Author

Interest in environmental issues was the motivation behind Maggie Walser's research pursuits. Her interest in atmospheric chemistry led to her current study of agricultural emissions of trace gases, which allowed her to learn and apply aspects of earth science and biology and gain a variety of new skills. Maggie has taken on various roles during her undergraduate years, including guiding, advising and helping fellow students. She has been a University Studies discussion leader, a House Assistant in Arroyo Vista, and a Peer Academic Advisor for the School of Physical Sciences. Her advice to others is "Find something you care about and get involved!"

Key Terms

- Agricultural Emissions
- Global Budgets
- Inhibition
- Methyl Halides
- Methyl Transferase
- S-adenosyl-L-methionine

Metabolic Pathways of Methyl Halide Production in Terrestrial Plants

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Chemical Engineering and Chemistry

Abstract

Inorganic halogen radicals in the atmosphere play a role in stratospheric ozone destruction. Methyl halides act as transport vectors for halogen radicals from surface sources to the atmosphere, making knowledge of their budgets necessary. Atmospheric concentrations of methyl halides have been measured, but their fluxes are not yet fully quantified. Better estimation of methyl halide sources and atmospheric budgets can be gained from the measurement of emissions from agricultural and other terrestrial plants. This study sought an understanding of the metabolic mechanism(s) that produces methyl halides. The role of methyl transferases in methyl halide biosynthesis in rice was examined using leaf-disk enzyme inhibition assays with known methyl transferase substrates as possible competitive inhibitors. Only thiocyanate had a significant impact ($p < 0.05$) on methyl bromide generation, while methyl iodide synthesis was not significantly inhibited by any of the methyl transferase substrates surveyed. In all assays, methyl bromide production was inhibited more than that of methyl iodide, suggesting that either the enzyme(s) responsible for methyl halide synthesis binds iodide preferentially, or a suitable competitive substrate was not found. Methyl halide emissions from barley, corn, soybean, and wheat leaf disks were also investigated. The findings of this investigation increase knowledge of the biochemical production pathway(s) of methyl halides.

Faculty Mentor

Maggie Walser's research topic was unusually original so we could only guess what her experiments would show. Methyl bromide is a chemical that is produced industrially and used as an agricultural and structural fumigant. Its use is being banned worldwide due to its potential impact on the ozone layer. The ban was conceived before natural sources were investigated. We have found that methyl bromide and methyl iodide are emitted by rice plants, and our group set out to learn the metabolic mechanism of biochemical production of methyl halide gases by plants. Maggie used potential chemical inhibitors to see if they would impede the activity of methyl halide transferase enzymes in plant leaves. She made many difficult measurements that were true experiments, and she found that one of the chemicals inhibited methyl bromide production and that none of them significantly impeded formation of methyl iodide. Her research unveiled the complexity of this new topic.

Ralph J. Cicerone, Chancellor
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Introduction

The study of methyl halide (MeX) production processes in agricultural plants will aid the development of responsible environmental policy through the identification of potentially significant terrestrial plant sources of atmospheric MeX. Investigation of MeX will also lead to an increased understanding of their atmospheric budgets as well as the anthropogenic impacts of reducing natural ecosystem coverage while increasing cropland area. The focus of this project is the determination of which methyl transferases (MTs) are responsible for MeX synthesis in plants. This may aid in the identification of other significant terrestrial sources, as plants with high activity of the enzyme(s) can be surveyed for MeX emissions.

Inorganic halogen radicals in the atmosphere are chemically important because of their role in stratospheric ozone destruction and tropospheric chemistry (Anderson et al., 1991; Platt and Janssen, 1996). Methyl halides transport halogens from Earth's surface to the atmosphere, making quantification of their fluxes necessary for the prediction of effects of human actions on the global budgets of MeX.

Atmospheric concentrations of MeX are well quantified, and while the sources and sinks of these gases have been studied, they are not completely understood. Nearly half of atmospheric methyl chloride (MeCl) sources, as well as one quarter of methyl bromide (MeBr) sources, remain unknown, while neither the sources nor the sinks of methyl iodide (MeI) are well understood (Butler, 2000). Although methyl fluoride (MeF) is a potent greenhouse gas, it has no known natural sources, due to the scarcity of C-F bonds in biological systems.

Recently, terrestrial sources of MeX have been implicated. Saini *et al.* (1995) showed that many plants have the ability to form MeX through a reaction involving s-adenosyl-L-methionine (SAM), a biological methyl donor (SAM + X- →MeX + Salt). The investigation of agriculture as a possible source of MeX emissions stemmed from the identification of this metabolic capability as well as the large planted area of specific crops. Quantification of emissions from agricultural crops, such as rice, and other terrestrial plants will lead to a better understanding of the atmospheric budgets of MeX.

