2003
Bar	Mush	B5 1.5 MYBDA		*or Collybia veluptipes*	8/21/2003	9/16/2003
Bar	Mush	B5 2.0 MYBDA			8/21/2003	9/8/2003
Bar	Mush	B6		*Russula*	8/21/2003	tossed
Bar	Mush	B7		*Amanita*	8/21/2003	tossed
Bar	Mush	B8 1.5 mybda		*Boletus*	8/21/2003	9/16/2003
Bar	Mush	B9 .5 mybda		*Boletus pulverulentus*	8/21/2003	9/16/2003
Bar	Mush	B9 2.0 mybda2 cmea MYBDA			8/21/2003	12/3/2003
Bar	Mush	B10 .5 mybda		*Boletus pulverulentus*	8/21/2003	10/7/2003
CW	Mush	CWM1 MEA D mybda		*Xeromphalina tenuipes*	5/20/2003	7/31/2003
CW	Mush	CWM1 mybda A		*or X. campanella*	5/20/2003	7/11/2003
CW	Mush	CWM1 mybda A			5/20/2003	7/1/2003
CW	Mush	CWM2 mybda A		*Cerena unicolor*	5/20/2003	7/1/2003
CW	Mush	CWM2 mybda A			5/20/2003	7/17/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM2 mybda B			5/20/2003	7/1/2003
CW	Mush	CWM2 mybda B			5/20/2003	7/17/2003
CW	Mush	CWM2 mybda C			5/20/2003	7/1/2003
CW	Mush	CWM2 mybda C			5/20/2003	7/17/2003
CW	Mush	CWM2 mybda C mybda			5/20/2003	8/26/2003
CW	Mush	CWM3 mybda B		*Crepidotus*	5/20/2003	7/1/2003
CW	Mush	CWM3 mybda B			5/20/2003	7/17/2003
CW	Mush	CWM3 mybda A mybda			5/20/2003	7/17/2003
CW	Mush	CWM3 mybda C mybda	cl		5/20/2003	7/14/2003
CW	Mush	CWM3 mybda C mybda CMEA			5/20/2003	3/28/2006
CW	Mush	CWM4 .5 mybda2	cl		5/20/2003	10/7/2003
CW	Mush	CWM4 .5 mybda2			5/20/2003	7/31/2003
CW	Mush	CWM4 1.5 mybda			5/20/2003	7/11/2003
CW	Mush	CWM4 2.0 mybda			5/20/2003	7/23/2003
CW	Mush	CWM5 .5 mybda	cl	*Peiza badioconfusa*	5/20/2003	7/11/2003
CW	Mush	CWM5 1.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM5 2.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM5 1.5 mybda			5/20/2003	7/11/2003
CW	Mush	CWM5 mybda A1			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda A1			5/20/2003	7/14/2003
CW	Mush	CWM5 mybda A2			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda A2			5/20/2003	7/14/2003
CW	Mush	CWM5 mybda A			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda A	cl		5/20/2003	7/17/2003
CW	Mush	CWM5 mybda B	cl		5/20/2003	7/14/003
CW	Mush	CWM5 mybda B			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda C	cl		5/20/2003	7/14/2003
CW	Mush	CWM5 mybda C			5/20/2003	10/12/2005
CW	Mush	CWM5 mybda C			5/20/2003	10/7/2003
CW	Mush	CWM5 mybda C1			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda C1			5/20/2003	7/14/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM5 mybda C2			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda C2			5/20/2003	7/14/2003
CW	Mush	CWM5 mybda D			5/20/2003	7/1/2003
CW	Mush	CWM5 mybda D			5/20/2003	7/14/2003
CW	Mush	CWM5 mybda E			5/20/2003	7/14/2003
CW	Mush	CWM5 mybda E			5/20/2003	7/1/2003
CW	Mush	CWM6 .5 mybda		*Polyporus alveolaris*	5/20/2003	7/11/2003
CW	Mush	CWM6 1.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM6 2.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM6 mybda A			5/20/2003	7/1/2003
CW	Mush	CWM6 mybda A			5/20/2003	7/14/2003
CW	Mush	CWM6 mybda A mybda			5/20/2003	9/8/2003
CW	Mush	CWM6 mybda B			5/20/2003	7/1/2003
CW	Mush	CWM6 mybda B			5/20/2003	7/11/2003
CW	Mush	CWM6 mybda E			5/20/2003	7/14/2003
CW	Mush	CWM6 mybda E			5/20/2003	7/1/2003
CW	Mush	CWM6 mybda E MYA			5/20/2003	8/20/2003
CW	Mush	CWM7 .5 mybda		*Phebia radiata*	5/20/2003	7/22/2003
CW	Mush	CWM7 1.0 mybda			5/20/2003	7/23/2003
CW	Mush	CWM7 1.5 mybda			5/20/2003	7/22/2003
CW	Mush	CWM7 2.0 mybda			5/20/2003	7/31/2003
CW	Mush	CWM7 mybda DC mybda			5/20/2003	7/31/2003
CW	Mush	CWM7 mybda E			5/20/2003	7/11/2003
CW	Mush	CWM7 mybda E			5/20/2003	7/1/2003
CW	Mush	CWM8 0 mybda	cl		5/20/2003	7/11/2003
CW	Mush	CWM8 1.5 mybda			5/20/2003	7/11/2003
CW	Mush	CWM8 2.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM8 mybda A	cl		5/20/2003	7/1/2003
CW	Mush	CWM8 mybda A			5/20/2003	7/14/2003
CW	Mush	CWM8 mybda B	cl		5/20/2003	7/14/2003
CW	Mush	CWM8 mybda B			5/20/2003	7/1/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM8 mybda C	cl		5/20/2003	7/14/2003
CW	Mush	CWM8 mybda C			5/20/2003	7/1/2003
CW	Mush	CWM9		*Polyporus squamosus*	5/20/2003	tossed
CW	Mush	CWM10 mybda D		*X. tenuipes same as CWM1*	5/20/2003	7/1/2003
CW	Mush	CWM10 mybda D			5/20/2003	7/11/2003
CW	Mush	CWM11 mea A			5/20/2003	7/14/2003
CW	Mush	CWM11 mea A			5/20/2003	7/1/2003
CW	Mush	CWM11 mea D			5/20/2003	7/14/2003
CW	Mush	CWM11 mea D			5/20/2003	7/1/2003
CW	Mush	CWM11 mea E			5/20/2003	7/14/2003
CW	Mush	CWM11 mea E			5/20/2003	7/1/2003
CW	Mush	CWM11 mea E mybda			5/20/2003	9/8/2003
CW	Mush	CWM11 0 mybda2			5/20/2003	7/31/2003
CW	Mush	CWM11 .5 mybda			5/20/2003	7/23/2003
CW	Mush	CWM11 1.5 mybda			5/20/2003	7/22/2003
CW	Mush	CWM11 1.5 mybda A mybda2 MYA			5/20/2003	8/5/2003
CW	Mush	CWM12 0 mybda			5/20/2003	7/22/2003
CW	Mush	CWM12 mybda C			5/20/2003	7/1/2003
CW	Mush	CWM12 mybda C			5/20/2003	7/17/2003
		13 and 14 not used				
CW	Mush	CWM15 0 mybda		*Ceratiomyxa fruticulosa*	5/20/2003	7/1/2003
CW	Mush	CWM15 0 mybda		*v flexuosa*	5/20/2003	7/11/2003
CW	Mush	CWM15 .5 mybda			5/20/2003	7/1/2003
CW	Mush	CWM15 .5 mybda			5/20/2003	7/14/2003
CW	Mush	CWM15 1.0 mybda			5/20/2003	7/1/2003
CW	Mush	CWM15 1.0 mybda			5/20/2003	7/11/2003
CW	Mush	CWM15 1.5 mybda			5/20/2003	7/1/2003
CW	Mush	CWM15 1.5 mybda			5/20/2003	7/11/2003
CW	Mush	CWM15 2.0 mybda			5/20/2003	7/1/2003

