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Physiological and biochemical controls over methyl halide emissions from rice plants

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[1] This paper investigates physiological and biochemical aspects of methyl halide production in rice plants over two growing seasons. Multiple separate mechanisms appear to be responsible for production of methyl halides in rice plant tissues. Evidence for multiple mechanisms is found in timing of peak emissions of methyl halides from rice, inconsistent effects of competitive inhibitors on methyl halide emissions, and large differences in methyl halide emission rates when compared to plant tissue halide concentrations. Other results show that chloride, bromide, and iodide ion concentrations in plant tissue appear to be regulated throughout the season, and observed changes in leaf tissue concentration cannot explain observed methyl halide emissions. The K_m for methyl iodide formation in leaf tissue cell-free extract is 0.018 mM, suggesting a very efficient mechanism. Of the seven competitive inhibitors used, only thiol had a consistently strong effect on both methyl iodide and methyl bromide. *INDEX TERMS:* 0315 Atmospheric Composition and Structure: Biosphere/atmosphere interactions; 1610 Global Change: Atmosphere (0315, 0325); 1615 Global Change: Biogeochemical processes (4805); *KEYWORDS:* biochemistry, methyl halide, rice

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1. Introduction

[2] Inorganic halogen radicals are recognized as important components of global atmospheric processes. Enhanced aerosol formation, ozone loss, and regional oxidative capacity modification have all been linked to increased halogen radical concentrations [*Weisenstein et al.*, 1992; *Schauffler et al.*, 1993; *Davis et al.*, 1996; *Alicke et al.*, 1999; *Vogt et al.*, 1999; *McFiggans et al.*, 2000; *O'Dowd et al.*, 2002]. Methyl halides are the primary natural source of tropospheric and stratospheric inorganic halogens [*Weisenstein et al.*, 1992; *Schauffler et al.*, 1993; *Davis et al.*, 1996; *Khalil*, 1999]. Despite their acknowledged significance, methyl halide budgets remain incomplete, with identified atmospheric removal mechanisms larger than quantified production processes [*Butler*, 2000]. Recent research suggests that terrestrial ecosystems may be capable of generating enough methyl halides to balance these budget discrepancies [*Saini et al.*, 1995; *Watling and Harper*, 1998; *Varner et al.*, 1999; *Redeker et al.*, 2000; *Rhew et al.*, 2000, 2001; *Dimmer et al.*, 2001; *Yokouchi et al.*, 2002]. While the importance of quantifying emissions from terrestrial ecosystems for methyl halide budgets is recognized, studies that focus on the biological processes that produce methyl halides in terrestrial plants are sparse. Of the few studies that have investigated biogenic methyl halide production, only three have focused on terrestrial plants, while others have described white-rot fungi and marine algae [*White*, 1982; *Wuosmaa and Hager*, 1990; *Attieh et al.*, 1995; *Saini et al.*, 1995; *Ni and Hager*, 1998; *Watling and Harper*, 1998; *Harper*, 2000].

[3] Several putative methyl halide transferases, (the term methyl halide transferase suggests that the primary role of the enzyme is transfer of a methyl group to a halogen) have been implicated in terrestrial plant production of methyl halides. This nomenclature may be premature, as specific functionality of methyl halide-producing enzymes from terrestrial plants remains uncertain. Several s-adenosyl-L-methionine (SAM) utilizing methyltransferases (MTs) have been isolated from terrestrial plants and fungi [*Attieh et al.*, 1995; *Saini et al.*, 1995; *Watling and Harper*, 1998], yet only one specific function for methyl halides synthesized by SAM-dependent methyl transferases has been identified; for many species of white-rot fungi from the Hymenochaetaceae family, methyl chloride plays a role in veratryl alcohol

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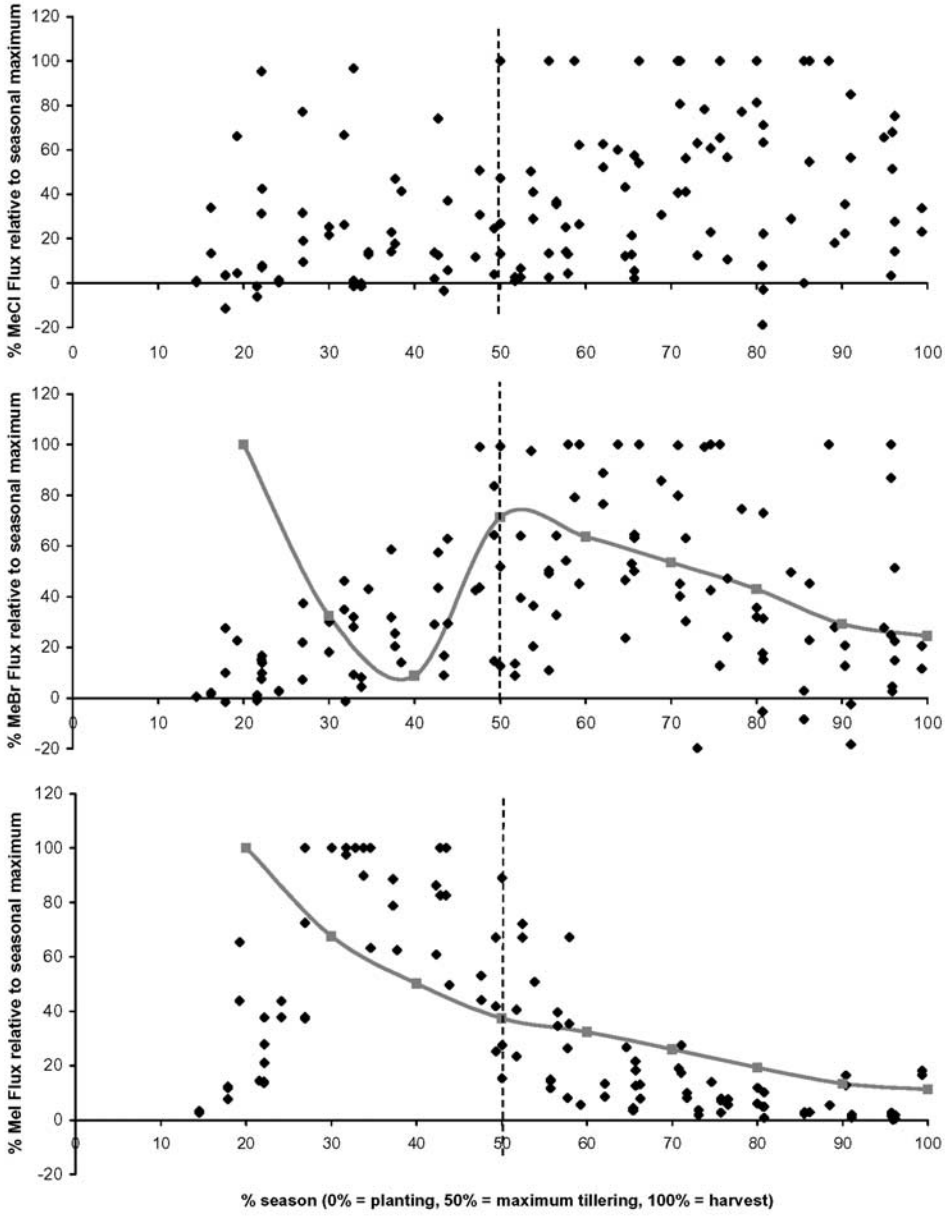

Figure 1. Seasonal methyl chloride, methyl bromide, and methyl iodide emission patterns from rice normalized to the maximum emission of each respective methyl halide per season. Shaded line indicates the calculated emission of methyl bromide and methyl iodide based on the product of plant tissue emission potentials and fresh weight plant tissue biomass.

synthesis [*Harper*, 2000]. Other suggested roles for methyl halide biosynthesis include removal mechanisms for either methyl compounds or halogens from leaf tissue [*Attieh et al.*, 1995] and production of protective pesticides against herbivory by microbes, insects, and nematodes. These possible roles have yet to be confirmed through experimentation.

[4] We suggest that methyl halide production may be a by-product of the many *O*-methyl transferase reactions that occur in plants. Methyl transferases are ubiquitous, serving to create many compounds, from lignins to sterols, flavinoids to floral scents. A few methyl transferase enzymes have been purified, including several purported methyl halide transferases, and their general sequences determined [*Attieh et al.*, 1995; *Joshi and Chiang*, 1998; *Ni and Hager*, 1998; *Ross et al.*, 1999; *Schroder et al.*, 2002]. Methyl transferases may be membrane bound and range in size between 22,500 and 40,000 Daltons [*Attieh et al.*, 1995; *Joshi and Chiang*, 1998; *Ni and Hager*, 1998; *Ross et al.*, 1999].

