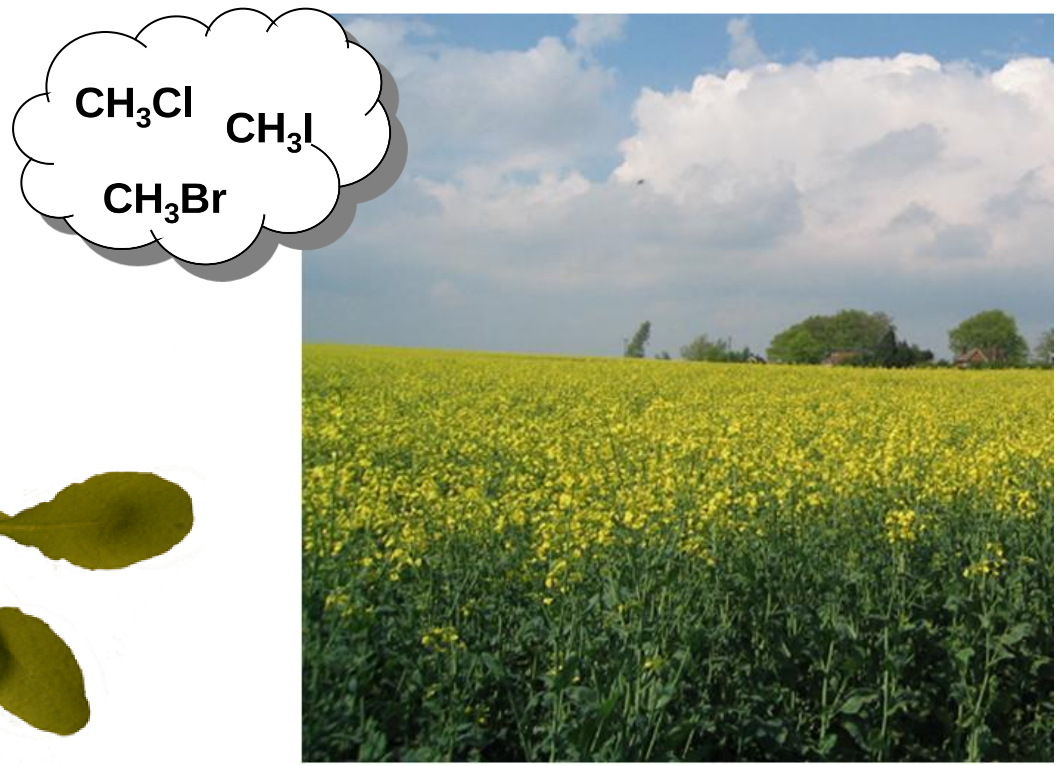
<u>Methyl halide emissions from *Arabidopsis* and *Brassica* crops</u>