Rice is the staple food of nearly two billion people worldwide, and its crops cover over one percent of continental surface area (Swaminathan, 1984). In addition, rice has been shown to be a significant source of methane (Cicerone et al., 1983). These considerations made rice a good candidate for further investigation of MeX emissions. Because rice covers such a large area, small methyl halide emissions from individual plants could have atmospheric significance. Research has shown that methyl halide emissions depend on a variety of factors, including rice growth stage, rice cultivar, straw management practices, soil halide concentrations, and ambient temperatures. Field measurements in rice paddies show that MeI emissions peak before MeBr emissions, and the seasonal variations of neither of these species correlate with variations in methyl chloride production (Redeker et al., 2000; Redeker et al., in press). These results suggest that different metabolic processes may be responsible for the generation of MeBr and MeI in rice tissue.

The discovery of globally significant MeX emissions from rice agriculture (Redeker et al., 2000), as well as reported emissions from broccoli and rapeseed (Gan et al., 1998), has prompted the current investigation of other, primarily agricultural, plant sources. Crops with large planting areas may be significant sources of MeX despite low emissions from individual plants; similarly, large emitters with small crop areas are also of interest.

The current study sought a greater understanding of the metabolic mechanism(s) that leads to MeX production in plants. Because field measurements show that MeCl emissions have little seasonal variation, and emissions in planted and unplanted fields are similar, it has been proposed that MeCl emissions from rice paddies may not be caused primarily by the rice plants themselves (Redeker et al., 2000; Redeker et al., in press). Therefore, MeBr and MeI were targeted in this study. It is postulated that halides that are present in plant tissue enter the active sites of MTs where they have the capability to accept a methyl carbocation from the methyl donor SAM via catalysis by the MT. There are several plant MTs with known reaction specificity (Attieh et al., 2000; Dudareva et al., 1998; Dudareva et al., 2000). Possibly, MeX are a by-product of MT catalysis intended for other purposes. Other evidence suggests MeX are produced as a defensive insecticide (Chen and Chang, 1985) or as a methylating agent for creation of methyl benzoic and furoic acids (Harper, 1998).

This investigation included the quantification of leaf disk MeX generation through several enzyme inhibition assay experiments. Using a method modified from Saini *et al.* (1995), disks of leaf tissue were incubated in a sodium halide bath and the headspace was analyzed using gas chromatography. The results of these assays showed that incubations of rice tissue yield quantifiable MeX production

rates. In a further effort to gain understanding of the mechanisms of methyl halide generation and the role of MTs, the impact of seven possible competitive inhibitors was tested. These species are all known to accept methyl carbocations from SAM through specific MT-catalyzed reactions.

A novel SAM:benzoic acid carboxyl methyl transferase (BAMT) has been identified in snapdragons. As the final enzyme in the biosynthesis of methyl benzoate, an important floral scent compound, it acts on benzoate as its substrate (Dudareva et al., 2000). Caffeic acid is a substrate in a lignin-creation pathway involving a methyl transferase reaction (Bugos et al., 1992). Lignin has a high degree of strength and is an important component of plant cell walls. Both catechol and myo-inositol are substrates for SAM-MTs that have been shown to have conserved sequences with the MT that uses caffeic acid as a substrate (Joshi and Chiang, 1998). Quercetin showed activity with purified recombinant MTs, suggesting that it also acts as a substrate in plant MT reactions (Schröder et al., 2002). Salicylic acid is a substrate in the MT catalyzed biosynthesis of methylsalicylate, another common component of floral scent (Dudareva et al., 1998). Finally, an MT was found to catalyze SAM methylation of thiocyanate. It is possible that the same enzyme may also accept halide ions as substrates (Attieh et al., 2000). If MeX are formed as side products of MT-catalyzed reactions, substrates such as those summarized above should competitively inhibit MeX production. Using this rationale, inhibitory studies were performed using benzoate, caffeic acid, catechol, myo-inositol, quercetin, salicylic acid, and thiocyanate.