[5] Our previous research has shown that rice and other members of the Poaceae family (wheat and barley) show distinct seasonal emission profiles for methyl bromide and methyl iodide (Figure 1, *Redeker and Cicerone* [2003],

Redeker et al. [2000], and unpublished data). Methyl iodide emissions maximize during the early season, while the plant is rapidly increasing in biomass and height. Maximum methyl bromide emissions occur during the flowering period in the reproductive phase. Methyl chloride emissions from the rice plant are usually masked by emissions from paddy water; however, when soil halide concentrations are high, maximum emissions of methyl chloride occur near the same time as maximum emissions of methyl bromide [*Redeker and Cicerone*, 2003]. This may indicate that emissions of methyl halides are connected with processes involved in plant growth and reproduction.

[6] Methyl halide emissions should be dependent on factors that influence the activity of the halide methylating enzyme(s). The level of methyl transferases present in the plant tissue and therefore the methyl halide production probably changes with growth stage due in part to induction and repression of synthesis of methyl transferases. Activity is also likely to be affected by the amount of halide ion and methyl groups available to the enzyme, as well as the presence of any competitive substrates.

2. Methods

[7] The methods describing cultivar selection, soil preparation, and greenhouse conditions are described by *Redeker and Cicerone* [2003]. Soil halide concentrations were obtained as described by *Redeker et al.* [2002].

[8] Tissue halide concentrations were determined by drying components of harvested plants to constant weight, then milling the biomass to <0.1 mm. Plant tissue (0.5 g ground dust) was boiled in 50-mL DI H_2O for 10 min then filtered through a #1 Whatman filter. A second extraction was performed for each tissue sample; the combination of extractions was sufficient to capture 96 ± 2, 94 ± 5, and 90 ± 10% of the available chloride, bromide, and iodide, respectively, when compared to a subset of samples that were extracted three times. Halide concentrations of the extracted solutions were measured using the same method as that described for soil extracts by *Redeker et al.* [2002].

[9] Emission potentials (defined as the rate of production of methyl halide per gram fresh weight (FW) of whole plant tissue per day) were obtained through a method modified from *Saini et al.* [1995]. Rice portions (leaf, panicle, root, or stem), 16 mm in length, and between 0.0025 and 0.0422 g FW, were placed in 10-mL serum vials with 1 mL 0.1M sodium bromide or iodide in 0.1M HEPES buffer solution (pH = 7.00 ± 0.05). The sealed vials were incubated for 1 hour at 20°C, with shaking and constant irradiance (650 μE/m^2/s). Headspace samples (2 mL) were injected onto a Shimadzu 17A GC/ECD running isothermally with a PoraPlot Q column. Leaf emissions did not correlate well with surface area (data not shown). The possibility of stem tissue emitting as much methyl halide as leaf tissue based on surface area is unlikely as stem tissue areas were consistently larger than leaf disk areas. Root areas were not calculated, but are likely to have been much larger than leaf or stem surface areas as the sum of the root diameters per vial was approximately equivalent to leaf disk widths. Owing to chromatographic interference, methyl chloride emissions were not detectable for any plant tissue type using this method.

[10] To ensure that we did not preferentially sample more active/immature leaves during the early season we derived emission potentials for the first four sampling dates based on effective leaf thickness (leaf weight divided by leaf surface area, g/mm^2). Immature leaves would be expected to be somewhat less thick than older leaves as the vascular tissue is less developed. We did not observe a distinct trend toward lower emission potentials with increased thickness for either methyl bromide or methyl iodide (Figure 2). As a further precaution, leaf disks were not cut from tissue within 2 cm of the tip or the base of the rice leaf. During inhibition tests, leaf tissues portions were randomly selected from the available mid-section of the leaf.

[11] Inhibition studies included a preliminary step in which leaf disks were soaked (at 20°C with constant shaking and irradiance) for an hour in solution containing 5 mM of an alleged competitive inhibitor. They were then placed in a 1-mL solution containing both inhibitor and 0.1 M bromide or iodide for an hour, then sampled as described above. Plant tissue for both emission potential and inhibition studies was cut from whole plants within 12 hours of collection from the greenhouse. Collected plants were kept with their roots placed in an ice water bath to maintain plant tissue turgidity prior to use.

[12] Cell-free extracts were acquired by crushing 2 g frozen (liquid N_2) leaf tissue with 2 g sand (grinding agent), 1 g polyvinylpolypyrrolidine (phenol compound binder), and buffer (0.1 M HEPES pH = 7, 10% glycerol, and 4% dithiothreitol). The slurry was passed through four layers of cheesecloth and centrifuged at 2200 rpm for 10 min. The supernatant was further homogenized and subjected to ultrafiltration (>10,000 NWML). The concentrated extract was diluted to 20 mL with buffer and frozen at −25°C until analysis. All extraction steps were performed at or near 4°C and all analyses occurred within 48 hours of extraction.

[13] Cell-free extract emissions were analyzed by placing 500 μL extract along with 50 μL of both 0.11M NaX (X = Br or I) and 55 mM SAM solutions into a 10-mL serum vial (final solution concentrations 0.1M halide and 5 mM SAM). The vial was shaken for 1 hour and headspace air (∼2 mL) was taken and injected onto a Shimadzu 17A GC/ECD (fixed volume sample loop = 0.5 mL). Cell-free inhibition experiments were performed at the same inhibitor concentration as leaf disk inhibition analyses (5 mM). Protein analyses were performed according to methods of *Smith et al.* [1985].

3. Results and Discussion

3.1. Biochemical Parameters

3.1.1. Cell-Free Extracts

[14] The effect of halide concentration on emissions of leaf tissue cell-free extracts is shown in Figure 3. Iodide concentrations were saturated at 0.1 M while bromide emissions still showed linear response. K_m and V_{max} values for methyl iodide production based on this concentration

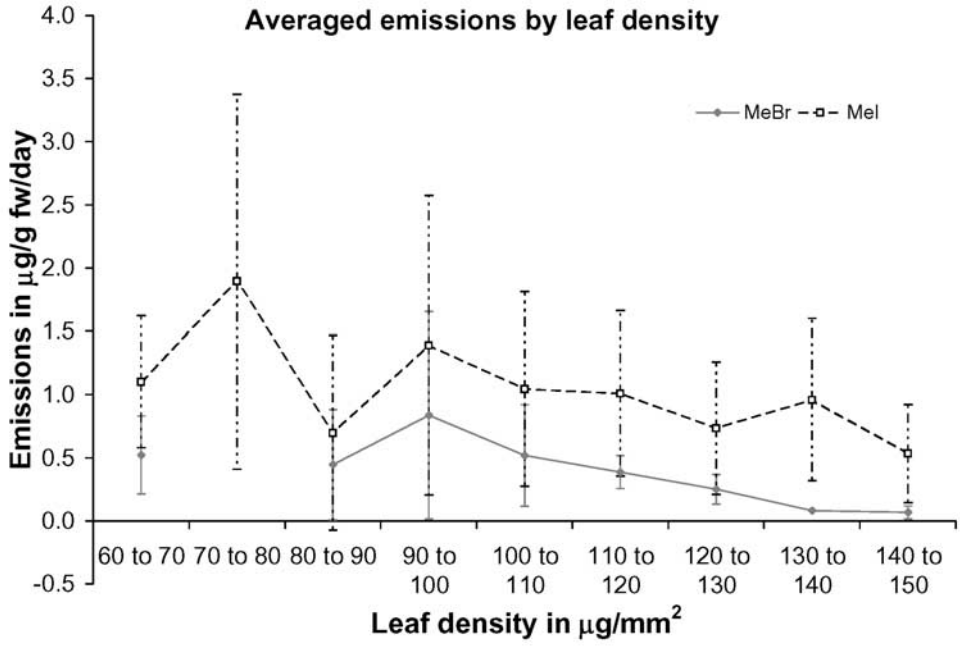

Figure 2. Methyl bromide and methyl iodide emissions versus effective leaf thickness (g FW/surface area leaf tissue).

gradient and processed using an Eadie-Scatchard plot (r^2 = 0.88) were 0.018 mM and 4.94×10^{-10} g MeI/mg protein/day, respectively. These results can not be compared to previously published V_{max} values as our cell-free extract was not fully purified (Table 1). The unpurified, cell-free extract K_m value was at least an order of magnitude smaller than any other reported (purified) iodide value (0.018 mM for *O. sativa* versus 0.25 mM and 1.3 mM for *P. pomaceus* and *B. Oleracea*). A lower K_m implies a higher methyl transferase affinity for iodide. High emissions of methyl iodide by rice, especially relative to iodide taken up, may be a result of a methyl transferase with high affinity for iodide and high catalytic efficiency. These would be properties of a methyl iodide specific methyl transferase that may remove iodide from the soil as a detoxification mechanism.