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM15 2.0 mybda			5/20/2003	7/11/2003
		16 and 17 not used				
						7/23/2003
CW	Mush	CWM18 0 mybda		*Mycena haematopus*	5/20/2003	
CW	Mush	CWM19			5/20/2003	tossed
		20 not used				
CW	Mush	CWM21 .5 A mybda2	cl	*Crepidotus herbarum*	5/20/2003	8/20/2003
CW	Mush	CWM21 .5 B mybda2	cl		5/20/2003	8/20/2003
CW	Mush	CWM21 .5 mybda	cl		5/20/2003	8/20/2003
CW	Mush	CWM21 2.0 mybda			5/20/2003	8/20/2003
CW	Mush	CWM21 2.0 A mybda2	cl		5/20/2003	8/20/2003
CW	Mush	CWM21 2.0 B mybda2	cl		5/20/2003	8/20/2003
CW	Mush	CWM22 .5 mybda			7/3/2003	7/31/2003
CW	Mush	CWM23 1.0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM23 1.0 mybda2	cl		7/3/2003	7/23/2003
CW	Mush	CWM24			7/3/2003	tossed
CW	Mush	CWM25 0 mybda			7/3/2003	8/5/2003
CW	Mush	CWM25 .5 mybda			7/3/2003	8/20/2003
CW	Mush	CWM25 1.0 A mybda MYA			7/3/2003	10/7/2003
CW	Mush	CWM25 2.0 mybda MYA			7/3/2003	10/7/2003
CW	Mush	CWM26 .5 mybda A MYA CMEA			7/3/2003	9/8/2003
CW	Mush	CWM26 1.5 mybda A MYA			7/3/2003	7/31/2003
CW	Mush	CWM26 1.5 mybda B MYA mybda			7/3/2003	9/8/2003
CW	Mush	CWM26 1.5 mybda B MYA			7/3/2003	7/31/2003
CW	Mush	CWM27			7/3/2003	tossed
CW	Mush	CWM28 2.0 mybda2			7/3/2003	7/31/2003
CW	Mush	CWM29 0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM29 1.0 mybda	cl		7/3/2003	7/17/2003

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CW	Mush	CWM29 2.0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM30 0 mybda			7/3/2003	7/23/2003
CW	Mush	CWM30 .5 mybda2 A			7/3/2003	7/31/2003
CW	Mush	CWM30 .5 mybda2 B			7/3/2003	7/31/2003
CW	Mush	CWM30 1.0 mybda mybda 1	cl		7/3/2003	8/5/2003
CW	Mush	CWM30 1.0 mybda mybda 2	cl		7/3/2003	8/5/2003
CW	Mush	CWM30 1.5 mybda	cl		7/3/2003	7/17/2003
CW	Mush	CWM31 1.0 A mybda			7/3/2003	7/23/2003
CW	Mush	CWM31 1.0 A mybda mybda 1	cl		7/3/2003	8/26/2003
CW	Mush	CWM31 1.0 A mybda mybda 2	cl		7/3/2003	8/26/2003
CW	Mush	CWM31 1.0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM31 0.5 mybda			7/3/2003	7/22/2003
CW	Mush	CWM32 0 mybda2	cl		7/3/2003	8/5/2003
CW	Mush	CWM32 .5 mybda2	cl		7/3/2003	8/5/2003
CW	Mush	CWM32 2.0 A mybda2	cl		7/3/2003	8/5/2003
CW	Mush	CWM33			7/3/2003	tossed
CW	Mush	CWM34 0 mybda	cl		7/3/2003	7/22/2003
CW	Mush	CWM34 0 mybda2			7/3/2003	7/23/2003
CW	Mush	CWM34 .5 mybda A mybda	cl		7/3/2003	7/31/2003
CW	Mush	CWM34 1.0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM34 1.0 mybda A	cl		7/3/2003	7/22/2003
CW	Mush	CWM34 1.0 mybda			7/3/2003	10/12/2005
CW	Mush	CWM34 1.0 A mybda			7/3/2003	7/22/2003
CW	Mush	CWM34 1.0 mybda A mybda			7/3/2003	7/23/2003
CW	Mush	CWM34 1.0 mybda B mybda			7/3/2003	7/31/2003
CW	Mush	CWM34 2.0 mybda			7/3/2003	7/22/2003
CW	Mush	CWM35 0 mybda	cl		7/3/2003	7/23/2003
CW	Mush	CWM35 .5 mybda	cl		7/3/2003	7/17/2003
CW	Mush	CWM35 1.0 mybda A mybda	cl		7/3/2003	8/5/2003
CW	Mush	CWM35 1.0 mybda B mybda	cl		7/3/2003	8/5/2003
CW	Mush	CWM35 1.0 mybda A mybda CMEA			7/3/2003	3/28/2006

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM36 0 mybda	cl		7/3/2003	7/31/2003
CW	Mush	CWM36 1.0 mybda	cl		7/3/2003	7/17/2003
CW	Mush	CWM36 1.0 A mybda	cl		7/3/2003	7/23/2003
CW	Mush	CWM36 1.0 mybda MYA			7/3/2003	12/19/2003
CW	Mush	CWM37 2.0 mybda A mybda	cl		7/3/2003	7/31/2003
CW	Mush	CWM38 0 mybda			7/3/2003	7/31/2003
CW	Mush	CWM38 .5 mybda mybda 1			7/3/2003	8/20/2003
CW	Mush	CWM38 .5 mybda mybda 2			7/3/2003	8/20/2003
CW	Mush	CWM38 2.0 mybda			7/3/2003	8/5/2003
CW	Mush	CWM39 0 mybda			7/3/2003	7/31/2003
CW	Mush	CWM39 1.0 mybda			7/3/2003	7/31/2003
CW	Mush	CWM39 2.0 mybda			7/3/2003	7/31/2003
CW	Mush	CWM40 1.5 mybda B mybda			7/3/2003	7/31/2003
CW	Mush	CWM40 1.5 mybda A MYA			7/3/2003	7/31/2003
CW	Mush	CWM40 2.0 mybda A mybda	cl		7/3/2003	7/31/2003
CW	Mush	CWM40 2.0 mybda B mybda	cl		7/3/2003	7/31/2003
		CWM41 - CWM54 not used				
CW	Mush	CWM55		*Paxillus panuoides*	10/2/2003	12/19/2003
CW	Mush	CWM56 0 mybda		*Spongipellis pachydon*	10/2/2003	12/19/2003
CW	Mush	CWM56 1.0 mybda MYA			10/2/2003	11/17/2003
CW	Mush	CWM57 0 mybda2	cl	*Boletinellus merulioides*	10/2/2003	10/30/2003
CW	Mush	CWM57 .5 mybda	cl	*assoc. w/ maple roots*	10/2/2003	10/30/2003
CW	Mush	CWM58 .5 mybda2		*Lycoperdon perlatum*	10/2/2003	10/30/2003
CW	Mush	CWM59 .5 mybda mybda mybda 1		*Mycena*	10/2/2003	11/17/2003
CW	Mush	CWM59 .5 mybda mybda 2			10/2/2003	10/30/2003
CW	Mush	CWM59 1.0 mybda MYA			10/2/2003	11/17/2003
CW	Mush	CWM59 1.5 mybda2			10/2/2003	7/2/2004
CW	Mush	CWM60 0 mybda MYA			10/2/2003	11/17/2003
CW	Mush	CWM60 0.5 mybda MYA			10/2/2003	11/17/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM60 1.0 mybda MYA		*Coriolellus albidus*	10/2/2003	11/17/2003
CW	Mush	CWM60 1.5 mybda2			10/2/2003	11/17/2003
CW	Mush	CWM61			10/2/2003	tossed
CW	Mush	CWM62 1.5 mybda	cl	*Grifola frondosa*	10/2/2003	10/30/2003
CW	Mush	CWM62 2.0 mybda2		*at base of hemlock tree*	10/2/2003	12/19/2003
CW	Mush	CWM62-B 1.0 mybda2	cl		10/2/2003	11/17/2003
CW	Mush	CWM62-B 1.5 mybda	cl		10/2/2003	10/30/2003
CW	Mush	CWM63 .5 mybda			10/2/2003	10/30/2003
CW	Mush	CWM63 1.5 mybda A mybda			10/2/2003	11/17/2003
CW	Mush	CWM64 0 mybda		*Amanita muscaria*	10/2/2003	12/19/2003
CW	Mush	CWM65 0 mybda A mybda	cl		10/2/2003	11/17/2003
CW	Mush	CWM65 0 mybda B mybda	cl		10/2/2003	11/17/2003
CW	Mush	CWM65 .5 mybda MYA			10/2/2003	11/17/2003
CW	Mush	CWM65 2.0 mybda2 A mybda			10/2/2003	11/17/2003
CW	Mush	CWM66 2.0 mybda2		*Xeromphalina campanella*	5/19/2004	6/10/2004
CW	Mush	CWM66 2.0 mybda			5/19/2004	6/10/2004
CW	Mush	CWM67 0 mybda	cl	*Laetiporus sulphureus*	5/19/2004	7/2/2004
CW	Mush	CWM67 2.0 mybda2	cl		5/19/2004	6/9/2004
CW	Mush	CWM68 0 mybda A mybda			5/19/2004	6/10/2004
CW	Mush	CWM68 0 mybda B mybda	cl		5/19/2004	6/9/2004
CW	Mush	CWM68 1.0 mybda A mybda			5/19/2004	6/10/2004
CW	Mush	CWM68 2.0 mybda A mybda			5/19/2004	6/9/2004
CW	Mush	CWM68 2.0 mybda B mybda			5/19/2004	6/9/2004
CW	Mush	CWM69 2.0 mybda	cl	*Ganoderma tsugae*	5/19/2004	7/2/2004
CW	Mush	CWM70 1.0 mybda2		*Trametes versicolor*	5/19/2004	6/10/2004
CW	Mush	CWM70 2.0 mybda			5/19/2004	6/10/2004
CW	Mush	CWM71 0 mybda2		*Mycena alkalina*	5/19/2004	6/9/2004
CW	Mush	CWM71 1.0 mybda			5/19/2004	6/9/2004
CW	Mush	CWM71 2.0 mybda			5/19/2004	6/9/2004
CW	Mush	CWM75 tossed; *bacteria*			5/25/2004	tossed
CW	Mush	CWM76 tossed; bacteria		*Stereum*	5/25/2004	tossed