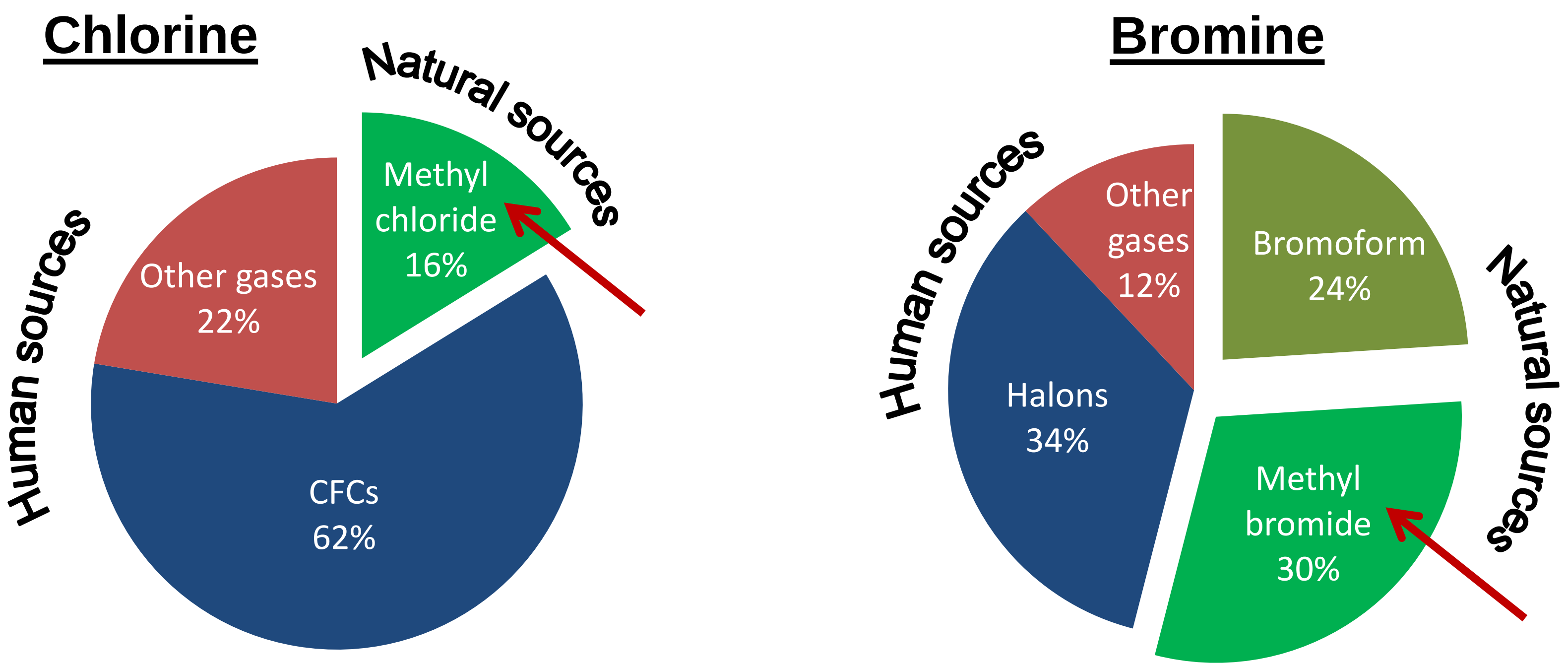


John Innes Centre

Evelyn Körner

University of East Anglia

Ozone-depleting substances (ODS)



Sources of chlorine and bromine for the stratosphere in 2004 (WMO, 2006)

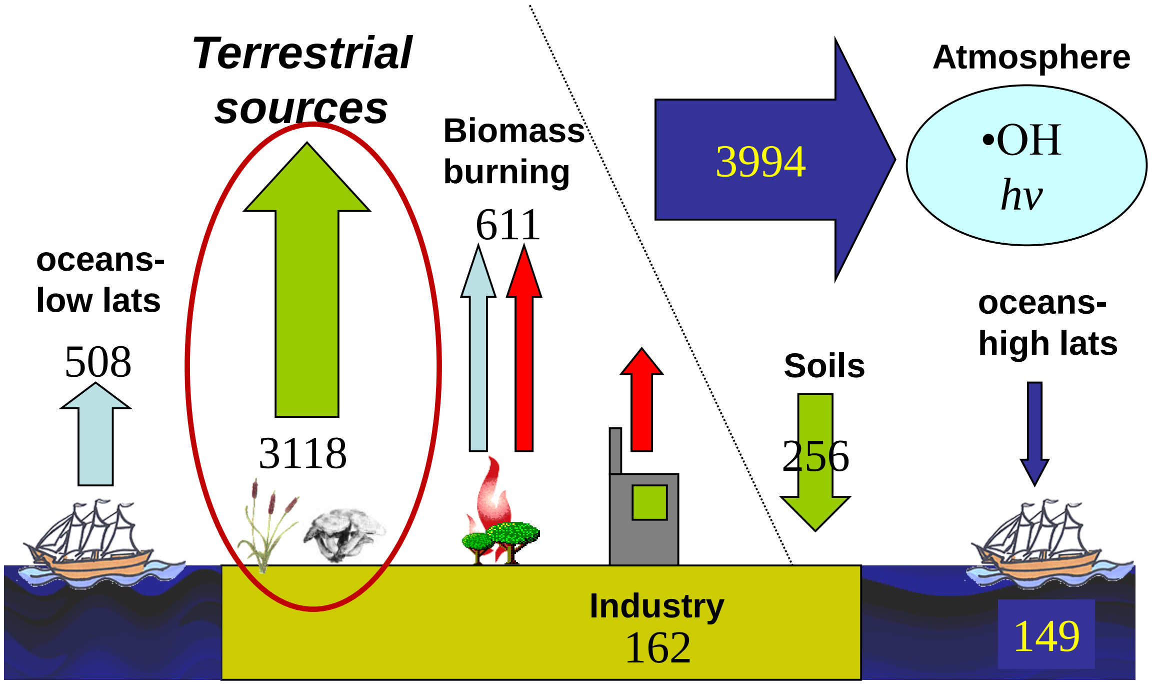
→ Thanks to the Montreal Protocol signed in 1987, the production and consumption of most of the human made ODS has ended in 2010

→ Quantities of methyl halides will remain constant based on the assumption of **unchanged contributions from natural sources**

Natural sources of methyl halides

Methyl chloride budget in Gg/year in 2006

Plants are important emitters!



Source type	% of total CH_3Cl emissions
Tropical and subtropical plants	66%
Oceans	12%
Salt marshes	4%
Wetlands	1%