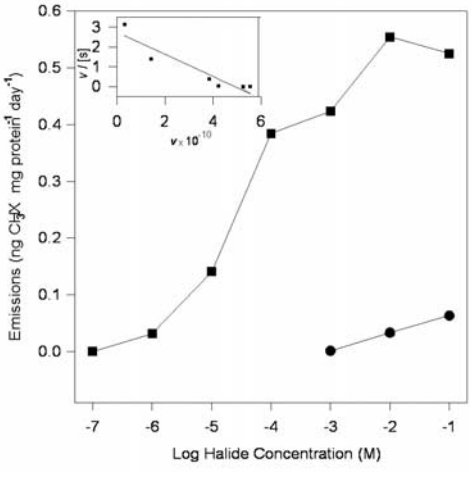

Figure 3. Cell-free methyl bromide and methyl iodide emissions versus solution halide concentration. MeI emissions are denoted by squares, and MeBr emissions are denoted by solid circles. Error bars show 1 standard error. R^2 of Eadie-Scatchard diagram is equal to 0.88.

[15] The results from the cell-free experiment that examined halide concentrations may explain why methyl iodide emissions are consistently observed from leaf disks in HEPES buffer (Figure 4). Assuming that all leaf tissue halides (Table 2) were in solution, and using the observed percent dry weights (Figure 5), leaf tissue halide solution concentrations can be calculated. During the 2001 greenhouse tillering phase (60 days after seeding) leaf tissue solutions contained approximately 0.15 mM, 2.0 μM, and 0.02 μM Cl^-, Br^-, and I^-. Leaf tissue iodide concentrations were quite likely to be near the 0.1-μM threshold of detection of our experiment. In contrast, bromide leaf tissue concentrations were much lower than 1 mM, which was the threshold for the limit of methyl bromide detection.

[16] These cell-free extractions may not fully represent rice plant cellular methyl transferase activity since previous research has identified membrane-bound methyl transferases that denature quickly when separated from the membrane during extraction [*Attieh et al.*, 1995]. Our methods cannot guarantee that these types of methyl transferases were included.

3.1.2. Methyl Transferases and Inhibition Studies

[17] Inhibition experiments utilized eight putative competitive inhibitors. Of the inhibitors, thiol (HS) and thiocyanate (SCN) have been identified as competitive substrates in a possible halide/bisulfide methyl transferase reaction [*Attieh et al.*, 1995; *Saini et al.*, 1995]. Caffeic acid (CA) is a potential lignin precursor that appears to be a substrate for a group of methyl transferases which are

Table 1. Reported K_m and V_{max} Values

Author	Species	V_{max}, g-MeI day^{-1} mg protein^{-1}	K_m, mM	Notes
Saini et al. [1995]	*B. Oleracea* (broccoli)	13.99	1.3	Lineweaver-Burk method, also has SH-data purified extract
Saxena et al. [1998]	*P. Pomaceus* (white-rot fungus)	1.86 E-5	0.25	Lineweaver-Burk method, also has Cl, Br, and SAM data, purified extract
Harper [2000]	*E. Muricata* (microalgea)	0.0154	n.d.	also has Cl, Br, HS, and SAM data, purified extract
	B. Martima (saltwort)	0.0613	8.55	
Redeker et al. [2002]	*O. Sativa* (rice)	4.94 E-10	0.018	Eadie-Scatchard unpurified enzyme extract

also able to react rapidly with other compounds including quercetin and catechol (CAT) [*Joshi and Chiang*, 1998; *Schroder et al.*, 2002]. Benzoic acid (BA) and salicylic acid (SA) have previously been identified as flavinoid and floral scent precursors [*Ross et al.*, 1999], and inositol (Ino) is implicated in plant tissue osmotic stress tolerance reactions [*Joshi and Chiang*, 1998]. Because methyl iodide emissions are at a maximum during the early season,

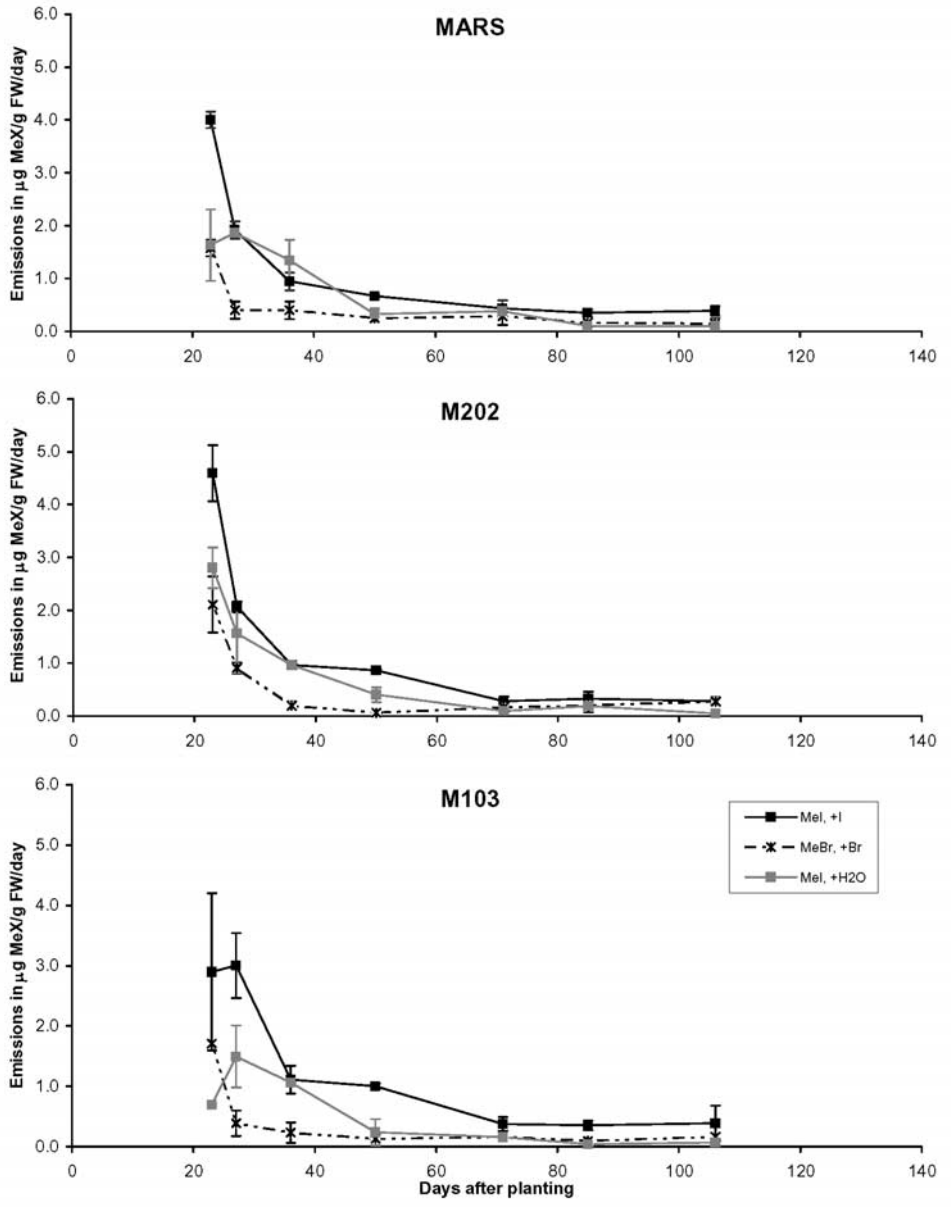

Figure 4. Leaf disk emission potential for M103, M202, and Mars during the 2001 greenhouse experiment.