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM77 tossed;bacteria		*Favolus*	5/25/2004	tossed
CW	Mush	CWM78 tossed; bacteria		*Favolus*	5/25/2004	tossed
CW	Mush	CWM79 tossed; bacteria		*white polypore*	5/25/2004	tossed
CW	Mush	CWM80 tossed; bacteria		*jelly*	5/25/2004	tossed
CW	Mush	CWM83 0 mybda		*LBM*	6/8/2004	8/4/2004
CW	Mush	CWM83 1.0 mybda A mybda			6/8/2004	8/4/2004
CW	Mush	CWM83 1.0 mybda B mybda			6/8/2004	8/4/2004
CW	Mush	CWM84 1.5 mybda2			6/8/2004	8/4/2004
CW	Mush	CWM84 2.0 mybda2			6/8/2004	8/4/2004
CW	Mush	CWM85 1.5 mybda2			6/8/2004	8/4/2004
CW	Mush	CWM85 0 mybda3			6/8/2004	8/13/2004
CW	Mush	CWM85 1.0 mybda3			6/8/2004	8/13/2004
CW	Mush	CWM87 1.0 mybda CMEA mya			6/8/2004	6/6/2005
CW	Mush	CWM89 0 mybda b mybda			2004	7/6/2005
CW	Mush	CWM89 0 mybda a mybda			2004	7/6/2005
CW	Mush	CWM90 1.0 mybda2 PDA			2004	6/2/2005
CW	Mush	CWM91 1.0 mybda2			2004	2/10/2005
CW	Mush	CWM92 1.0 mybda2 mya			2004	6/2/2005
CW	Mush	CWM93 1.0 mybda PDA			2004	6/2/2005
CW	Mush	CWM94 0 mybda a mybda			2004	6/6/2005
CW	Mush	CWM96 0 mybda2			2004	6/30/2005
CW	Mush	CWM97 1.0 mybda C mybda PDA			2004	6/2/2005
CW	Mush	CWM98 2.0 II B mybda			2004	6/2/2005
CW	Mush	CWM98 2.0 II A mybda			2004	6/2/2005
CW	Mush	CWM98 0 mybda a mybda	cl		2004	1/3/2005
CW	Mush	CWM98 0 mybda2 CMEA mybda PDA			2004	6/2/2005
CW	Mush	CWM98 0 mybda a mybda CMEA mybda	cl		2004	2/10/2005
CW	Mush	CWM98 1.0 II A mybda			2004	6/2/2005
CW	Mush	CWM99 1.0 mybda			2004	2/10/2005
CW	Mush	CWM100 0 mybda			2004	1/3/2005
CW	Mush	CWM100 0 mybda			2004	3/7/2005

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM100 1.0 mybda Czp MEA			2004	6/22/2005
CW	Mush	CWM100 0 mybda2			2004	2/10/2005
CW	Mush	CWM100H 0 mybda B mybda			2004	7/15/2005
CW	Mush	CWM101 2.0 mybda2			9/1/2004	2/10/2005
CW	Mush	CWM101 1.0 mybda2 C mybda			9/1/2004	7/15/2005
CW	Mush	CWM101 1.0 mybda2 b mybda			9/1/2004	6/6/2005
CW	Mush	CWM101 1.0 mybda2 a mybda2 MEA			9/1/2004	6/2/2005
CW	Mush	CWM101 1.0 mybda2 mybda2 MEA			9/1/2004	6/22/2005
CW	Mush	CWM101 0 mybda2			9/1/2004	7/6/2005
CW	Mush	CWM102 1.0 mybda2 PDA MYA			9/1/2004	6/22/2005
CW	Mush	CWM103 1.0 mybda2			9/1/2004	7/15/2005
CW	Mush	CWM104 2.0 mybda2 a mybda			9/1/2004	7/6/2005
CW	Mush	CWM104 1.0 mybda2 Al mybda			9/1/2004	6/6/2005
CW	Mush	CWM105 2.0 (mybda A)2 mybda			9/1/2004	7/6/2005
CW	Mush	CWM105 2.0 mybda B mybda			9/1/2004	2/10/2005
CW	Mush	CWM106 0 mybda2 A mybda			9/1/2004	6/30/2005
CW	Mush	CWM107 0 mybda3			9/1/2004	6/6/2005
CW	Mush	CWM107 2.0 mybda2 b mybda			9/1/2004	6/2/2005
CW	Mush	CWM107 1.0 mybda2 a mybda A mybda			9/1/2004	6/2/2005
CW	Mush	CWM107 1.0 mybda2 a mybda B mybda			9/1/2004	7/15/2005
CW	Mush	CWM108 1.0 mybda2			9/1/2004	3/7/2005
CW	Mush	CWM109 0 mybda2			9/1/2004	7/15/2005
CW	Mush	CWM110 1.0 mybda2 a mybda			9/1/2004	6/6/2005
CW	Mush	CWM110 2.0 mybda2			9/1/2004	2/10/2005
CW	Mush	CWM110 2.0 mybda			9/1/2004	3/7/2005
CW	Mush	CWM111 1.0 mybda2 a mybda			9/1/2004	6/6/2005
CW	Mush	CWM111 1.0 mybda2 b mybda PDA			9/1/2004	6/2/2005
CW	Mush	CWM112 2.0 mybda B mybda2			9/1/2004	6/2/2005
CW	Mush	CWM112 2.0 mybda A mybda			9/1/2004	2/10/2005
CW	Mush	CWM113 1.0 mybda2 MYA			9/1/2004	6/6/2005
CW	Mush	CWM113 .5 (mybda A)3 MEA			9/1/2004	6/22/2005