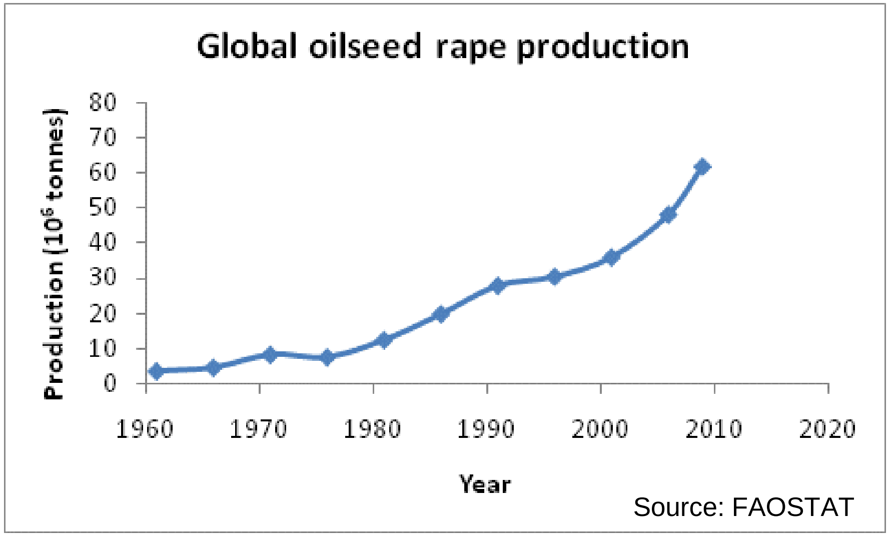
WMO: *Scientific Assessment of Ozone Depletion* (2006)

Natural sources of methyl halides

Methyl bromide

Source type	% of total CH_3Br emissions
Oceans	39%
Salt marshes	7%
Oilseed rape	5%
Rice paddies	1%

Source: WMO, 2010



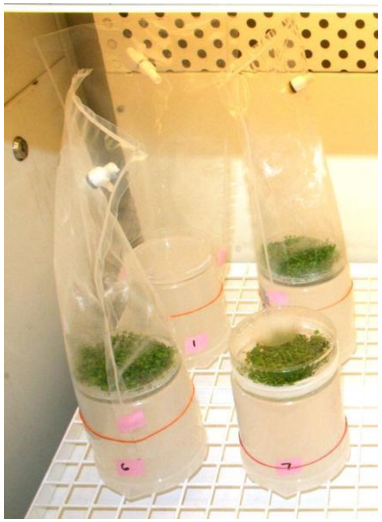
→ Quantities of methyl bromide will remain constant based on the assumption of **unchanged contributions from natural sources**

"...For every 1°C rise in ambient temperature, modelled CH_3Br and CH_3Cl emission from rice rise by 10%..."

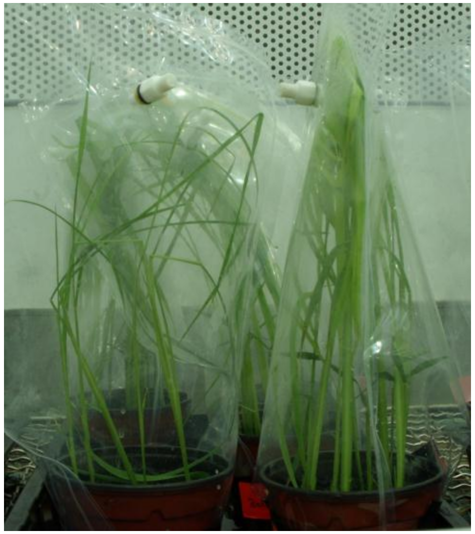
Lee-Taylor & Redeker (2005)