Methods and Materials

Plant Material and Growth Conditions

Plant tissue was obtained from barley (*H. vulgare*), corn (*Z. mays*), rice (*O. sativa*), soybean (*G. max*), and wheat (*T. aestivum*) plants grown in a greenhouse located on the University of California, Irvine campus. The relative humidity and the temperature in the greenhouse ranged from 60-95% and 19-32 °C, respectively. Lights were timed such that the greenhouse was in darkness or the equivalent of full sunlight for 12 hr each day. The plants were grown in 12 glass bins, each with an area of 1 yd^2 and a depth of 2 ft, and filled with approximately 350 kg of soil. Fertilizer was added to achieve nitrogen levels similar to those applied commercially: 150 lb-N/acre or 15 g-N/bin. This amount of fertilizer also supplied 1.5 g-P/bin and 1.5 g-S/bin. In the first growing season (2001) all 12 bins were planted with rice. The exterior of each bin was covered in tarpaper to keep light from reaching the soil. In 2002, 6 bins were planted with rice. Barley, corn, oak, soybean, and wheat were planted in 1 bin each, and the remaining bin served as a control to allow for the determination of the contribution of soil and water to MeX production. In both growing seasons, the bins containing rice were flooded to a depth of 10 cm after the plants sprouted. In 2002, the bins containing rice were lined with a commercially available rubber pond liner to prevent leakage.

Leaf-disk Enzyme Inhibition Assays

Samples were collected several times throughout the growing season. Leaf tissue disks with a 16 mm diameter were cut and placed in a 0.1 M sodium halide bath. Each bath was also 5.0 mM in one of seven known methyl transferase substrates and possible competitive inhibitors: benzoate, caffeic acid (3,4-dihydroxy-cinnamic acid), catechol (1,2-benzenediol), myo-inositol (1,2,3,4,5,6-hexahydroxy-cyclohexane), quercetin (3,3',4',5,7-pentahydroxy-flavone), salicylic acid (2-hydroxy-benzoic acid), and thiocyanate. The solvent for all inhibitors but caffeic acid and salicylic acid was water. Due to limited water solubility, ethanol was used as the solvent for caffeic acid and salicylic acid.

The disks were soaked in the solution for 1 hr to allow the inhibitor under investigation to diffuse into the plant cells. The disks were then incubated in the solution, with the bottom of the leaf facing down, for 1 hr at 22 °C in a stoppered 10 ml vial to allow the MeX produced within the tissue to accumulate in the headspace. Control leaf disks were incubated in a 0.1 M sodium halide bath without an inhibitor. The control for caffeic acid and salicylic acid was a 0.1 M sodium halide/2.5% ethanol bath. During the second growing season, tissue samples were incubated under the same conditions; however, to separate possible effects of pH from the effects of the inhibitors, the pH of the sodium halide bath was maintained at 7.0 ± 0.3 using a HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (0.1 M).

Cell-free Enzyme Inhibition Assays

Leaf tissue (2 g) was frozen in liquid nitrogen and ground using a mortar and pestle on ice. Sand (2 g) was used to break the tissue and aid in mechanical grinding, and polyvinylpolypyrrolidone (1 g) was used to bind ketones and esters and prevent denaturation of proteins. Additionally, a buffer mixture of 0.1 M HEPES, 10% glycerol, and 4% dithiothreitol (to protect sulfur bonds) was added. The entire mixture was filtered through four layers of cheesecloth and the filtrate was centrifuged under refrigeration for 10 min at 2200 rpm. The supernatant fluid was further

homogenized and filtered three times to separate all components greater than 10 kDa. The extract was diluted to 20 ml with buffer and stored at -25 °C until analysis. Within 48 hr the extract (500 ml) was mixed with 0.1 M sodium halide, SAM, and one of the seven inhibitors. The mixture was sealed in a stoppered 10 ml vial and incubated for 1 hr at 22 °C.

Analysis

Headspace gas (2 ml) was sampled using airtight glass syringes and analyzed via gas chromatography (Shimadzu GC-17A/ECD, VoCol column). The methyl halides were separated under isothermal column conditions (30 °C). The injection port and detector were maintained at 250 °C. The carrier gas was helium at a flow rate of 3 ml/min.