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM115 0 mybda2 b mybda A mybda			9/17/2004	6/22/2005
CW	Mush	CWM115 0 mybda2 b mybda B mybda			9/17/2004	7/15/2005
CW	Mush	CWM115 2.0 mybda			9/17/2004	2/10/2005
CW	Mush	CWM131		*Clitocybe candicans?*	10/6/2005	lost
CW	Mush	CWM132 1.0 mybda A mybda		*Suillus pictus*	10/6/2005	10/20/2005
CW	Mush	CWM132 0 mybda cmea			10/6/2005	3/28/2006
CW	Mush	CWM134 1.0 mybda2		*yellow/grn/tan cap, wht stalk*	10/6/2005	10/20/2005
CW	Mush	CWM135 0 mybda A mybda CMEA		*Marasmius rotula on dead twig*	10/6/2005	3/28/2006
CW	Mush	CWM135 0 mybda B mybda CMEA			10/6/2005	3/28/2006
CW	Mush	CWM137 1.0 mybda A mybda		*Cortinarius iodes?*	10/6/2005	10/20/2005
CW	Mush	CWM138 0 mybda A mybda			10/6/2005	3/28/2006
CW	Mush	CWM139 1.0 mybda2		*Cantharellus or Hygrophorus?*	10/6/2005	3/21/2006
CW	Mush	CWM140 0 mybda E mybda		*Gymnopilus?*	10/6/2005	3/21/2006
CW	Mush	CWM140 0 (mybda A)2 mybda	cl		10/6/2005	4/6/2006
CW	Mush	CWM140 0 mybda B mybda CMEA			10/6/2005	3/23/2006
CW	Mush	CWM141 1.0 B mybda2		*Armillariella*	10/6/2005	3/23/2006
CW	Mush	CWM142 0 mybda B mybda A mybda	cl	*Trametes?*	10/6/2005	4/6/2006
CW	Mush	CWM142 0 MYBDA A mybda CMEA			10/6/2005	3/23/2006
CW	Mush	CWM143 0 mybda C mybda		*Collybia tuberosa?*	10/6/2005	10/20/2005
CW	Mush	CWM143 1.0 B mybda B mybda2			10/6/2005	4/6/2006
CW	Mush	CWM144 0 mybda A mybda		*sm gilled;dk olive cap,gray stk*	10/6/2005	10/20/2005
CW	Mush	CWM144 0 mybda B mybda CMEA			10/6/2005	10/20/2005
CW	Mush	CWM144 1.0 mybda			10/6/2005	3/21/2006
CW	Mush	CWM145 0 mybda B mybda		*Trametes?*	10/6/2005	3/23/2006
CW	Mush	CWM145 0 mybda D mybda A mybda	cl		10/6/2005	4/6/2006
CW	Mush	CWM148 0 A mybda2 A mybda	cl	*chantrelle type*	10/6/2005	4/6/2006
CW	Mush	CWM151 0 mybda B mybda2 A mybda CMEA		*Boletus*	10/6/2005	3/23/2006
CW	Mush	CWM152 1.0 mybda A mybda CMEA		*Marasmius?*	10/6/2005	10/20/2005
CW	Mush	CWM159 1.0 mybda A mybda CMEA2	cl	*Russula?*	10/6/2005	3/28/2006
CW	Mush	CWM159 1.0 mybda D mybda			10/6/2005	3/21/2006
CW	Mush	CWM160 1.0 A myba2		*tan cap; buff stalk; attached*	10/6/2005	3/21/2006

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
CW	Mush	CWM161 0 mybda D mybda		*Russula?*	10/6/2005	3/21/2006
CW	Mush	CWM161 0 mybda A B mybda CMEA			10/6/2005	3/23/2006
CW	Mush	CWM161 0 mybda C A mybda2			10/6/2005	3/28/2006
CW	Mush	CWM166 0 A mybda2		*small tan gilled*	10/6/2005	3/21/2006
CW	Mush	CWM166 0 B mybda A mybda2	cl		10/6/2005	4/6/2006
CW	Mush	CWM185 1.0 A MEA mybda			10/6/2005	3/21/2006
CW2	Mush	CW2M 187 1.0 A MEA mybda			10/6/2005	3/23/2006
CW2	Mush	CW2M 187 1.0 B MEA mybda CMEA			10/6/2005	3/23/2006
CW	Root	CWM57 A .5 mybda 1				10/20/2003
CW	Root	CWM57 B .5 mybda CMEA 2				11/17/2003
CW	Root	CWM63 A .5 mybda				10/30/2003
CW	Root	CWM63 C .5 mybda				10/30/2003
CW	Root	CWM63 D .5 mybda				10/30/2003
CW1	Bait	2 P 1204 1.0 mybda2				8/15/2005
CW1	Bait	2 P 1204 2.0 mybda2 CMEA				10/12/2005
CW1	Bait	3 P 305 wood 1.0 mybda B mybda				10/18/2005
CW1	Bait	4 P 505 soil MEA A mybda2 CMEA				3/28/2006
CW1	Bait	23 M 305 soil mybda MEA A mybda2				3/23/2006
CW1	Bait	23 M 305 soil mybda3				10/20/2005
CW1	Bait	24 M 505 soil mybda B MEA2				10/20/2005
CW1	Bait	24 M 505 wood 1.0 mybda2				10/18/2005
CW1	Bait	62 Q 1204 2.0 root mybda2				8/15/2005
CW1	Bait	62 Q 1204 2.0 wood mybda2				8/15/2005
CW1	Bait	63 Q 305 wood 1.0 mybda2 A mybda				10/20/2005
CW1	Bait	63 Q 305 wood 1.0 mybda2 B mybda3				3/23/2006
CW1	Bait	64 Q 505 soil mybda C MEA				10/20/2005
CW1	Bait	72 Q 1204 0 mybda2 B mybda2				10/18/2005
CW1	Bait	72 Q 1204 0 mybda2 A mybda				10/18/2005
CW1	Bait	124 M 505 soil mybda C MEA mybda				3/28/2006
CW2	Bait	12 P 1204 0 mybda2				8/15/2005