Measuring methyl halide emissions

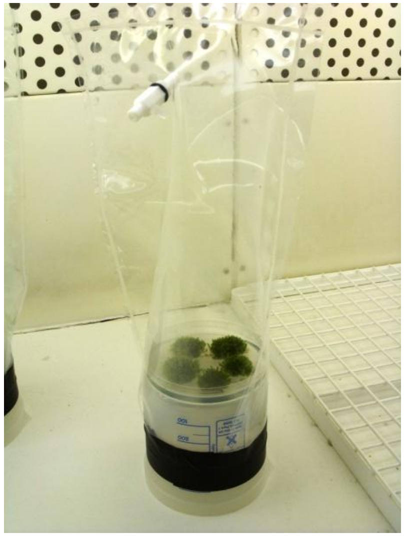
Oilseed rape



Arabidopsis



Rice



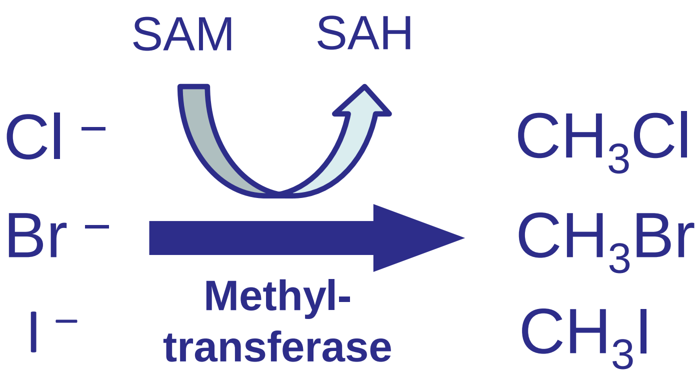
Moss

GC-MS

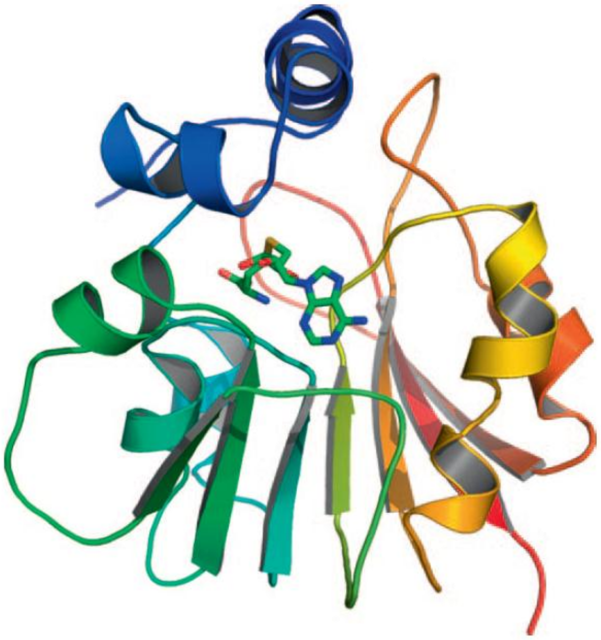
Collaboration with
Bill Sturges lab, UEA

How are methyl halides produced in plants?

S-adenosyl-L-methionine (SAM)-dependent methyltransferases

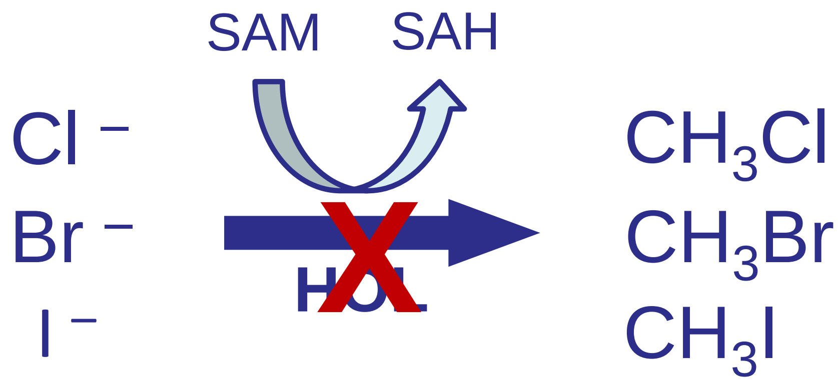


→ in ***Arabidopsis***: encoded by the ***H****ARMLESS TO* ***O****ZONE* ***L****AYER* ***(HOL)*** gene

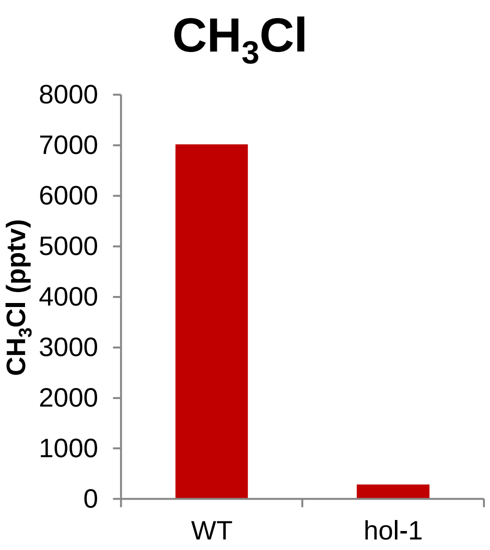


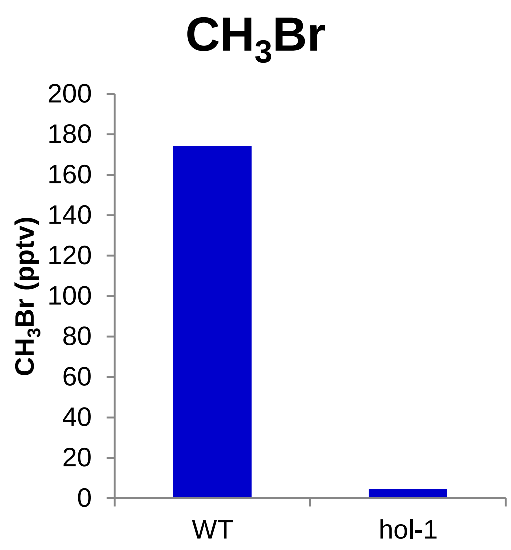
Structure of HOL in *A. thaliana* (Schmidberger, 2010)

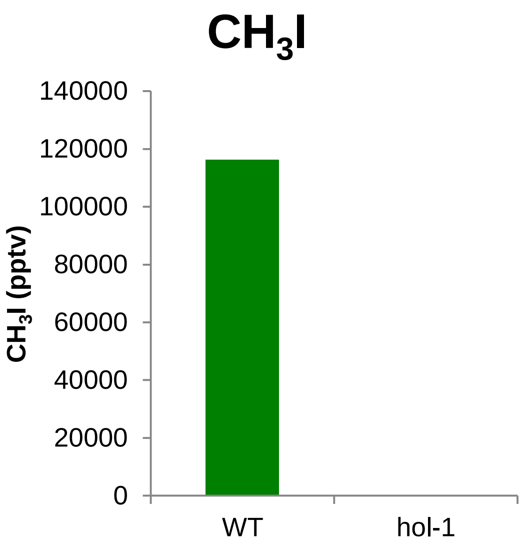
HOL and methyl halide emissions in *Arabidopsis*