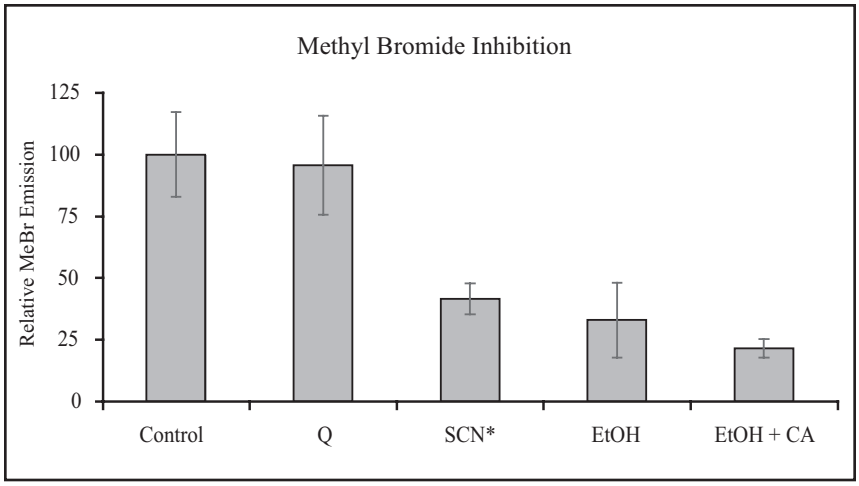

Figure 1
Relative MeBr emission during the tillering stage (Q = quercetin, SCN = thiocyanate, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid; error bars indicate one standard error of the mean, asterisk indicates significant inhibitory effect).

Results

Preliminary Enzyme Inhibition Assays

Enzyme-inhibition data were collected at two stages during the 2001 growing season. During growth, rice passes through two distinct phases: vegetative and reproductive. These categories can, in turn, be subdivided into separate growth stages. During the 2001 season, the first sampling occurred approximately one month after planting, when the rice was in the tillering stage of the vegetative phase. During this stage, rice grows rapidly, producing many new leaf shoots (tillers). The second sampling occurred near the end of the season, approximately four months after planting. At this time the rice was in the late reproductive phase and the plant biomass was actively senescing. This stage of growth occurs between maturity and death and is characterized by an accumulation of metabolic products.

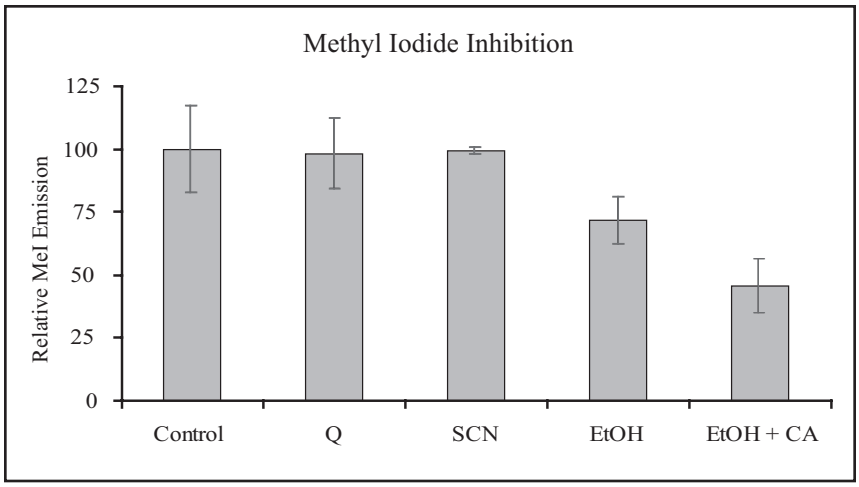

Figure 2
Relative MeI emission during the tillering stage (Q = quercetin, SCN = thiocyanate, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid; error bars indicate one standard error of the mean).

The results of the inhibition of MeBr production in leaf disks during the tillering stage are shown in Figure 1. All methyl halide emissions were normalized to the sodium halide control; that is, the relative emission rates shown in Figure 1 are the ratio of the amount of MeBr produced in the presence of an inhibitor to the amount produced in the absence of an inhibitor.

Although all emissions shown in Figure 1 are normalized to the control, the emission for the caffeic acid assay should be compared to ethanol. Quercetin did not significantly inhibit MeBr production (according to the Student's t-test; $p > 0.05$). The relative emission of MeBr in the presence of caffeic acid was slightly different than in the ethanol control, however this difference was not statistically significant ($p = 0.062$). Figure 1 also demonstrates that thiocyanate had a significant inhibitory effect ($p = 0.017$).

The production of MeI in rice tissue is different than that of MeBr. The rice tissue samples produced MeBr only when incubated in a bath containing bromide. However, the tissue produced MeI when incubated in either a bromide or iodide bath. Figure 2 shows the results of the MeI inhibition assays conducted during the tillering stage of rice