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Port	Mush	PSM41			7/31/2003	tossed
Hvd	Mush	HFM42			7/31/2003	tossed
Hvd	Mush	HFM43			7/31/2003	tossed
ME	Mush	M1			8/18/2003	tossed
ME	Mush	M2 0 mybda A			8/18/2003	9/8/2003
ME	Mush	M2 0 mybda B			8/18/2003	9/8/2003
ME	Mush	M2 2.0 mybda			8/18/2003	9/8/2003
ME	Mush	M3 0 mybda			8/18/2003	9/8/2003
Sall	Mush	SFM44 .5 mybda2			7/31/2003	9/8/2003
Sall	Mush	SFM44 1.0 mybda A mybda 1	cl		7/31/2003	8/26/2003
Sall	Mush	SFM44 1.0 mybda A mybda 2	cl		7/31/2003	8/26/2003
Sall	Mush	SFM44 1.0 mybda B mybda 1			7/31/2003	8/28/2003
Sall	Mush	SFM44 1.0 mybda B mybda 2			7/31/2003	8/28/2003
Sall	Mush	SFM45 1.0 mybda			7/31/2003	8/26/2003
Sall	Mush	SFM45 1.5 mybda2			7/31/2003	8/26/2003
Sall	Mush	SFM45 2.0 mybda			7/31/2003	8/26/2003
Sall	Mush	SFM45 2.0 mybda MYA			7/31/2003	8/26/2003
Sall	Mush	SFM46 0 mybda2		*Hygrophorus*	9/11/2003	10/30/2003
Sall	Mush	SFM46 .5 mybda	cl		9/11/2003	10/7/2003
Sall	Mush	SFM46 2.0 mybda	cl		9/11/2003	10/21/2003
Sall	Mush	SFM46-B 0 mybda2	cl		9/11/2003	10/7/2003
Sall	Mush	SFM46-B 1.0 mybda 1			9/11/2003	10/30/2003
Sall	Mush	SFM46-B 1.5 mybda	cl		9/11/2003	10/7/2003
Sall	Mush	SFM46-B 1.5 mybda2	cl		9/11/2003	10/7/2003
Sall	Mush	SFM46-B 2.0 mybda2			9/11/2003	11/17/2003
Sall	Mush	SFM47		*Hygrophorus*	9/11/2003	tossed
Sall	Mush	SFM48 .5 mybda2			9/11/2003	11/17/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Sall	Mush	SFM48 1.0 mybda2			9/11/2003	10/30/2003
Sall	Mush	SFM48 1.0 mybda 2 mybda			9/11/2003	10/30/2003
Sall	Mush	SFM48 2.0 mybda			9/11/2003	10/7/2003
Sall	Mush	SFM49 .5 mybda	cl		9/11/2003	11/17/2003
Sall	Mush	SFM49 1.0 mybda			9/11/2003	10/7/2003
Sall	Mush	SFM50 1.0 mybda MYA	cl	*Collybia pollustrus*	9/11/2003	10/19/2003
Sall	Mush	SFM51		*Collybia*	9/11/2003	tossed
		52 not used			9/11/2003	
Sall	Mush	SFM53 1.0 mybda B mybda	cl	*tiny white under Alder*	9/11/2003	11/17/2003
Sall	Mush	SFM53 2.0 mybda	cl		9/11/2003	11/17/2003
Sall	Mush	SFM54 0 mybda2	cl	*sm brown under Alder*	9/11/2003	10/7/2003
Sall	Mush	SFM54 1.5 mybda2	cl		9/11/2003	10/7/2003
Sall	Mush	SFM86 2.0mybda MYA C mybda CMEA2			7/21/2004	1/13/2005
Sall	Mush	SFM86 2.0 mybda MYA A MYBDA CMEA			7/21/2004	3/7/2005
Sall	Mush	SFM86 2.0 mybda MYA B MYBDA CMEA MYBDA			7/21/2004	7/6/2005
Sall	Mush	SFM86 1.0 mybda MYA B mybda CMEA2 B mybda2			7/21/2004	6/6/2005
Sall	Mush	SFM116 2.0 MYBDA3 A MEA			10/19/2004	6/6/2005
Sall	Mush	SFM116 2.0 MYBDA3 B MEA			10/19/2004	6/6/2005
Sall	Mush	SFM116 0.5 hyphae mybda			10/19/2004	6/2/2005
Sall	Mush	SFM116 1.0 a mybda			10/19/2004	6/2/2005
Sall	Mush	SFM126 0 A mybda2			6/28/2005	8/15/2005
Sall	Mush	SFM126 0 B mybda2			6/28/2005	8/15/2005
Sall	Mush	SFM127 1.0 mybda2 A mybda			6/28/2005	10/18/2005
Sall	Mush	SFM127 0 B mybda2 A mybda			6/28/2005	10/18/2005
Sall	Mush	SFM127 0 B mybda2 C mybda			6/28/2005	8/15/2005
Sall	Mush	SFM127 0 A mybda B			6/28/2005	7/15/2005
Sall	Mush	SFM127 1.0 mybda2 CMEA			6/28/2005	10/12/2005
Sall	Mush	SFM128 1.0 mybda4 A mybda			6/28/2005	10/18/2005
Sall	Mush	SFM128 1.0 mybda4 B mybda			6/28/2005	10/18/2005

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Sall	Mush	SFM129 1.0 mybda3			6/28/2005	10/18/2005
Sall	Mush	SFM129 0 A mybda3 B mybda			6/28/2005	8/15/2005
Sall	Mush	SFM129 0 A mybda3 A mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 0 A mybda2 A mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 0 A mybda2 B mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 0 B mybda2			6/28/2005	8/15/2005
Sall	Mush	SFM130 1.0 A mybda2			6/28/2005	8/15/2005
Sall	Mush	SFM130 1.0 B (mybda A)3 mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 1.0 B mybda B mybda C mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 1.0 B mybda A mybda B mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 1.0 B (mybda A)2 mybda B mybda			6/28/2005	10/18/2005
Sall	Mush	SFM130 1.0 B (mybda B)2 mybda A mybda			6/28/2005	10/12/2005
Sall	Mush	SFM130 1.0 B (mybda B)2 mybda MEA mybda3			6/28/2005	3/21/2006
Sall	Plant	SFM126 1.0 moss mybda B mybda2 A MYBDA			6/28/2005	10/18/2005
Sall	Plant	SFM127 1.0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM127 0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM127 1.0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM128 1.0 moss mybda			6/28/2005	7/15/2005
Sall	Plant	SFM128 1.0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM129 1.0 moss mybda2 A mybda			6/28/2005	8/15/2005
Sall	Plant	SFM129 1.0 moss mybda2 C mybda			6/28/2005	8/15/2005
Sall	Plant	SFM129 0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM129 1.0 moss mybda2 B mybda			6/28/2005	8/15/2005
Sall	Plant	SFM130 0 moss mybda2			6/28/2005	7/15/2005
Sall	Plant	SFM130 1.0 moss mybda2			6/28/2005	7/15/2005
Sall	Bait	SF 43 P 305 0 mybda CMEA mybda			6/28/2005	8/15/2005
Sall	Bait	SF 43 P 305 1.0 mybda2			6/28/2005	8/15/2005
Sall	Bait	SF 44 P 505 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 53 P 305 0 mybda CMEA mybda			6/28/2005	8/15/2005
Sall	Bait	SF 53 P 305 1.0 mybda4 B mybda			6/28/2005	10/18/2005

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Sall	Bait	SF 54 P 505 0 MYBDA2 A MYBDA			6/28/2005	10/18/2005
Sall	Bait	SF 54 P 505 1.0 MYBDA2 B MYBDA2			6/28/2005	10/18/2005
Sall	Bait	SF 54 P 505 0 MYBDA2 B MYBDA2			6/28/2005	10/18/2005
Sall	Bait	SF 102 Q 1204 1.0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 102 Q 1204 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 103 Q 305 1.0 mybda3			6/28/2005	10/18/2005
Sall	Bait	SF 103 Q 305 0 mybda CMEA mybda			6/28/2005	8/15/2005
Sall	Bait	SF 112 Q 1204 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 113 Q 305 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 113 Q 305 1.0 mybda2			6/28/2005	10/18/2005
Sall	Bait	SF 152 P 1204 1.0 mybda2			6/28/2005	8/15/2005
Sall	Bait	SF 152 P 1204 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 152 M 1204 1.0 mybda2			6/28/2005	10/18/2005
Sall	Bait	SF 152 M 1204 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 153 M 305 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 153 M 1.0 MYBDA2 CMEA			6/28/2005	10/20/2005
Sall	Bait	SF 154 M 505 0 mybda CMEA mybda			6/28/2005	8/15/2005
Sall	Bait	SF 154 M 505 1.0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 162 M 1204 1.0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 162 M 1204 0 mybda			6/28/2005	7/15/2005
Sall	Bait	SF 163 M 305 0 mybda CMEA mybda			6/28/2005	8/15/2005
Sall	Bait	SF 163 M 305 1.0 mybda CMEA mybda			6/28/2005	10/12/2005
Sall	Bait	SF 164 M 505 0 mybda B MYBDA4			6/28/2005	10/18/2005
Sall	Bait	SF 164 M 505 1.0 mybda2 A MYBDA			6/28/2005	10/18/2005
Sall	Bait	SF 164 M 505 1.0 mybda2 B MYBDA			6/28/2005	10/18/2005
Sall	Plant	Alder1 D mybda			9/11/2003	10/21/2003
Sall	Plant	Alder1 A mybda CMEA			9/11/2003	11/17/2003
Sall	Plant	Alder1 E mybda mybda			9/11/2003	12/19/2003
Sall	Plant	Alder2 B mybda			9/11/2003	10/21/2003
Sall	Plant	Cranberry 46 A mybda a mybda			9/11/2003	10/21/2003