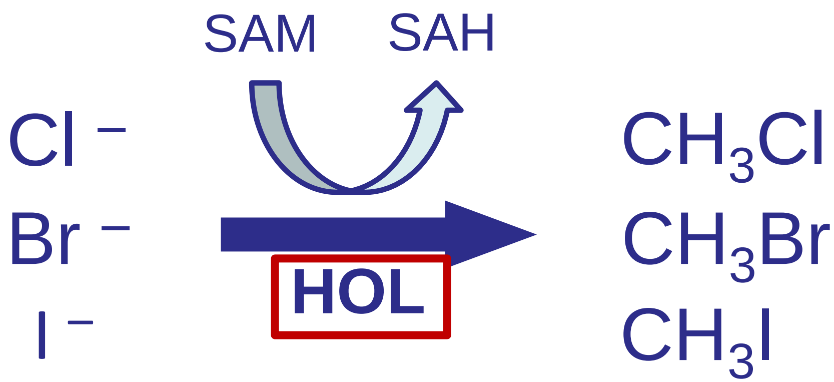
Reduced methyl halide emissions in *hol*-mutants



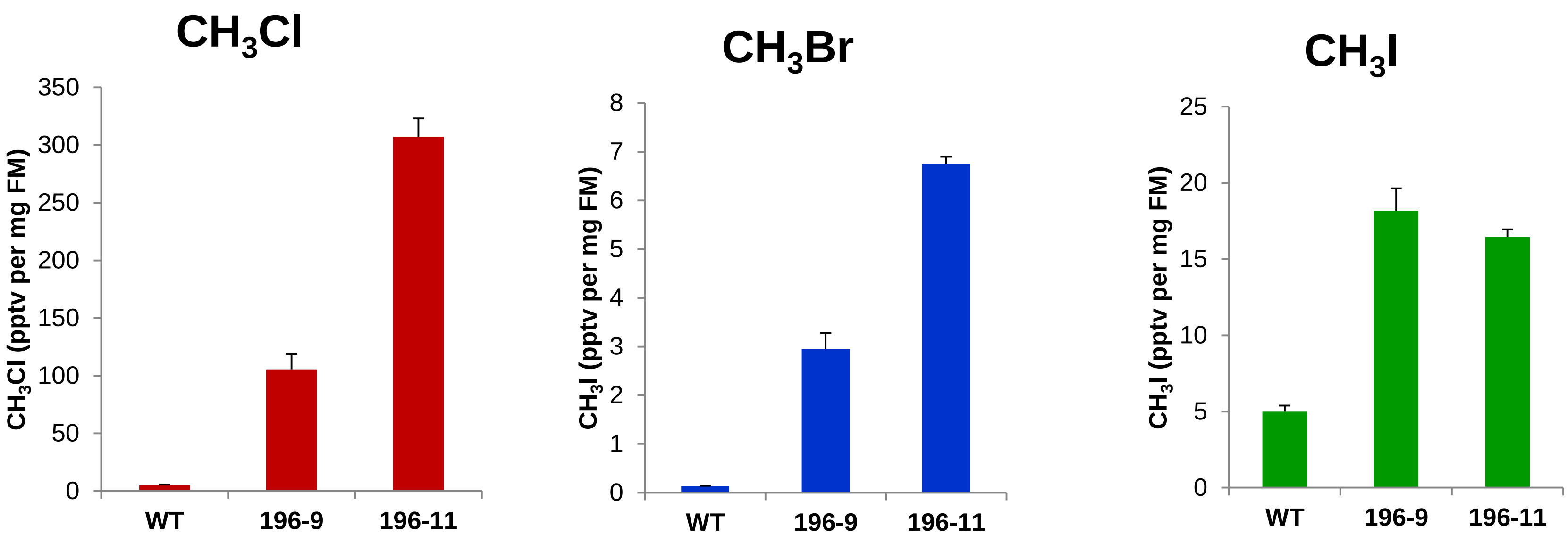




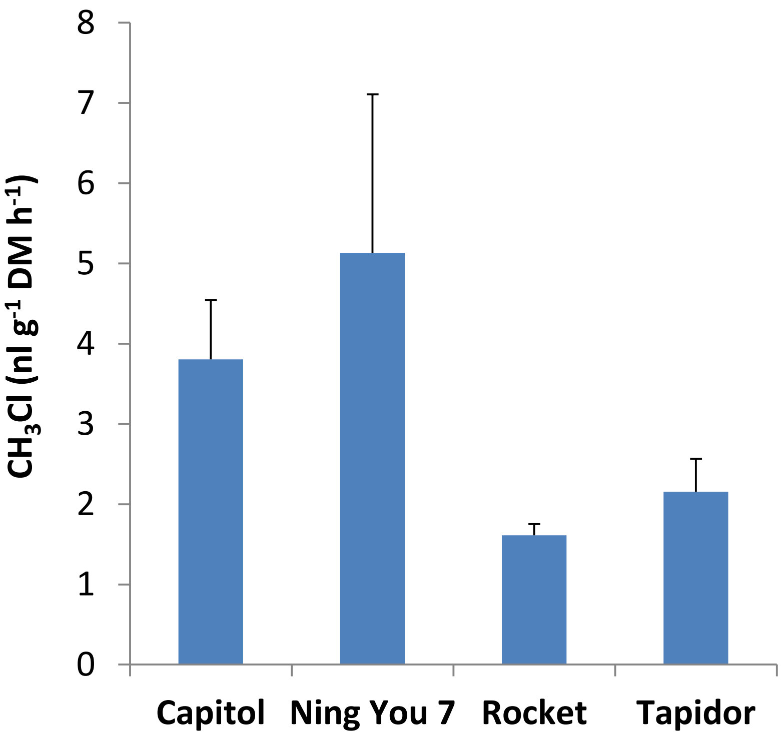
HOL and methyl halide emissions in *Arabidopsis*