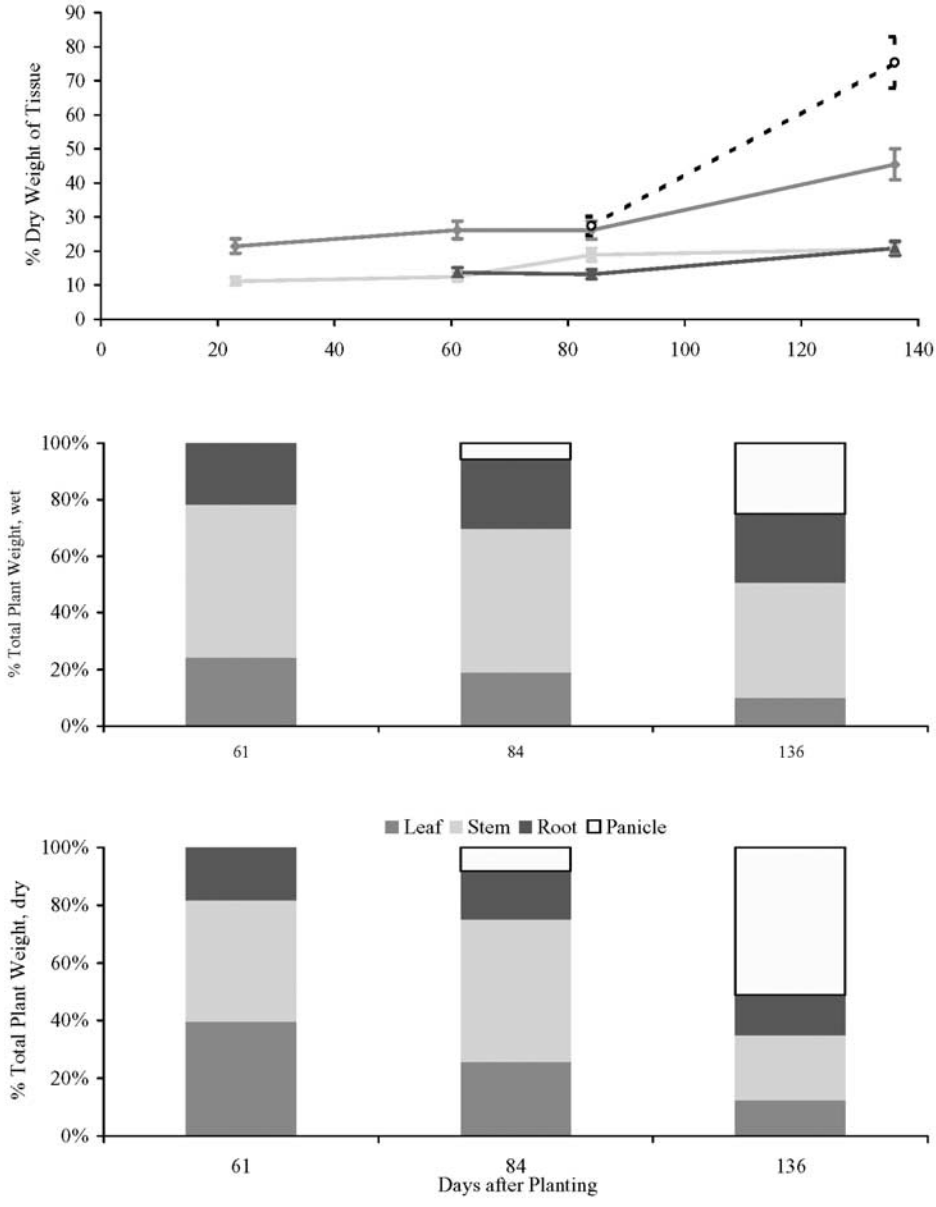

Figure 5. Percent dry weight of rice plant components throughout the season and percent plant biomass for each component when either wet or dry. Green bars indicate leaf tissue (diamonds), while root (triangles), stem (squares), and panicle (circles) tissues are marked by brown, gold, and pale yellow bars, respectively. See color version of this figure at back of this issue.

lignin and other structurally related methyl transferases would be expected to produce methyl iodide. Methyl bromide, maximally emitted during the reproductive phase, might be expected to be derived from floral scent and flavinoid specific methyl transferases. Given these seasonal emission patterns, caffeic acid and catechol would be expected to be potential methyl iodide inhibitors while methyl bromide would be expected to be most affected by benzoic and salicylic acids.

[18] Leaf disk inhibition studies were performed at three separate growth stages: tillering, reproductive, and ripening (Figure 6). Cell-free assays were performed along with the first set of leaf disk assays and gave similar results (data not shown), with somewhat higher inhibition efficiencies for catechol and caffeic acid in bromide solution.

[19] Methyl halide emission potentials demonstrated several different responses to the inhibitors tested (Figure 6). Changes in methyl bromide response over the course of the season were in several cases opposite methyl iodide reactions to the same inhibitor (inositol and caffeic acid). In other cases, compounds that efficiently inhibited methyl bromide were not successful inhibitors of methyl iodide (benzoic acid, thiocyanate, and salicylic acid). Some inhibitors became more efficient during the season (benzoic

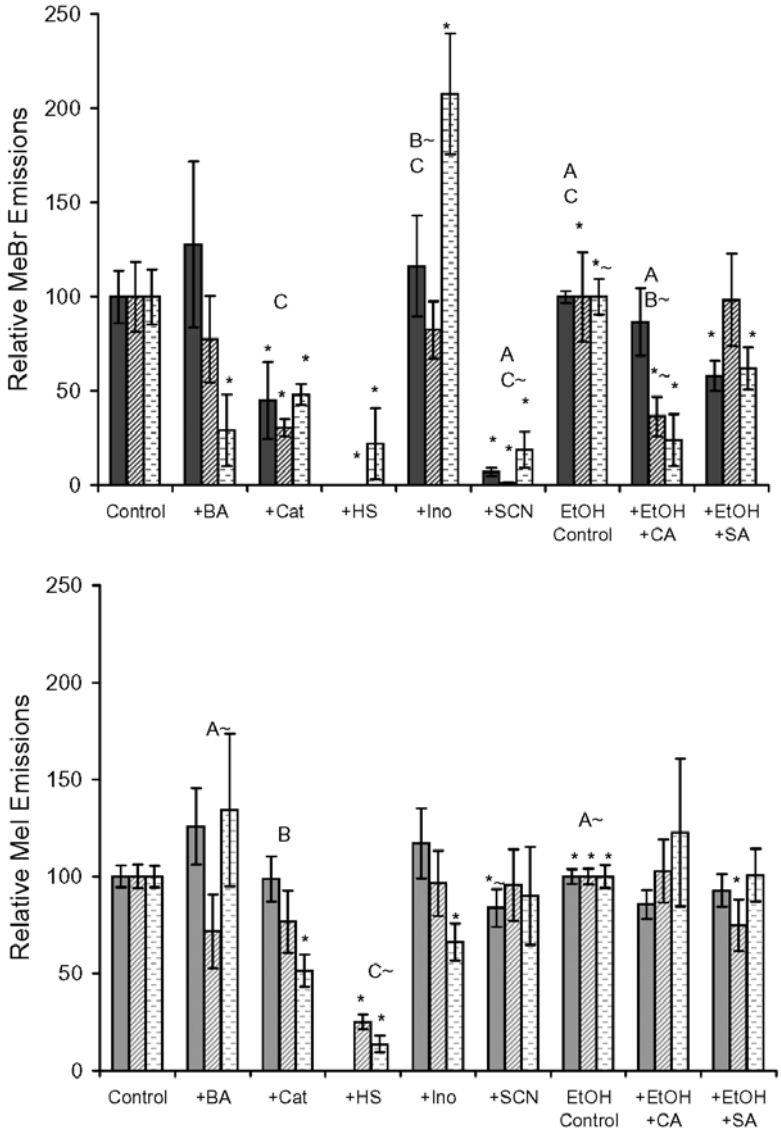

Figure 6. Relative efficiencies of methyl halide inhibitors. Solid bars show data from the tillering phase, bars with diagonal stripes show reproductive phase data, and dashed line bars indicate ripening phase data. The HEPES buffer control and the ethanol/HEPES buffer control data are set to 100% emissions for comparison with inhibitors. Asterisks indicate that emissions are significantly different from the respective control (95% confidence), while the letters A, B, and C indicate that there exists a significant difference between the tillering and reproductive, tillering and ripening, and reproductive and ripening phase data. Subscript tildes indicate that the differences noted are only at the 90% confidence level.

acid, catechol, inositol, caffeic acid), some became less efficient (inositol, thiol), and some remained constant in their efficacy (catechol, thiocyanate, caffeic acid and salicylic acid). Surprisingly, inositol appears to act as an accelerant for methyl bromide production during the late season. The strong inhibitory effect of thiol on methyl bromide and methyl iodide emission potentials may be due to a general enzyme inhibition from non-specific protein binding. These results suggest that there are several (at least two separate) processes that produce methyl halides in rice plant tissue. These enzymatic reactions are likely turned off and on as the season progresses and the plant shifts its metabolic requirements.