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Sall	Plant	Cranberry 46 A mybda b mybda			9/11/2003	10/21/2003
Sall	Plant	Cranberry 46 B mybda			9/11/2003	10/21/2003
Sall	Plant	Cranberry 46 C mybda2			9/11/2003	10/21/2003
Sall	Plant	Cranberry 46 C mybda			9/11/2003	10/21/2003
Sall	Plant	Carex 46 C mybda			9/11/2003	10/21/2003
Sall	Plant	Carex 46 B mybda			9/11/2003	11/17/2003
Sall	Plant	Carex 46 C mybda2			9/11/2003	10/21/2003
Sall	Plant	Leatherleaf 46 A mybda			9/11/2003	10/21/2003
Sall	Plant	Leatherleaf 46 B mybda			9/11/2003	10/21/2003
Sall	Plant	Leatherleaf 46 C mybda			9/11/2003	10/21/2003
Sall	Plant	Sphagnum 46 B mybda			9/11/2003	10/21/2003
KF	Mush	KM118 0 mybda	cl		6/2/2005	7/6/2005
KF	Mush	KM118 1.0 mybda	cl		6/2/2005	7/6/2005
KF	Mush	KM119 0 mybda	cl		6/2/2005	7/6/2005
KF	Mush	KM123 0 mybda2			6/2/2005	7/6/2005
KF	Mush	KM123 1.0 MEA B mybda2			6/2/2005	8/15/2005
KF	Soil	KS119 0 A mybda B mybda2			6/2/2005	6/30/2005
KF	Soil	KS119 0 A mybda B mybda6			6/2/2005	7/6/2005
KF	Soil	KS119 0 A mybda C mybda2			6/2/2005	8/15/2005
KF	Soil	KS124 0 B mybda B mybda 1 mybda			6/2/2005	7/15/2005
KF	Soil	KS125 0 A mybda A mybda3			6/2/2005	6/22/2005
KF	Soil	KS125 0 A mybda A mybda1 mybda2 A mybda2			6/2/2005	10/18/2005
Kit	Mush	KITM66 0 a mybda			3/5/2004	3/30/2004
Kit	Mush	KITM66 0 b mybda			3/5/2004	3/30/2004
Kit	Mush	KITM66 .5 mybda			3/5/2004	3/30/2004
Kit	Mush	KITM66 1.0 mybda			3/5/2004	3/30/2004
Kit	Mush	KITM66 1.5 mybda			3/5/2004	3/30/2004
Kit	Mush	KITM66 2.0 mybda			3/5/2004	3/30/2004

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
BBH	Plant	BBH1 2.0 mybda2 b mybda CMEA			8/2/2004	2/10/2005
BBH	Plant	BBH1 2.0 mybda2 a mybda			8/2/2004	3/7/2005
BBH	Plant	BBH1 0 mybda2 a mybda CMEA2 mybda			8/2/2004	3/7/2005
BBH	Plant	BBH1 1.0 mybda2 a mybda			8/2/2004	3/17/2005
BBH	Plant	BBH1 1.0 mybda2 b mybda4			8/2/2004	6/22/2005
BBH	Plant	BBH1 1.0 mybda2 c mybda			8/2/2004	7/6/2005
BBH	Plant	BBH1 0 mybda2 b mybda CMEA mybda			8/2/2004	7/15/2005
BBH	Plant	BBH2 0 dead mybda2			8/2/2004	1/13/2005
BBH	Plant	BBH2 0 green mybda2 II mybda MEA			8/2/2004	6/22/2005
BBH	Plant	BBH2 2.0 dead mybda2 (CMEA mybda)2 PDA			8/2/2004	6/6/2005
BBH	Plant	BBH2 0 green mybda2 I mybda			8/2/2004	7/15/2005
BBH	Plant	BBH2 2.0 dead mybda2 (CMEA mybda)2			8/2/2004	6/2/2005
BBH	Plant	BBH2 2.0 dead mybda2 (CMEA mybda)2 CMEA	cl		8/2/2004	8/15/2005
Chap	Root	CLR4 a mybda2			4/27/2004	5/14/2005
Chap	Root	CLR3 a mybda 4 mybda MEA			4/27/2004	5/28/2004
Chap	Root	CLR3 b MYA mybda			4/27/2004	5/14/2004
Chap	Root	CLR4 a MYA 1 mybda			4/27/2004	5/14/2004
Chap	Root	CLR3 a mybda 2 mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 11 MEA b mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 11 MEA c mybda MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 12 MEA mybda 4 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 12 MEA mybda 1 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 12 MEA mybda3 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 14 MEA c mybda 1 mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 14 MEA b mybda 2 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 14 MEA a mybda			4/27/2004	5/14/2004
Chap	Soil	CLS 14 MEA b mybda 1 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 14 MEA c mybda 1 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 14 MEA C mybda 2 MEA			4/27/2004	6/10/2004

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Chap	Soil	CLS 15 MEA mybda 1 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 15 MEA mybda 2 MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 21 MEA a mybda MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 21 MEA b mybda MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 21 MEA a mybda MEA CMEA mybda			4/27/2004	6/9/2004
Chap	Soil	CLS 23 MEA b mybda			4/27/2004	5/14/2004
Chap	Soil	CLS 23 MEA c mybda CMEA mybda			4/27/2004	6/10/2004
Chap	Soil	CLS 23 MEA C mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 24 MEA C mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 24 MEA D mybda CMEA mybda			4/27/2004	6/10/2004
Chap	Soil	CLS 24 MEA D mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 25 MEA b mybda MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 25 MEA c mybda			4/27/2004	5/28/2004
Chap	Soil	CLS 36 MEA b mybda MEA			4/27/2004	5/28/2004
Chap	Soil	CLS 36 MEA c mybda			4/27/2004	5/14/2004
Chap	Soil	CLS 36 MEA d mybda			4/27/2004	5/14/2004
Chap	Soil	CLS 45 MEA mybda MEA			4/27/2004	5/28/2004
Chap	Wood	1 A mybda4 A mybda2			2/10/2005	10/12/2005
Chap	Wood	1 B mybda4 A mybda2			2/10/2005	10/12/2005
Chap	Wood	1 B mybda4 B MEA			2/10/2005	10/12/2005
Chap	Wood	1 C mybda2			2/10/2005	7/15/2005
Chap	Wood	1 C mybda Czp			2/10/2005	6/2/2005
Chap	Wood	1 D mybda Czp mybda2			2/10/2005	10/18/2005
Chap	Wood	2 a mybda3 A mybda A mybda2			2/10/2005	10/18/2005
Chap	Wood	2 a mybda3 A mybda B mybda			2/10/2005	10/18/2005
Chap	Wood	2 a mybda3 B mybda			2/10/2005	10/18/2005
Chap	Wood	2 d mybda3 A mybda2			2/10/2005	10/18/2005
Chap	Wood	2 d mybda3 B mybda			2/10/2005	10/18/2005
Chap	Wood	3 b 1 mybda MYA			2/10/2005	6/6/2005
Chap	Wood	3 b 2 mybda2			2/10/2005	7/6/2005