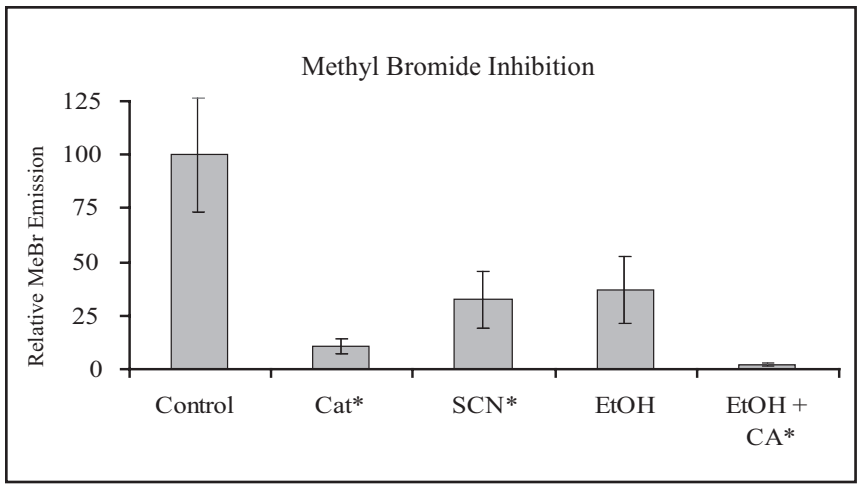

Figure 3
Relative MeBr emission during the reproductive phase (Cat = catechol, SCN = thiocyanate, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid; error bars indicate one standard error of the mean, asterisk indicates significant inhibitory effect).

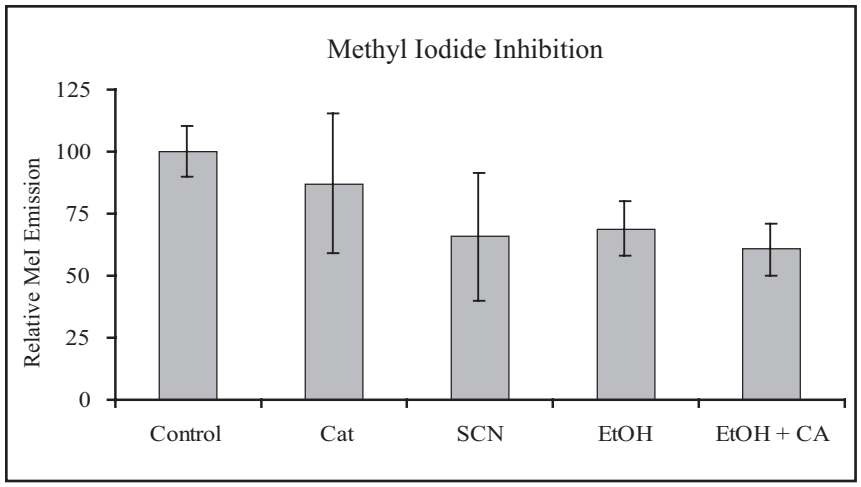

Figure 4
Relative MeI emission during the reproductive phase (Cat = catechol, SCN = thiocyanate, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid; error bars indicate one standard error of the mean).

Table 1
Relative MeBr and MeI emission during the vegetative phase

Inhibitor	Relative MeBr Production	Relative MeI Production
Control	100 ±14	100 ±6
Benzoate	92 ±21	116 ±23
Catechol	44 ±22	62 ±16
myo-Inositol	83 ±4	126 ±19
Thiocyanate	8 ±2	138 ±22
Ethanol	66 ±16	61 ±7
Ethanol and Caffeic Acid	63 ±11	57 ±5
Ethanol and Salicylic Acid	41 ±11	56 ±5

growth. Only the MeI data for tissue incubated in sodium iodide solutions are shown. As with MeBr inhibition, the caffeic acid data should be compared to the production of MeI in the ethanol control.

In general, the inhibitors examined in this phase of rice growth affected the production of MeI less than the production of MeBr. None of the inhibitors tested had a statistically significant impact on the emission of MeI by leaf disks. Although the absolute amounts of MeI produced by tissue incubated in sodium bromide were less than that produced by tissue incubated in sodium iodide, the trends in MeI production across inhibitors were similar.

The enzyme-inhibition assays described above were repeated in the late reproductive phase of the 2001-growing season. Because quercetin did not significantly affect the production of either MeBr ($p = 0.874$) or MeI ($p = 0.941$), catechol was examined in its place. The data obtained are summarized in Figures 3 and 4. Catechol ($p = 0.022$), thiocyanate ($p = 0.043$), and caffeic acid ($p = 0.028$) all had a significant inhibitory effect on MeBr formation. As during the tillering stage, MeI production was not significantly impacted by any of the inhibitors tested.