3.2. Physiological Parameters

3.2.1. Rice Plant Tissue Halide Concentrations and Percent Dry Weight Throughout the Season

[20] Rice is comprised of four main components: root, stem, panicle, and leaf tissue. Panicle tissue includes the stalk on which flowers bloom and are germinated, and where grain develops. All rice components followed well-defined seasonal successions where water-rich tissues

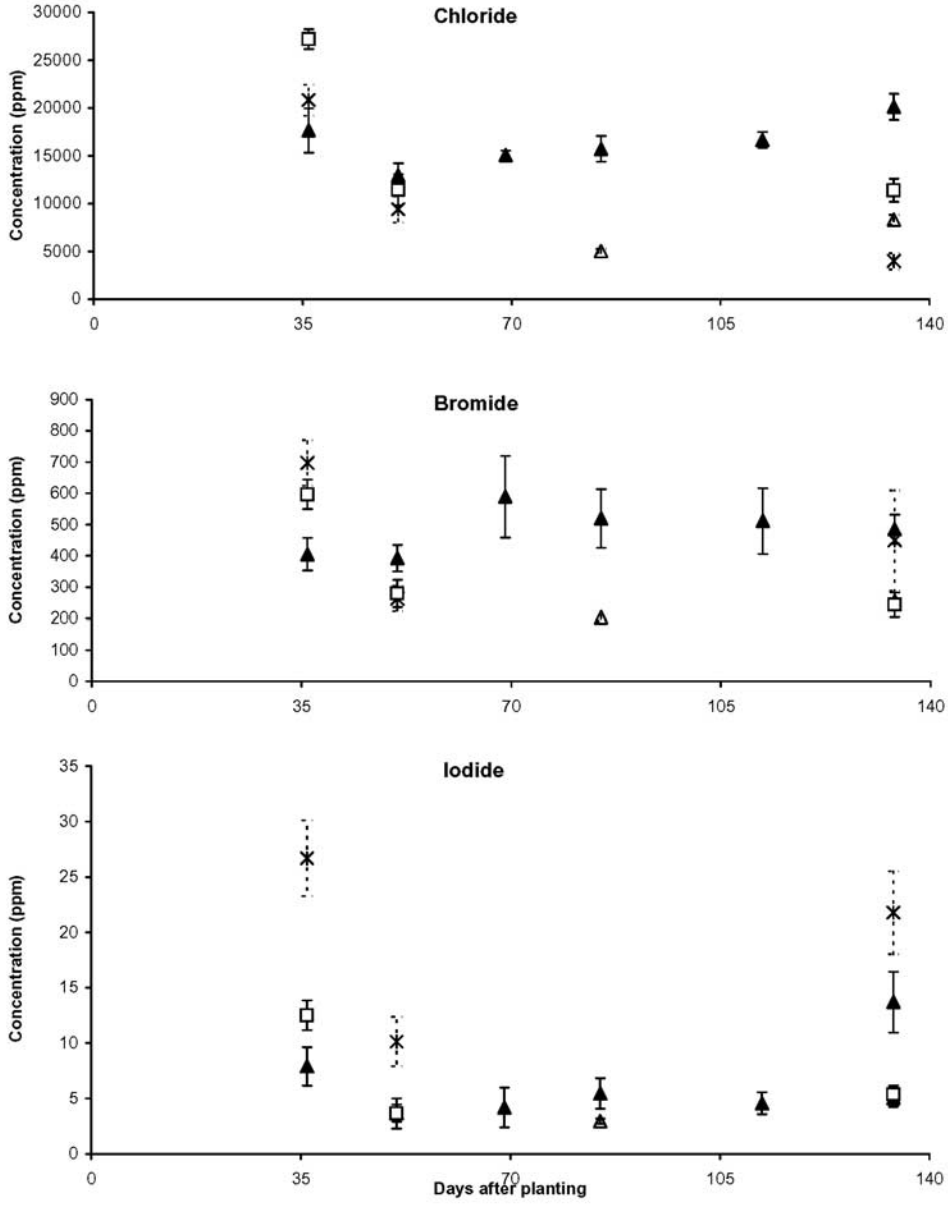

Figure 7. Rice plant tissue halide concentrations. Values shown are mg halide per kg tissue dry weight. Leaf tissue is shown by the solid triangles, panicle tissue by open triangles, stem tissue by open squares, and root tissue by asterisks. Note that the scale for each halide is different while the *x* axis scale remains the same. Error bars show 1 sample variance.

became progressively drier toward harvest. Dry weight of leaf and panicle tissue increased dramatically (20 and 30% to 45 and 75% dry weight, respectively) after the reproductive stage, while root and stem tissue remained relatively water saturated (from 10 to 20% dry weight, Figure 5). Stem tissue dominated wet biomass throughout the season, maintaining 40–50% of the plant biomass while leaf tissue biomass decreased from 25% to less than 10%. Dry plant tissue biomass was more evenly distributed at the beginning of the season (40% for both leaf and stem tissue types) due to differences in leaf and stem water percentage during the season. Later in the season, panicle tissue (including grain) dominated plant dry weight biomass (>50%), while the percentage of dry leaf tissue biomass diminished to less than 10% (Figure 5).

[21] Rice plant tissue halide concentrations varied over the course of the season (Figure 7; Tables 2a–2c). Near the beginning of the season (36 days after planting (DAP)) tissue chloride and iodide concentrations were at their highest, with leaf tissue concentrations up to 34,000 (3.4%) and 51 mg/kg dry weight, respectively. Leaf tissue concentrations dropped (despite differences in soil halide concentration) to stable concentrations of 16,000 ± 500 and 4.7 ± 0.7 mg/kg chloride and iodide during the

Table 2a. M202 Rice Plant Tissue Chloride Concentrations in mg/kg Dry Weight (ppm) for Various Soil Halide Concentrations[a]

DAP	Leaf	Panicle	Root	Stem
	2001 UCI Greenhouse Study (0–10 cm Concentrations of Cl = 40 mg/kg Soil)			
36	17600 (2300)	— (—)	20800 (1600)	27200 (1000)
85	15700 (1300)	5000 (200)	n.d.	n.d.
134	20100 (1400)	8300 (500)	4000 (800)	11400 (1200)
	2002 UCI Greenhouse Study (0–10 cm Concentrations of Cl = 65 mg/kg Soil)			
36	22600 (6800)	— (—)	43100 (11500)	47600 (5200)
85	16100 (2100)	5200 (300)	10900 (800)	12500 (2000)
134	14400 (3400)	6800 (300)	7400 (900)	13400 (2800)
	2002 Salt Amended UCI Greenhouse Study (0–10 cm Concentrations of Cl = 165 mg/kg Soil)			
36	33600 (4000)	— (—)	44700 (11400)	79600 (17900)
85	15500 (600)	4100 (300)	14500 (1600)	12800 (1000)
134	30900 (1000)	9100 (200)	10400 (2000)	18400 (3300)

[a]Sample variance is shown in parentheses.

reproductive stage. Post-reproductive phase leaf tissue concentrations generally rose for chloride and iodide, with the exception of the 2002 non-amended study plants (Figure 7; Table 2a). Leaf tissue bromide concentrations remained nearly constant at 480 ± 90 mg/kg throughout the season, although large concentrations (1000 mg/kg) were observed in late season rice grown in amended soil during the 2002 greenhouse study (Figure 7; Table 2b). Stem tissue concentrations began the growing season higher than leaf tissue concentrations for all halides. Chloride and bromide stem tissue concentrations dropped throughout the season until at harvest they were nearly a factor of 2 less concentrated than leaf tissue concentrations. Iodide stem tissue concentrations dropped during the reproductive period, but at harvest, relative concentrations of stem to leaf tissue did not follow a common pattern. Initial root tissue halide concentrations were higher than leaf tissue concentrations, but at harvest were equivalent to or less concentrated than stem and leaf tissue halide concentrations depending on the halide (Tables 2a–2c). Panicle halide concentrations were consistently lower than leaf tissue halide concentrations by approximately a factor of 2.