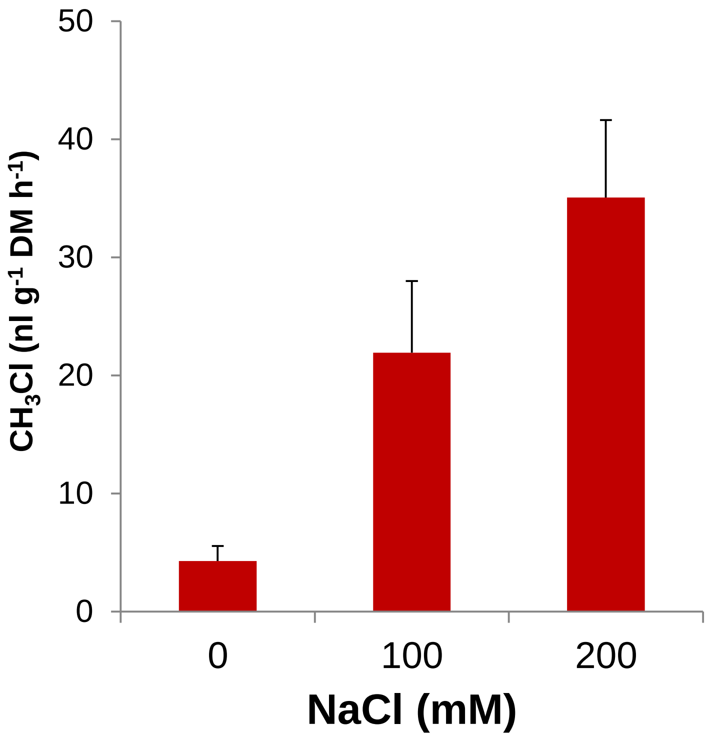
Increased methyl halide emissions in *35S::HOL lines*



Methyl chloride emissions in *Brassica*



Brassica napus



Brassica rapa

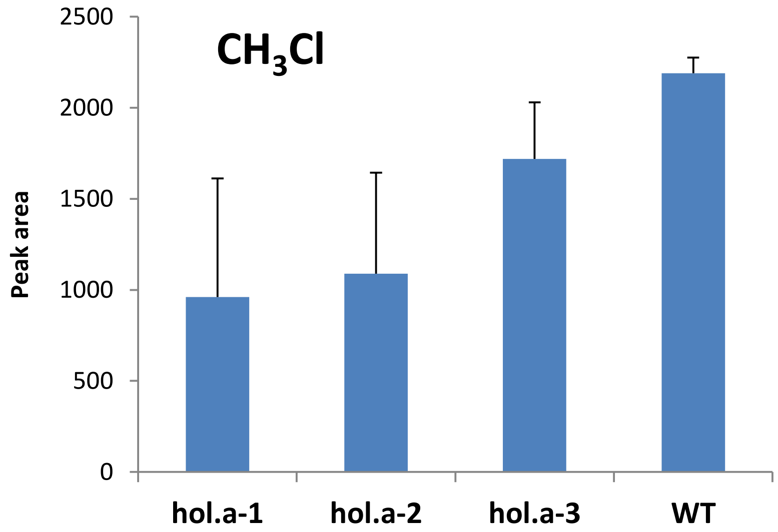
→ Salt stress increases methyl halide production!

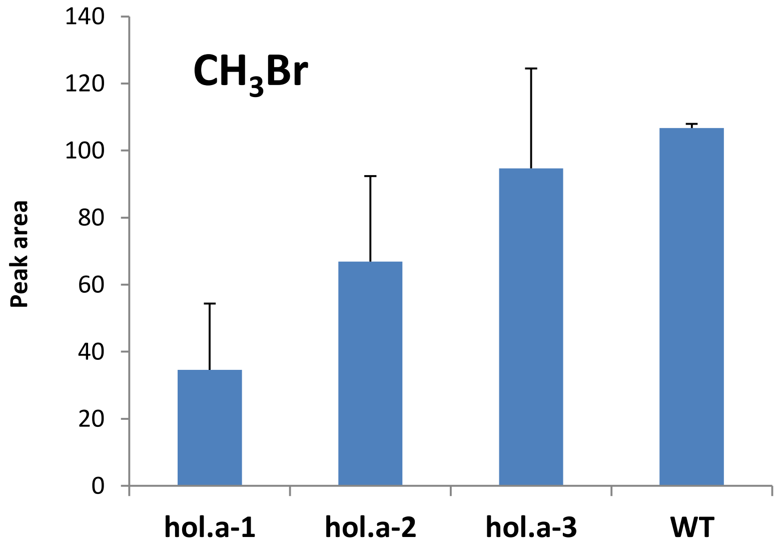
Katrin Armeanu-D'Souza

Genetic control of methyl halide production in *B. rapa*

There are 2 *HOL* genes in *B. rapa* (*BraA.HOL.a* and *BraA.HOL.b*) which have 84% and 85% similarity with *AtHOL* (AA sequence).