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Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
Chap	Wood	3 b 3 mybda2			2/10/2005	7/6/2005
Chap	Wood	3 a 2 mybda Czp mybda A mybda			2/10/2005	10/18/2005
Chap	Wood	3 a 2 mybda Czp mybda B mybda			2/10/2005	10/18/2005
Chap	Wood	3 a 1 mybda Czp mybda MEA			2/10/2005	10/12/2005
Chap	Wood	3 mybda3 A mybda MEA			2/10/2005	10/12/2005
Chap	Wood	3 mybda3 B mybda MEA			2/10/2005	10/12/2005
Chap	Wood	3 mybda3 C mybda A MEA			2/10/2005	3/21/2006
OD	Plant	OD1 0 dead culm & leaf mybda MYA CMEA2			8/2/2004	1/13/2005
OD	Plant	OD1 1.0 dead culm & leaf mybda2 CMEA2			8/2/2004	1/13/2005
OD	Plant	OD2 2.0 mybda3 CMEA			8/2/2004	3/17/2005
OD	Plant	OD2 0 mybda2 b mybda CMEA			8/2/2004	2/10/2005
OD	Plant	OD2 2.0 mybda3 b mybda			8/2/2004	6/2/2005
OD	Plant	OD2 2.0 mybda3 a mybda b mybda2			8/2/2004	6/22/2005
OD	Plant	OD2 2.0 mybda3 b mybda CMEA MEA			8/2/2004	6/22/2005
OD	Plant	OD2 1.0 mybda2 a mybda CMEA2 mybda MEA			8/2/2004	6/22/2005
OD	Plant	OD2 2.0 mybda3 a mybda2 MEA			8/2/2004	1/13/2005
OD	Plant	OD2 0 mybda2 a mybda CMEA			8/2/2004	1/13/2005
MP	Plant	MP1 0 mybda2 C mybda CMEA2			8/2/2004	1/13/2005
MP	Plant	MP1 1.0 mybda2 CMEA			8/2/2004	6/2/2005
MP	Plant	MP2 0 mybda3 b mybda			8/2/2004	3/17/2005
MP	Plant	MP2 1.0 mybda MYA mybda			8/2/2004	3/7/2005
MP	Plant	MP2 0 mybda3 b mybda			8/2/2004	7/15/2005
MP	Plant	MP2 0 mybda3 a mybda 2 mybda			8/2/2004	7/6/2005
UNH	Mush	UNHM1 1.0 mybda2	cl	*Russula*	9/30/2003	10/30/2003
UNH	Mush	UNHM72 dead; not cultured			5/24/2004	tossed
UNH	Mush	UNHM73 dead; not cultured		*Favolus alveolaris*	5/25/2004	tossed
UNH	Mush	UNHM74 dead; not cultured			5/26/2004	tossed
UNH	Mush	UNHM81 1.0 mybda2	cl	*tiny gilled in lawn*	5/27/2004	7/2/2004

Site	Type	Isolate Name		Putative ID	Collected:	Cryovial:
UNH	Mush	UNHM81 1.5 mybda	cl		5/27/2004	7/2/2004
UNH	Mush	UNHM81 2.0 mybda	cl		5/27/2004	7/2/2004
UNH	Mush	UNHM82 1.5 mybda2 B			5/27/2004	7/14/2004
UNH	Mush	UNHM82 1.0 mybda			5/27/2004	7/2/2004
UNH	Mush	UNHM82 1.5 mybda2 A mybda			5/27/2004	7/14/2004

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APPENDIX B

B.0 ION CHROMATOGRAPHY RESULTS

This appendix contains the ion chromatography analysis results for soils used in methyl halide assays. The soils were assayed as controls; they were not inoculated with fungi. The sterile water used for culturing fungi was also analyzed with water samples from the ion chromatography laboratory for comparison.

Soil samples were not tested for manganese; however, traces indicated the presence of manganese, similar to the magnesium levels.

Table B-1contains the data for control soils that were not autoclaved before assaying for methyl halides. The soils were collected from College Woods and Kingman Farm on 3-February-2006, placed directly in sterilized flasks, capped with sterile stoppers, and covered in aluminum foil in the field.

Upon return to the laboratory, the soils were immediately set up on the zero air flushing system. Samples were assayed for methyl halides between 4-February-2006 and 21-February-2006. Substrate induced respiration tests were conducted on 6-March-2006.

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Soils were dried 28-March-2006, prepared for ion chromatography on 13-April-2006, and analyzed on 18-April-2006.

Table B-1: Cation and Anion Contents for Soils Not Autoclaved. CW=College Woods; KF=Kingman Farm; u=untreated soils (soils were not autoclaved); all measurements are in nanomoles/liter.

Sample	Na	NH4	K	Mg	Ca	Cl	NO3	SO4	Oxalate
CW 1a	2.73E+04	1.40E+05	1.06E+05	5.00E+04	3.45E+04	4.68E+04	1.50E+03	5.47E+04	2.75E+04
CW 1b	2.83E+04	1.37E+05	1.09E+05	5.46E+04	3.49E+04	4.82E+04	1.18E+03	5.64E+04	2.55E+04
CW 2a	5.31E+04	1.29E+05	9.40E+04	5.52E+04	3.65E+04	6.14E+04	1.35E+03	6.97E+04	5.17E+04
CW 2b	5.41E+04	1.26E+05	9.60E+04	5.98E+04	3.70E+04	6.31E+04	6.83E+02	7.34E+04	5.14E+04
CW 3a	2.43E+04	8.01E+04	6.59E+04	4.11E+04	1.23E+04	2.76E+04	1.33E+03	3.41E+04	2.05E+04
CW 3b	2.81E+04	9.51E+04	7.77E+04	5.14E+04	1.50E+04	3.20E+04	1.56E+03	3.85E+04	2.18E+04
CW 3.1b	2.82E+04	9.33E+04	7.80E+04	5.21E+04	1.51E+04	3.18E+04	1.56E+03	3.85E+04	1.08E+04
CW 4a	2.22E+04	9.67E+04	8.17E+04	7.72E+04	1.30E+05	1.32E+04	2.19E+03	3.68E+04	6.07E+03
CW 4b	2.33E+04	1.00E+05	8.47E+04	8.21E+04	1.28E+05	1.34E+04	2.23E+03	3.72E+04	5.51E+03
CW 5a	6.74E+04	7.29E+05	2.15E+05	8.44E+04	3.76E+04	1.14E+05	8.56E+03	1.60E+05	1.32E+05
CW 5b	6.93E+04	6.62E+05	2.16E+05	9.05E+04	3.86E+04	1.09E+05	8.70E+03	1.62E+05	1.24E+05
Average:	**3.87E+04**	**2.17E+05**	**1.11E+05**	**6.35E+04**	**4.72E+04**	**5.09E+04**	**2.80E+03**	**6.92E+04**	**4.34E+04**
KF 1b	7.59E+03	5.80E+03	3.31E+04	7.42E+04	1.42E+05	3.53E+03	2.22E+05	9.95E+03	8.09E+02
KF 1a	7.42E+03	5.50E+03	3.26E+04	7.76E+04	1.50E+05	3.50E+03	2.28E+05	9.94E+03	4.69E+02
KF 2b	6.23E+03	7.89E+03	1.33E+04	5.35E+04	7.61E+04	3.22E+03	4.48E+04	1.86E+04	6.47E+02
KF 2a	6.05E+03	7.70E+03	1.29E+04	5.55E+04	7.99E+04	3.23E+03	4.55E+04	1.86E+04	3.26E+02
KF 3b	5.83E+03	1.08E+04	2.00E+04	4.49E+04	7.63E+04	2.72E+03	1.11E+04	1.47E+04	1.11E+03
KF 3a	5.82E+03	1.04E+04	1.90E+04	4.60E+04	7.96E+04	2.71E+03	1.14E+04	1.44E+04	6.13E+02
KF 4b	6.41E+03	3.56E+04	3.16E+04	3.38E+04	6.16E+04	3.83E+03	1.47E+03	1.50E+04	1.90E+03
KF 4a	6.56E+03	3.60E+04	3.10E+04	3.46E+04	6.39E+04	3.90E+03	1.31E+03	1.41E+04	1.02E+03
KF 4.1a	6.26E+03	3.30E+04	3.06E+04	3.48E+04	6.37E+04	3.76E+03	1.29E+03	1.40E+04	1.27E+03
KF 5b	4.06E+04	9.11E+04	7.57E+04	3.51E+04	2.29E+04	2.35E+04	1.13E+03	1.63E+04	3.04E+04
KF 5a	3.98E+04	8.99E+04	7.35E+04	3.53E+04	2.37E+04	2.20E+04	7.16E+02	1.53E+04	2.49E+04
Average:	**1.26E+04**	**3.03E+04**	**3.39E+04**	**4.78E+04**	**7.63E+04**	**6.91E+03**	**5.16E+04**	**1.46E+04**	**5.77E+03**