Constant pH Enzyme Inhibition Assays

In order to resolve the effects of pH and the suspected competitive inhibitors, the experimental method was modified for the 2002 growing season. The halide baths were maintained at a nearly constant pH using a 0.1 M HEPES buffer. In addition, three other possible inhibitors were studied, for a total of six: benzoate, caffeic acid, catechol, myo-inositol, salicylic acid, and thiocyanate.

The first sampling occurred approximately one month after planting, during the early part of the vegetative phase of growth. The results of the MeBr and MeI inhibition studies are given in Table 1.

Only thiocyanate had a significant inhibitory effect on the production of MeBr ($p = 0.000032$). Inhibition by catechol was marginally significant ($p = 0.054$); more data are needed

to clarify the effect of catechol. These data imply that previously measured inhibition of MeBr formation by caffeic acid was due to pH effects. The production of MeI by leaf-tissue disks was not significantly affected by any of the inhibitors tested.

Cell-free Enzyme Inhibition Assays

Cell-free data were collected during the 2002 growing season approximately one and a half months after planting, when the rice was still in the early part of the vegetative phase. The incubations were carried out in the same manner as the leaf-disk assays, however, methylation of the halides occurred outside the plant cells. Enzymes were separated from the rice tissue and mixed with SAM in a halide bath. The activities of the enzyme(s) in the presence of inhibitors were similar both within the plant tissue (leaf-disk assays) and outside the tissue (cell-free assays). The relative production of MeX by leaf disks and enzyme extracts are shown in Figures 5 and 6.

The relative emission rates of both MeBr and MeI in the presence of each inhibitor were comparable in the leaf-disk and cell-free assays. The only inconsistent result is the MeBr production by the cell-free extract in the presence of benzoate. The baseline in the chromatograph for this incubation was poor, which may have led to an incorrect integration of the MeBr peak area. It is likely that replicate analyses would yield a result similar to that measured in the leaf-disk assay.

Survey of Other Plants

Preliminary data indicate that MeX formation in leaf tissue from other agricultural plants is generally less than that measured in rice (Figure 7).

Only wheat emitted a detectable amount of MeBr, approximately 1/500 of that measured in rice. Barley, corn and soybean leaf disks all produced less MeI, with values approximately 13%, 3%, and 0.9% of those measured in rice, respectively. Wheat tissue, however, produced nearly 10 times more MeI than rice tissue incubated under the same conditions.

Discussion

Agricultural plants were chosen for investigation because of their large continental coverage. Rice,

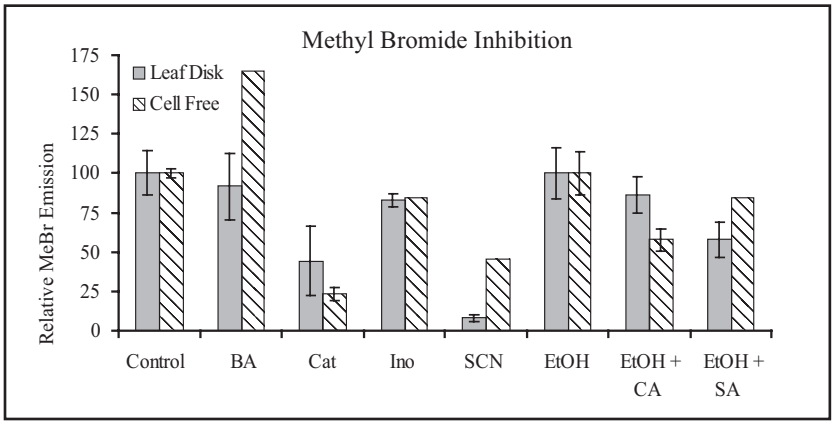

Figure 5
Relative MeBr emission by leaf disks and cell-free extracts (Cat = catechol, SCN = thiocyanate, BA = benzoate, Ino = myo-Inositol, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid, EtOH + SA = salicylic acid; error bars indicate one standard error of the mean).