From a TILLING mutant population we obtained three *braA.hol.a* mutant lines and measured methyl halide emissions.



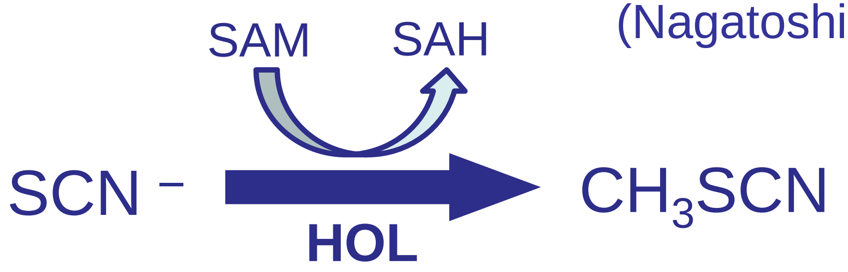


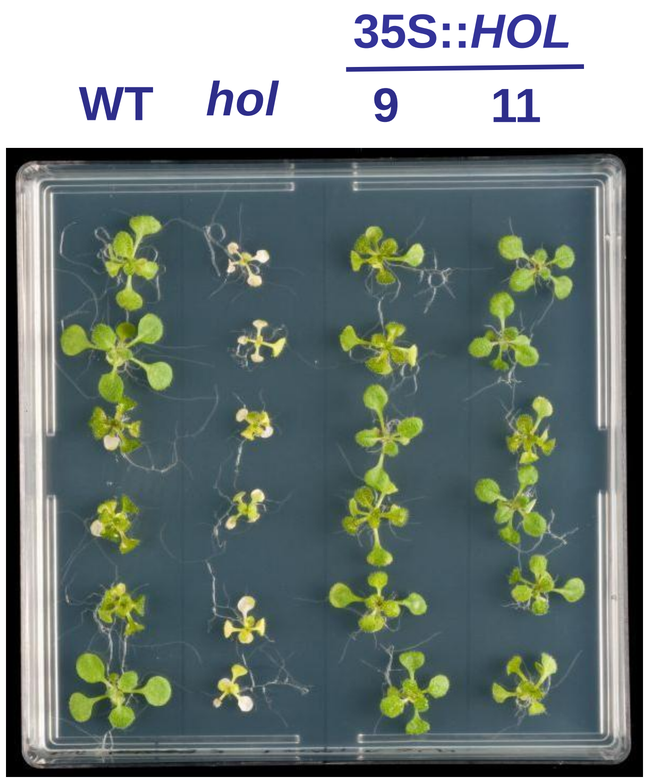
2 mutant lines show reduced emission!

→*HOL.a* and *HOL.b* are most likely redundant

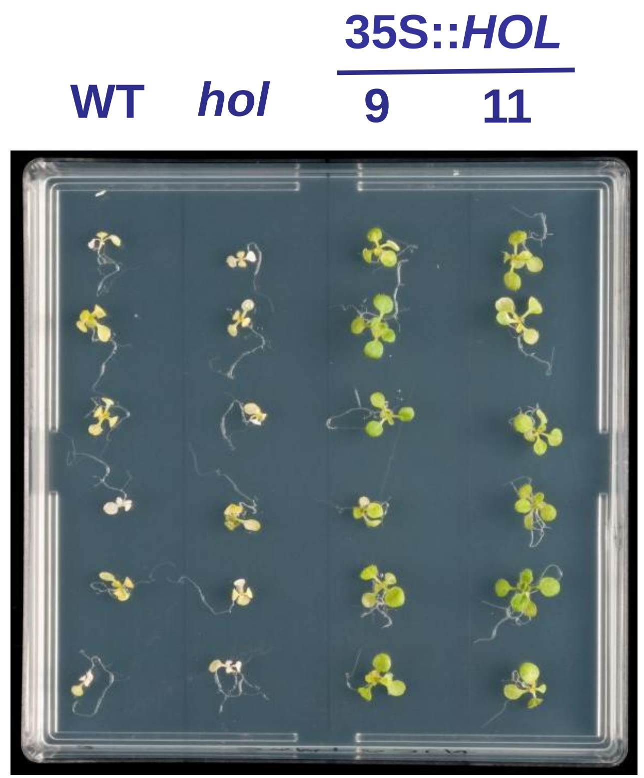
HOL and glucosinolate metabolism

(Nagatoshi & Nakamura, 2009)





1 mM KSCN media



5 mM KSCN media

Summary

- Methyl halides contribute to ozone degradation in the atmosphere
- *Brassica* crops produce large amounts of methyl halides which could have a negative impact on the ozone layer, e.g. if oilseed rape production continues to rise
- *BraA.HOL.a* gene contributes to methyl halide production in *B. rapa*
- We need to understand the physiological role of HOL and methyl halides in plants and the environmental factors promoting the production of these gases...