[22] Our reported plant tissue halide concentrations are within the same order of magnitude as those reported for rice grown in Japan [*Yuita*, 1994a], where chloride and iodide content of average rice plant leaf tissue (growth stage unknown) was measured at 4000 and 0.8 mg/kg (factors of 4 and 6 times less concentrated than our measured values during the reproductive stage). Bromide concentrations were measured to be 20 mg/kg, 25 times less than the values observed in these experiments. The differences between these data sets could be due to many variables, including soil halide concentrations, cultivar, growth stage at tissue collection, and analytical technique.

[23] The consistency of plant tissue chloride, bromide, and iodide concentrations during the reproductive period of the growing season for a broad range of soil halide concentrations suggests that rice plants monitor and conserve internal halide ion concentrations. This hypothesis is supported by relative rates of emissions for each methyl halide. Less than 1% of leaf tissue chloride or bromide

Table 2b. M202 Rice Plant Tissue Bromide Concentrations in mg/kg Dry Weight (ppm) for Various Soil Halide Concentrations[a]

DAP	Leaf	Panicle	Root	Stem
	2001 UCI Greenhouse Study (0–10 cm Concentrations of Br = 2.5 mg/kg Soil)			
36	410 (50)	— (—)	700 (70)	600 (50)
85	520 (90)	200 (10)	n.d.	n.d.
134	490 (50)	260 (20)	450 (160)	240 (40)
	2002 UCI Greenhouse Study (0–10 cm Concentrations of Br = 17.1 mg/kg Soil)			
36	610 (170)	— (—)	1320 (270)	730 (80)
85	400 (20)	190 (50)	290 (40)	220 (20)
134	360 (50)	200 (20)	460 (90)	270 (50)
	2002 Salt Amended UCI Greenhouse Study (0–10 cm Concentrations of Br = 14.0 mg/kg Soil)			
36	560 (160)	— (—)	1730 (820)	1520 (590)
85	540 (70)	430 (100)	1110 (140)	550 (110)
134	1000 (90)	350 (50)	620 (100)	600 (70)

[a]Sample variance is shown in parentheses.

Table 2c. M202 Rice Plant Tissue Iodide Concentrations in mg/kg Dry Weight (ppm) for Various Soil Halide Concentrations[a]

DAP	Leaf	Panicle	Root	Stem
2001 UCI Greenhouse Study (0–10 cm Concentrations of I = 0.3 mg/kg Soil)				
36	7.9 (1.7)	— (—)	26.7 (3.4)	12.5 (1.4)
85	5.5 (1.4)	3.0 (0.2)	n.d.	n.d.
134	13.7 (2.7)	5.0 (0.8)	21.8 (3.7)	5.4 (0.8)
2002 UCI Greenhouse Study (0–10 cm Concentrations of I = 1.9 mg/kg Soil)				
36	43.0 (8.9)	— (—)	167.0 (12.0)	105.1 (6.3)
85	4.2 (1.0)	1.6 (0.0)	458.2 (217.4)	1.8 (0.7)
134	3.8 (0.9)	2.9 (0.1)	126.2 (92.0)	7.1 (0.9)
2002 Salt Amended UCI Greenhouse Study (0–10 cm Concentrations of I = 1.3 mg/kg Soil)				
36	51.1 (23.0)	— (—)	451.6 (263.6)	81.3 (21.6)
85	4.4 (1.4)	6.6 (1.9)	388.0 (135.5)	7.1 (3.3)
134	12.0 (3.6)	3.2 (0.7)	31.9 (6.1)	24.4 (11.9)

[a]Sample variance is shown in parentheses.

was emitted as methyl halide, but over 90% of the iodide removed from the soil and processed by the rice plant was emitted as methyl iodide [*Redeker and Cicerone*, 2003; K. R. Redeker et al., unpublished data]. This may indicate that methyl iodide production in rice is a targeted iodide detoxification mechanism whereas methyl bromide and methyl chloride production is a ''metabolic accident'' [*Manley*, 2002]. Elevated iodide concentrations are known to adversely affect rice growth (Reclamation-Akagare disease [*Yuita*, 1994b]), while chlorine is an identified micronutrient for other Poaceae crops [*Christensen et al.*, 1981].

[24] If plant tissue halide concentrations are a result of their steady uptake from soil along with soil water, an increase (>50%) in plant tissue chloride and bromide concentrations would be expected over the course of the season, as these halides are not volatilized at the same relative rate as iodide. However, significant increases over initial concentrations for rice tissue chloride and bromide concentrations were not observed during either greenhouse study, suggesting that rice plants actively process soil halides through either a primary or secondary metabolic mechanism.

[25] Equally, if plant uptake of halides were passive, leaf tissue concentrations of halides might be expected to mimic soil halide concentration ratios. For the 2001 greenhouse experiment, soil Cl^-, Br^-, and I^- concentrations were in the ratio of 133:8:1, while leaf tissue concentrations at day 85 were in the ratio of 2850:95:1. It appears that rice plants preferentially remove chloride from the soil, most probably to maintain a positive osmotic balance between the rice plant and the flooded paddy soil as well as to maintain internal cellular electrical balance. Bromide and iodide are preferentially left in the soil, relative to chloride, and iodide that makes its way into the plant tissue is removed in the form of methyl iodide. The bromide that is taken into plant tissue is stored there throughout the season.

[26] Tissue halide concentrations alone do not explain the seasonal pattern of emissions shown in Figure 1. Rice leaf tissue bromide concentrations did not change significantly and stem bromide concentrations dropped over the course of the season. Neither of these patterns fits the peak in methyl bromide emissions that occurs during the reproductive phase, which has been demonstrated over several growing seasons [*Redeker and Cicerone*, 2003]. Iodide concentrations in leaf and stem tissue began the season highly concentrated, dipped to low values during the reproductive phase, then increased until harvest. A U-shaped seasonal emission pattern was not observed. Also, rice tissue iodide concentrations do not correspond with the slight increase in emissions during the flowering period, while leaf and stem iodide concentrations were at a minimum.

3.2.2. Rice Tissue Emission Potentials

[27] Leaf tissue showed a bi-modal distribution of emission potentials (g MeX/g FW/day) with the majority of leaf tissue emitting methyl halides at the more rapid rate (Table 3, Figure 8). Active leaves were more prevalent than less active leaves (67 ± 17% and 80 ± 20% for MeBr and MeI, respectively, with a possible trend toward more completely active leaf tissue during the later season). Large variability in leaf-based methyl halide emissions has been observed previously in bean plants [*Amiro and Johnston*, 1989].

Table 3. Number of Active Versus Inactive Leaf Tissue[a]

	MeBr		MeI	
DAP	Active	Inactive	Active	Inactive
23	7	4	20	15
27	12	11	41	19
36	18	5	36	24
50	9	7	18	6
71	14	9	24	0
85	14	10	24	0
106	5	0	6	0
	MeBr		MeI	
Percent active	67.1%		80.1%	
Standard deviation	16.7%		19.5%	

[a]Leaves were assigned as less active if they emitted more than 2 standard deviations less methyl halide than the average of the more active group (Figure 3). All three cultivars are represented in the data shown below, with no statistical difference in relative activity between cultivars. DAP is days after planting.