Table B-2 contains the cation and anion data for autoclaved soils that were assayed for methyl halides from 31-Jan-2006 to 9-Feb-2006. The soils were collected in November, 2005 and autoclaved once on the same day as collected.

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The soils were autoclaved again, three consecutive times on 30-January-2006, before assaying for methyl halides. Soils were assayed from 31-January-2006 to 9-February-2006. Substrate induced respiration tests were conducted on 10-Feb-2006. Soils were dried 28-Feb-2006, prepared for ion chromatography on 9-March-2006, and analyzed 24-March-2006.

Table B-2: Ion Chromatography for Autoclaved Soils. CW=College Woods; KF=Kingman Farm; u=untreated soils (soils were not autoclaved); all measurements are in nanomoles/liter.

Sample	Na	NH4	K	Mg	Ca	Cl	NO3	SO4	Oxalate
CW 1a	1.07E+05	7.16E+05	1.76E+05	1.87E+05	2.58E+05	2.04E+05	1.27E+04	2.53E+05	8.79E+05
CW 2a	1.03E+05	8.07E+05	1.97E+05	2.00E+05	2.77E+05	2.59E+05	1.30E+04	2.39E+05	9.03E+05
CW 3a	1.21E+05	7.13E+05	1.79E+05	1.85E+05	2.53E+05	2.25E+05	1.26E+04	2.42E+05	8.85E+05
CW 4a	1.19E+05	7.50E+05	1.90E+05	2.13E+05	2.81E+05	2.51E+05	1.30E+04	2.69E+05	9.79E+05
CW 5a	1.09E+05	7.62E+05	1.90E+05	1.97E+05	2.56E+05	2.36E+05	1.34E+04	2.53E+05	9.06E+05
CW 1b	1.09E+05	7.31E+05	1.80E+05	1.98E+05	2.63E+05	1.98E+05	1.23E+04	2.43E+05	8.61E+05
CW 2b	1.05E+05	8.22E+05	2.00E+05	2.10E+05	2.83E+05	2.48E+05	1.24E+04	2.29E+05	8.87E+05
CW 3b	1.23E+05	7.28E+05	1.81E+05	1.93E+05	2.56E+05	2.17E+05	1.17E+04	2.31E+05	8.58E+05
CW 4b	1.21E+05	7.52E+05	1.92E+05	2.21E+05	2.85E+05	2.42E+05	1.22E+04	2.62E+05	9.55E+05
CW 5b	1.09E+05	7.53E+05	1.88E+05	2.02E+05	2.59E+05	2.27E+05	1.27E+04	2.39E+05	8.81E+05
Average:	**1.13E+05**	**7.53E+05**	**1.87E+05**	**2.01E+05**	**2.67E+05**	**2.31E+05**	**1.26E+04**	**2.46E+05**	**8.99E+05**
KF 1a	3.02E+04	5.82E+04	3.08E+04	1.49E+05	2.62E+05	2.88E+04	2.26E+04	7.36E+04	2.25E+04
KF 2a	4.09E+04	7.43E+04	3.62E+04	1.86E+05	3.25E+05	4.68E+04	3.60E+04	8.98E+04	2.65E+04
KF 2a.1	-	-	-	-	-	4.80E+04	3.70E+04	9.33E+04	2.90E+04
KF 3a	3.48E+04	7.19E+04	3.56E+04	1.79E+05	3.11E+05	3.61E+04	2.87E+04	8.58E+04	2.47E+04
KF 4a	4.37E+04	6.86E+04	3.95E+04	1.57E+05	2.61E+05	4.00E+04	3.20E+04	7.91E+04	2.17E+04
KF 5a	3.45E+04	7.00E+04	4.06E+04	1.44E+05	2.36E+05	3.49E+04	2.95E+04	7.25E+04	2.01E+04
KF 1b	3.04E+04	6.34E+04	3.17E+04	1.55E+05	2.66E+05	2.77E+04	2.19E+04	7.11E+04	2.01E+04
KF 2b	4.14E+04	7.66E+04	3.67E+04	1.93E+05	3.30E+05	4.41E+04	3.38E+04	8.49E+04	2.86E+04
KF 2b.1	3.48E+04	6.61E+04	3.15E+04	1.62E+05	2.72E+05	-	-	-	-
KF 3b	3.10E+04	6.65E+04	3.24E+04	1.61E+05	2.71E+05	3.35E+04	2.65E+04	7.94E+04	2.37E+04
KF 4b	4.38E+04	7.14E+04	3.97E+04	1.63E+05	2.64E+05	3.87E+04	3.10E+04	7.58E+04	2.18E+04
KF 5b	3.47E+04	7.16E+04	4.08E+04	1.49E+05	2.40E+05	3.34E+04	2.76E+04	6.91E+04	1.56E+04
Average:	**3.64E+04**	**6.90E+04**	**3.60E+04**	**1.63E+05**	**2.76E+05**	**3.75E+04**	**2.97E+04**	**7.95E+04**	**2.31E+04**
diH_2O unfiltered	2.64E+03	2.02E+03	1.53E+03	5.98E+02	1.97E+03	4.31E+03	3.70E+02	2.87E+02	6.66E+01
diH_2O filtered	1.61E+03	1.71E+03	8.74E+02	4.49E+02	1.25E+03	1.32E+03	2.44E+02	2.05E+02	-
dH_2O sterile	1.42E+04	4.14E+03	1.62E+03	2.44E+02	1.60E+03	1.73E+03	4.96E+02	3.74E+02	-

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Table B-3 contains the ion chromatography analysis for iodide contained in the autoclaved and non-autoclaved soils.

Table B-3: Iodide Analysis of Soils. CW=College Woods; KF=Kingman Farm; u=untreated soils (soils were not autoclaved); all measurements are in nanomoles/liter; nd=not detected.

Sample	Iodide
CW 1a	130.91
CW 2a	276.70
CW 3a	148.51
CW 3b	176.90
CW 4a	125.00
CW 5a	227.81
CW 5b	92.90
Average:	**168.39**
uCW 1a	9.60
uCW 1b	51.60
uCW 2a	9.50
uCW 2b	30.16
uCW 3a	38.50
uCW 3b	24.51
uCW 4a	124.30
uCW 4b	38.77
uCW 5a	24.78
uCW 5b	nd
Average:	**39.08**

Sample	Iodide
KF 1a	433.34
KF 2a	540.65
KF 3a	534.07
KF 3	667.29
KF 4a	734.92
KF 5a	515.87
KF 5	593.99
Average:	**574.30**
uKF 1	nd
uKF 2	22.62
uKF 3	9.69
uKF 4	13.03
uKF 5	193.70
Average:	**59.76**
unfiltered diH_2O	nd
Filtered diH_2O	nd
sterile dH_2O	nd

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