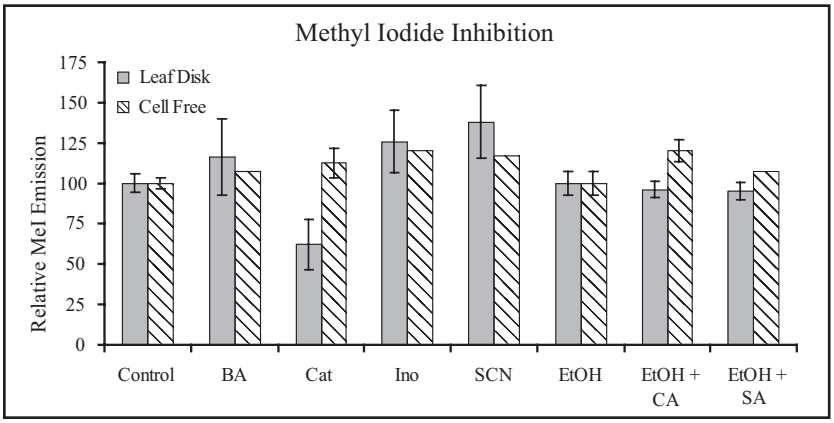

Figure 6
Relative MeI emission by leaf disks and cell-free extracts (Cat = catechol, SCN = thiocyanate, BA = benzoate, Ino = myo-Inositol, EtOH = ethanol, EtOH + CA = ethanol and caffeic acid, EtOH + SA = salicylic acid; error bars indicate one standard error of the mean).

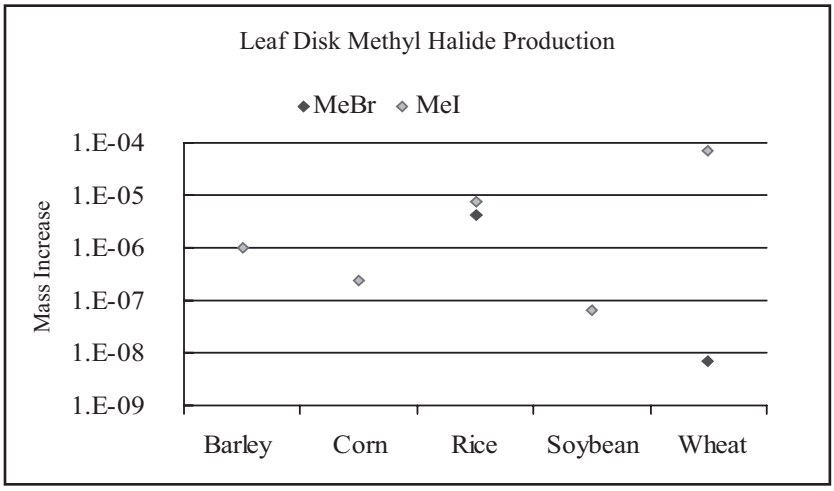

Figure 7
Comparison of MeX emissions in five agricultural plants (mass increase was measured in g MeX per hour per g fresh weight).

which was the primary focus of this study, has a global planted area of 400 million acres and is expected to increase with growing global populations. The annual production of rice grain is nearly 600 million tons worldwide (Statistics Norway, 2000). Because rice covers such a large amount of continental surface area, over one percent, emissions from individual plants could play a significant role in global MeX budgets. Wheat and barley crops cover 520 (1.3%) and 140 (0.25%) million acres of continental surface area, respectively. The respective annual yields of these grains are 580 and 130 million tons (Statistics Norway, 2000). In the United States alone, corn and soybean crops each cover approximately 75 million acres. In 2001, the United States produced 9.5 billion bushels of corn and 2.9 billion bushels of soybeans (USDA). A bushel is an agricultural unit of measure equivalent to 8 dry U.S. gallons. Thus, barley, corn, soybean, and wheat were good candidates for an initial survey of MeX production. Not only do they cover a large amount of continental surface area, their agricultural planting areas are known. This knowledge will aid in the quantification of the contributions of these crops to global methyl halide budgets.

The metabolic mechanism(s) that leads to MeX production in rice and other plants was investigated through the use of leaf-disk enzyme inhibition assays. It was hypothesized that the enzymes responsible for MeX generation in plants such as rice are MTs. To confirm this theory, known MT substrates were chosen for examination. Seven possible inhibitors were studied: benzoate, caffeic acid, catechol, myo-inositol, quercetin, salicylic acid, and thiocyanate. Because the enzymes responsible for the methylation of all these substrates are known, their inhibition of MeX generation in plant tissue leads to a greater understanding of which MTs produce MeX in rice.

Of the seven possible inhibitors surveyed, only tissues incubated in caffeic acid, catechol, and thiocyanate showed a statistically significant difference from the control samples. These data indicate that the MTs described above, for which caffeic acid, catechol, and thiocyanate are substrates, may be responsible for MeX synthesis in rice plants. Three sets of inhibition assays were conducted: early and late growth stage without pH control, and early growth stage with pH control. Only thiocyanate had a statistically significant inhibitory effect in all three sets of assays. In the non-pH controlled experiments, catechol inhibited MeBr production in the late reproductive phase, but not during the tillering stage. These data suggest that the activity of the MT that uses catechol as a substrate may vary throughout the season. Catechol also inhibited MeBr production in the pH-controlled assay, although the difference from the control was only marginally significant ($p = 0.057$). Caffeic acid acted as an inhibitor in the assays without pH control, but not during the pH-controlled assay. Therefore, the measured decrease in MeBr generation in the first two assays may have been an effect of pH, rather than caffeic acid inhibition. Most biochemistry occurs in the physiological pH range: between 6.5 and 8.0. This is because conditions outside this range can cause denaturation of proteins. The functions of enzymes, such as the MTs in this investigation, depend strongly upon their structure. The non-physiological pH of the halide/caffeic acid baths used in the 2001 growing season likely caused some denaturation of MTs and may have caused the measured inhibition of MeBr production.