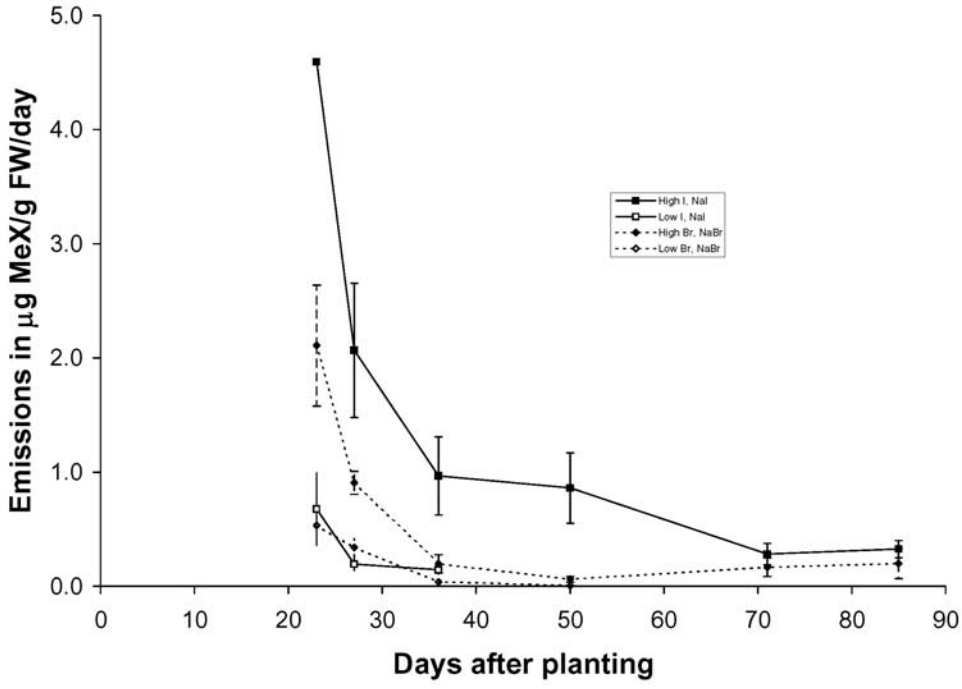

Figure 8. Variability in M202 leaf disk emission potentials over the course of one season. Data were sorted into high and low categories if two sigma separations existed between emission rates.

[28] Only data from the more active tissues are compared in Figures 4 and 9. Rice leaf tissue is most efficient at producing methyl iodide on a per-weight basis (Figure 9). The emission potential of methyl iodide from panicle tissue was highly variable, but with an average value of approximately 60% the emission potential of leaf tissue, while root and stem tissue potentials were only 20% that of leaves. Methyl bromide emission potentials were nearly equivalent between leaf and panicle tissue, while root and stem potentials again were 20% compared to leaves (Figure 9). All further studies were performed on leaf tissue only.

[29] A seasonal comparison of emission potential for three different cultivars is shown in Figure 4. Leaf emission potentials were at their highest, between 2.0 and 5.0 μg MeI and 1.5 and 2.5 μg MeBr/g FW/day, near the beginning of the season (on day 25, approximately 16% of the season's duration, the plants were ~40 cm tall with leaves ~5 mm wide). Leaf tissue emission potentials dropped rapidly during the season until they reached 0.5 and 0.1 μg/g FW/day MeI and MeBr, respectively. Once emission potentials reached this plateau they remained constant, from 70 DAP (~50% season duration) until mid-ripening for methyl iodide and from 30 DAP until mid-ripening for methyl bromide. All cultivars showed similar seasonal emission potentials, both in scale and pattern, for methyl bromide and methyl iodide (Figure 4). This is consistent with the observed daily fluxes of methyl halides from these cultivars during the 2001 greenhouse experiment [*Redeker and Cicerone*, 2003].

[30] Cell-free emissions were dependent on the halide concentration of the bath solution in which they were studied (Figure 3). If leaf emission potentials are equally halide sensitive, then changes in the relative water weight of rice tissues with constant halide content may measurably affect the observed potential. However, changes in emission potential of rice leaf tissue cannot be explained by changes in leaf tissue water content. The changes in percent dry weight between day 20 and day 60, during the rapid decline in methyl bromide and methyl iodide emission potentials, were too gradual to explain the observed shifts in methyl halide emission potentials. It is also difficult to imagine a scenario where a dilute leaf tissue solution would be more productive than a more concentrated leaf tissue solution.

[31] It seems more reasonable to assume that plant tissue halide concentrations would control methyl halide emission potentials, especially if the plant was actively processing halogens through a methyl transferase mechanism. Tissue halide concentrations for M202 cultivar grown in three separate soil halide concentrations at tillering, reproductive, and ripening growth stages are shown in Tables 2a–2c, while average tissue halide concentrations for three cultivars (MARS, M103, and M202) grown in identical soil are shown in Figure 7. Rice tissue halide concentrations for the three cultivars were statistically identical during the reproductive period (~85 DAP) although instantaneous methyl halide emissions were not [*Redeker and Cicerone*, 2003]. Methyl iodide leaf emission potentials declined between days 35 and 60. This was mirrored by a drop in leaf tissue iodide concentration during the same period, although bromide concentrations remained constant throughout the early stages of growth when methyl bromide emission potentials were dropping (Tables 2 and Figure 7).

[32] Throughout the season, plant tissues bathed in HEPES (a halogen free organic buffer) solution showed

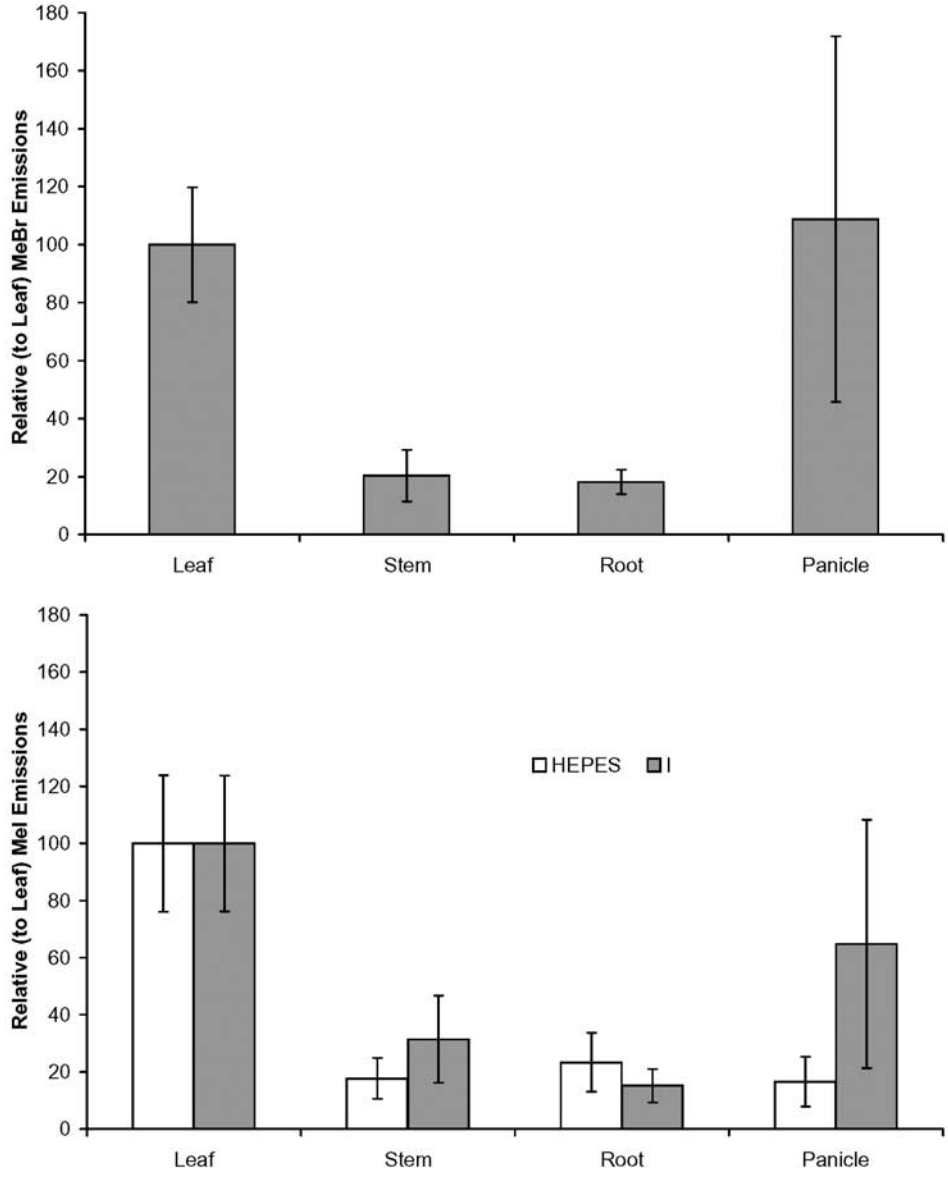

Figure 9. Leaf versus root, stem, and panicle emission potential for methyl bromide and methyl iodide. Rates are in g MeX/g FW/day and are normalized to those of leaf tissue. No methyl bromide was produced with buffer only.

the potential to emit methyl iodide, but not methyl bromide (Figures 4 and 9). Interestingly, leaf disks soaked in HEPES showed the same MeI emission potential as leaves soaked in 0.1 M iodide from 25 to 35 DAP. After day 35, the emission potential of leaves soaked in buffer grew progressively smaller relative to the leaves soaked in 0.1 M iodide (Figure 4).