... Then we could attempt to engineer “ozone-friendly” Brassicas!

Acknowledgements

JIC

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UEA

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- Bill Sturges
- Claire Reeves
- David Oram
- Stephen Humphrey

Thanks for your attention!

Earth & Life Systems Alliance

ELSA

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BoTMT2        ---------------------------MAEVQQNSGNSNGE-NIIP-PEDVAKFLPKTVDE 32
BraA.HOL.     ---------------------------MAEVQQNSAHINGE-NIIP-PEDVAKFLPKTVEE 32
BoTMT1        ---------------------------MAEEQQKAGHSNGE-NIIP-PEEVAKFLPETVEE 32
HOL           ---------------------------MAEEQQNSDQSNGG-NVIPTPEEVATFLHKTVEE 33
At2g43920     ---------------------------MAEEQQNSSYSIGG-NILPTPEEAATFQPQVVAE 33
BmMCT         ---------------------------MSTVANIAPVFTGDCKTIPTPEECATFLYKVVNS 34
GhHOL         MLRSLFFSPRIRR--VAVLSSPLGPTLAMDNNNRSRTTDSSVQTNPRIQKLQQIVKTDAS 58
HvMCT         MRRVLPSGPVVSRGARAAAMGSSA--GAGAGARGP--GGNPAVGRLREIFRGGGGGDAA 56
ZmMCT         --------------------MGSSAPVRAAGGTRDP--GENPAVGRLRELFTG-----DAA 34
OsMCT         --------------------MASAIVDVAGGGRQQALDGSNPAVARLRQLIGGGQ---ESS 38
                                          :

             splice 31015b
             |33G>D 30402b
             ||
BoTMT2       *GGWEKCWEDGVTPWDQGRATPLVVHLVESSSLPLGRG---LVPGCGGGHDVVAMASPERY 89
BraA.HOL.a    GGWEKCWEDGVTPWDQGRATPLVVHLVESSSLPLGRA---LVPGCGGGHDVVAMASPERY 89
BoTMT1        GGWEKCWEDGITPWDQGRATPLVVHLVDSSSLPLGRA---LVPGCGGGHDVVAMASPERF 89
HOL           GGWEKCWEEEITPWDQGRATPLIVHLVDTSSLPLGRA---LVPGCGGGHDVVAMASPERF 90
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BmMCT         GGWEKCWVEEVIPWDLGVPTPLVLHLVKNNALPNGKG---LVPGCGGGYDVVAMANPERF 91
GhHOL         AGWEESWKQGVTPWDLGRPTPVILHLHHSGSLPMGRV---LVPGCGTGYDVVAMACPGRY 115
HvMCT         DGWEKSWESGVTPWDLGKPTPIIEHLVKSGTLPKGRA---LVPGCGMGYDVVALASPERF 113
ZmMCT         DGWEKSWEFGVTPWDLGKPTPVIEHLARSGTLPKGRA---LVPGCGMGYDVVALASPERF 91
OsMCT         DGWSRCWEEGVTPWDLGQRTPAVVELVHSGTLPAGDATTVLVPGCGAGYDVVALSGPGRF 98
               **...*   : *** *  ** : .*  ..:** *     ****** *:****:: * *:

                 92G>S 30882a        111P>S 31132b            128P>L 31357a
                 |                   |                        |
BoTMT2        VVGLDISESALEKAAETYGSSP--------KAKYFTFVKEDFFTWRPNELFDLIFDYVVF 141
BraA.HOL.a    VVGLDISESALEKAAETYGSSP--------KAKYFTFVKEDFFTWRPNELFDLIFDYVVF 141
BoTMT1        VVGLDISESALEKAAETYGSSP--------KAKYFTFVKEDFFTWRPNELFDLIFDYVVF 141
HOL           VVGLDISESALAKANETYGSSP--------KAEYFSFVKEDVFTWRPTELFDLIFDYVFF 142
At2g43920     VVGLDISDKALNKANETYGSSP--------KAEYFSFVKEDVFTWRPNELFDLIFDYVFF 142
BmMCT         MVGLDISENALKKARETFSTMP--------NSSCFSFVKEDVFTWRPEQPFDFIFDYVFF 143
GhHOL         VVGLDISKEAIKKAKQMSSSLP--------NADDFTFIKADFFSWRPTDLFDLIFDYTFF 167
HvMCT         VVGLEISNIAAEKAKQWSLSLP--------NTDCFTFLVADFFKWRPSEPFDLIFDYTFF 165
ZmMCT         VVGLDISDMAVKKAKQWSSSLP--------NADYFTFLAEDFFKWIPSEQFDLIFDYTFF 143
OsMCT         VVGLDICDTAIQKAKQLSAAAAAADGGDGSSSFFAFVADDFFTWEPPEPFHLIFDYTFF 158
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