In addition to differences in the effectiveness of the seven inhibitors surveyed, differences in MeBr and MeI generation were also measured. In all three sets of inhibition assays, none of the inhibitors effected a significant decrease in MeI production from the control. If the same enzyme catalyzes both MeBr and MeI formation, it is likely that it binds iodide preferentially. If the enzymes are different, these data suggest that the MT responsible for MeI synthesis has a higher affinity for iodide than the MT responsible for MeBr synthesis has for bromide. The postulation that iodide is bound more strongly is supported by the observation that leaf disks produce MeI when incubated in either a bromide or iodide bath, while MeBr was produced only by leaf disks incubated in bromide baths. Ion analysis of tissue extracts shows that both bromide and iodide are present in rice tissue (125-750 and 5-75 ppm, respectively). Even in the presence of a solution containing excess bromide, the MT binds iodide already present in the tissue to produce MeI, as well as measurable quantities of MeBr. Leaf disks in iodide solutions produce MeI but do not form quantifiable amounts of MeBr.

The results of the cell-free enzyme inhibition assay support the results of the leaf-disk enzyme inhibition assays. The inhibitors tested had similar effects in both sets of assays. This indicates that the measured differences in activities were due to the enzymes themselves, and not diffusion processes within the leaf tissue.

Preliminary measurements of MeBr emissions from barley, corn and soybean leaf disks were below detection limits. Wheat leaf disks produced a measurable amount of MeBr, however, the value was less than 0.2% of that produced in rice. These results could indicate that the other agricultural plants surveyed are not significant producers of MeBr. Previous studies have shown, however, that MeBr produc-

tion in rice peaks later in the season. Although the data for barley, corn, soybean, and wheat were collected at the same time, wheat, which did produce a detectable amount of MeBr, was further along in its growing season, suggesting that barley, corn, and soybean leaf disks may emit measurable amounts of MeBr later in the season.

MeI production in barley, corn and soybean was significantly lower than in rice leaf disks, with differences ranging from one to two orders of magnitude. While the production of MeI in these plants is low, because they cover such large amounts of continental surface area, they could still play a significant role in the global MeI budget. Wheat leaf disks produced over 600 times as much MeI as rice, suggesting that wheat could possibly contribute to the global budget of MeI. These data are still preliminary; therefore, further investigation is needed to determine whether barley, corn, soybean, and wheat have a significant impact on the global budgets of MeX. While leaf disk experiments can demonstrate the ability of a particular plant to produce MeX, gas chamber measurements are necessary to accurately quantify seasonal emissions.

The outer layer of leaf tissue contains pores, or stomata, that allow for the exchange of gases between internal air spaces and the surrounding environment. Stomata are hydraulically operated valves, and the size of the opening can be controlled based on the environment around the tissue. These pores control two very important processes: uptake of carbon dioxide for photosynthesis and transpiration of water vapor. This latter process makes stomata crucial in protecting plant tissue against excessive water loss. When plants are water stressed, their stomata are only partially open; after watering they are fully open. In rice, which is grown under constantly flooded conditions, the stomata in the leaf tissue are always open. This is not the case in barley, corn, soybean, and wheat. Thus the variations between MeX emission in these plants and in rice could be due to stomata effects. Preliminary gas chamber measurements have shown that MeX production peaks after watering. Future work will investigate the effects of watering, and thus opening of stomata, on MeX emission from other agricultural plants.

Conclusion

The results of this investigation lead to a greater understanding of the biosynthesis of methyl halides in agricultural crops, particularly rice. Methyl bromide and methyl iodide production rates in both leaf disks and cell-free extracts responded differently to possible competitive inhibitors, indicating that different metabolic pathways may be responsible for the synthesis of these two methyl halides. The identification of possible methyl transferases responsible for methyl halide generation, as well as preliminary results from barley, corn, soybean, and wheat, will aid in predicting which other plants may be significant methyl halide producers.

Acknowledgements

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