3.2.3. Emission Potential and Observed Field Flux

[33] The observed pattern of MeI emission potential suggests that the seasonal pattern of commercial rice paddy emissions may be a function of rice leaf biomass and tissue emission potential. Calculated daily emissions of methyl bromide and methyl iodide, the sum of the products of tissue emission potentials (Figure 9) and fresh weight tissue biomass (Figure 5), are shown in Figure 1 (shaded line). Both methyl bromide and methyl iodide emissions maxima, driven by high early season emissions potentials, would be expected to occur before 20% of the season had passed. It is unlikely that preferential sampling of leaf tissue artificially created the high rates observed in the early season (Figure 2), which suggests that rice may actively process methyl halides generated within plant tissue during the early season, and that these consumption mechanisms may become less active/efficient over time.

[34] After the early season, however, the product of methyl halide emission potentials and plant tissue biomass acts as a reasonable predictor of the seasonal emission pattern of methyl iodide. Both methyl bromide and methyl

iodide emissions based on this method give daily emission rates equivalent to several mg/m^2/day. These fluxes are within a factor of 2 of the observed methyl iodide emissions however, they are two orders of magnitude larger than observed emissions of methyl bromide.

3.2.4. Effects of Temperature and Light

[35] The effects of temperature and light (diurnal response when combined) were studied and the results are presented in full by *Redeker and Cicerone* [2003]. As these are also significant physiological parameters, we present here a brief review of the observed responses of rice plants to changes in ambient temperature and light levels.

[36] Changes in light levels, either instantaneously or over the course of the day, appear to have a very limited effect (<10%). Emissions of methyl chloride from rice paddies did not appear to be significantly affected by changes in temperature. Emissions of methyl bromide and methyl iodide showed strong positive response to changes in temperature.

[37] While a strong positive response was observed in both methyl bromide and methyl iodide with increased temperature, these responses changed according to the growth stage of the rice plant. The increase in relative rate of methyl bromide generation from rice plants with elevated temperature decreased as the season progressed, until during the ripening phase, elevated temperature had no effect on methyl bromide emission rates. In contrast, methyl iodide emissions remained strongly affected by temperature throughout the season. This provides further evidence that methyl bromide and methyl iodide are produced through separate mechanisms.

4. Summary

[38] Several data sets strongly suggest that more than a single methyl transferase is involved in methyl bromide and methyl iodide production in rice: (1) the strikingly different seasonal patterns in production corresponding to growth stage (Figure 1), (2) the different effects that specific inhibitors have on the production of the two methyl halides (Figure 6), (3) the high production rate of methyl iodide relative to tissue iodide concentrations as compared to methyl bromide emissions relative to tissue bromide concentrations (Figures 3 and 4), and (4) the different seasonal responses of methyl bromide and methyl iodide to temperature.

[39] We show that plant tissue halide concentration and percent water weight in rice leaf, panicle, root, and stem tissues are highly variable throughout the season. Halide concentrations, percent dry weight, and methyl halide emission potential for these tissues changed significantly from planting to harvest. Cell-free extract analysis shows that the K_m value for unpurified cell-free extract is 0.018 mM I^-. This K_m value is an order of magnitude smaller than the smallest previously identified system.

[40] In terrestrial ecosystem research it is difficult to definitively assign emissions of a substance to one component, i.e., the soil, the water column, or the plant. This study, combined with the control chamber studies described by *Redeker and Cicerone* [2003], provides conclusive evidence that the rice plant itself produces methyl bromide and methyl iodide. The product of leaf disk emission potential and leaf tissue biomass provides fluxes that indicate that the rice plant alone is capable of generating the methyl bromide and methyl iodide fluxes observed in chamber experiments. While these results do not entirely rule out the possibility of methyl bromide and methyl iodide generation in the water or soil column, they do appear to limit these sources to minor roles.

[41] These results should caution against using leaf disk assays at single stages in plant growth to assess plant methyl halide production for three reasons: (1) Inhibition studies show that the metabolic processes that drive methyl halide generation within leaf tissue change throughout the season, (2) leaf tissue emission potentials for methyl bromide and methyl iodide change dramatically during the season, and (3) emission potentials do not accurately mimic in situ emissions profiles. Leaf disk assays should not be used as a proxy for in situ methyl halide emissions measurements.

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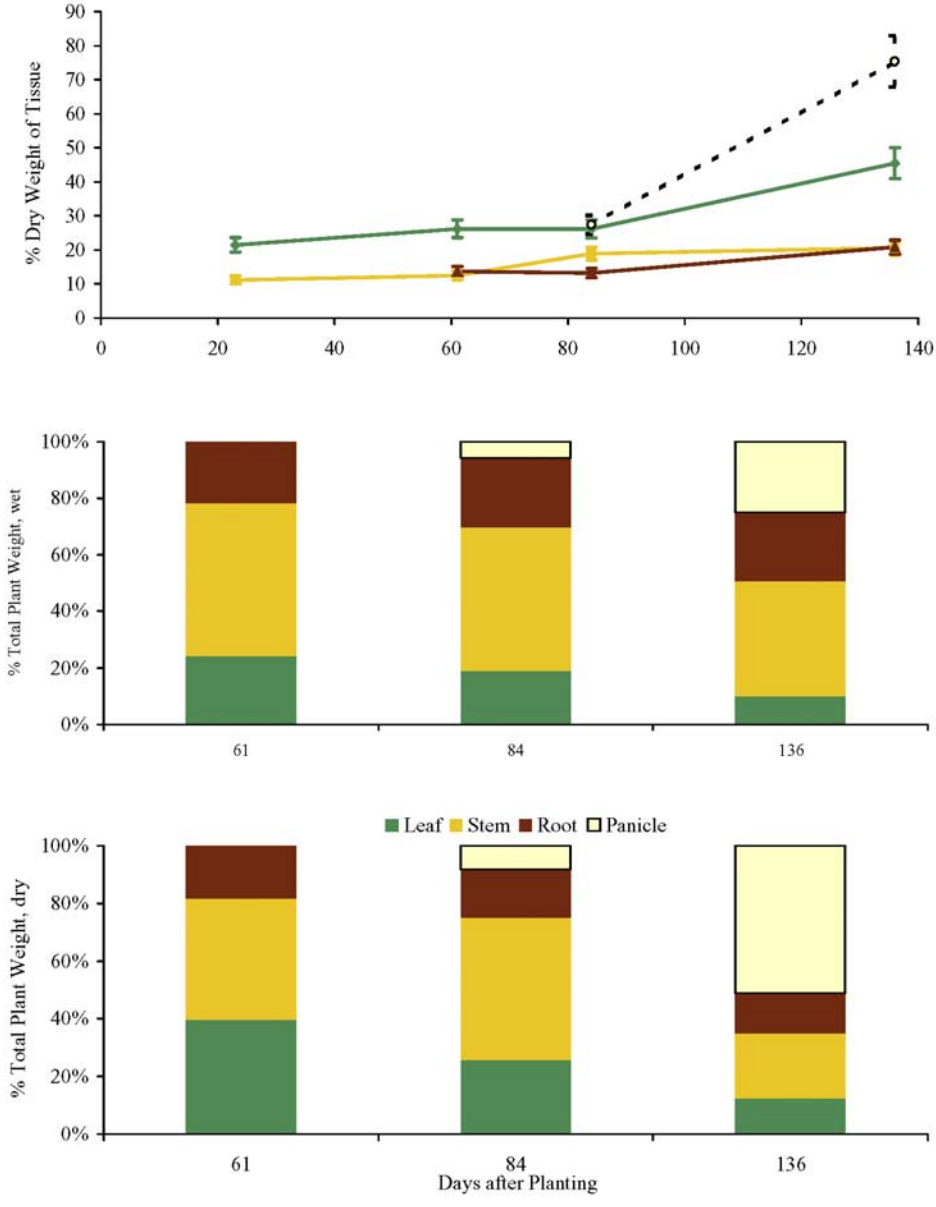

Figure 5. Percent dry weight of rice plant components throughout the season and percent plant biomass for each component when either wet or dry. Green bars indicate leaf tissue (diamonds), while root (triangles), stem (squares), and panicle (circles) tissues are marked by brown, gold, and pale yellow bars, respectively.