Influence of Abiotic Stress Factors on VOCs Emission from Portuguese Rice Paddy Fields

Relation with increased Climate Change

Mestrado Integrado em Engenharia do Ambiente
Gestão de Sistemas Ambientais

Catarina Oliveira
FCT, 18th October 2013

Presentation layout

- **Scope**
- **Objectives**
- **Introduction**
  - VOCs behavior on atmospheric chemistry
  - VOCs annual variation
  - Europe scale
- **Materials and methods**
  - COTArroz location
  - Sampling layout
  - VOCs extraction tecnhniques
  - VOCs analysis methods
  - VOCs identification process
- **Results and discussion**
  - GC/MS DB-5
  - VOCs main zone classes
  - GC/MS DB-5 *vs.* GC/MS DB-WAX
  - Rice cycle phases (same T°, CO_2 and soil texture)
  - Soil textures (same T°, CO_2 and cycle phase)
  - Temperature and atmospheric CO_2 concentration (same cycle stage and soil textures)
  - Statistical significance – Student's t-test
  - Rice behaivior under climate change scenarios
- **Conclusions I and II**
- **Further researches**
- **Acknowledgements**

Scope

Rice is the main staple food crop	Abiotic stress factors °C and CO_2	*Oryza sativa* L. cv. Aríete (2012 cycle) VOCs emission study[1]
Growth population trend	VOCs response to abiotic factors	Under different soil textures, °C and CO_2 concentration
Portugal 4th EU producer and 1st consumer	VOCs influence tropospheric O_3 formation	Worldwide 1st study in the scope

Key-words: Portugal; abiotic stress factors; VOCs; *Oryza sativa* L. cv. Aríete.

[1]Project: PTDC/AGR-AAM/102529/2008 "Trace gas emission from Portuguese irrigated rice fields in contrasting soils, by the influence of crop management, climate and increased concentration of CO_2 and temperature in the atmosphere"

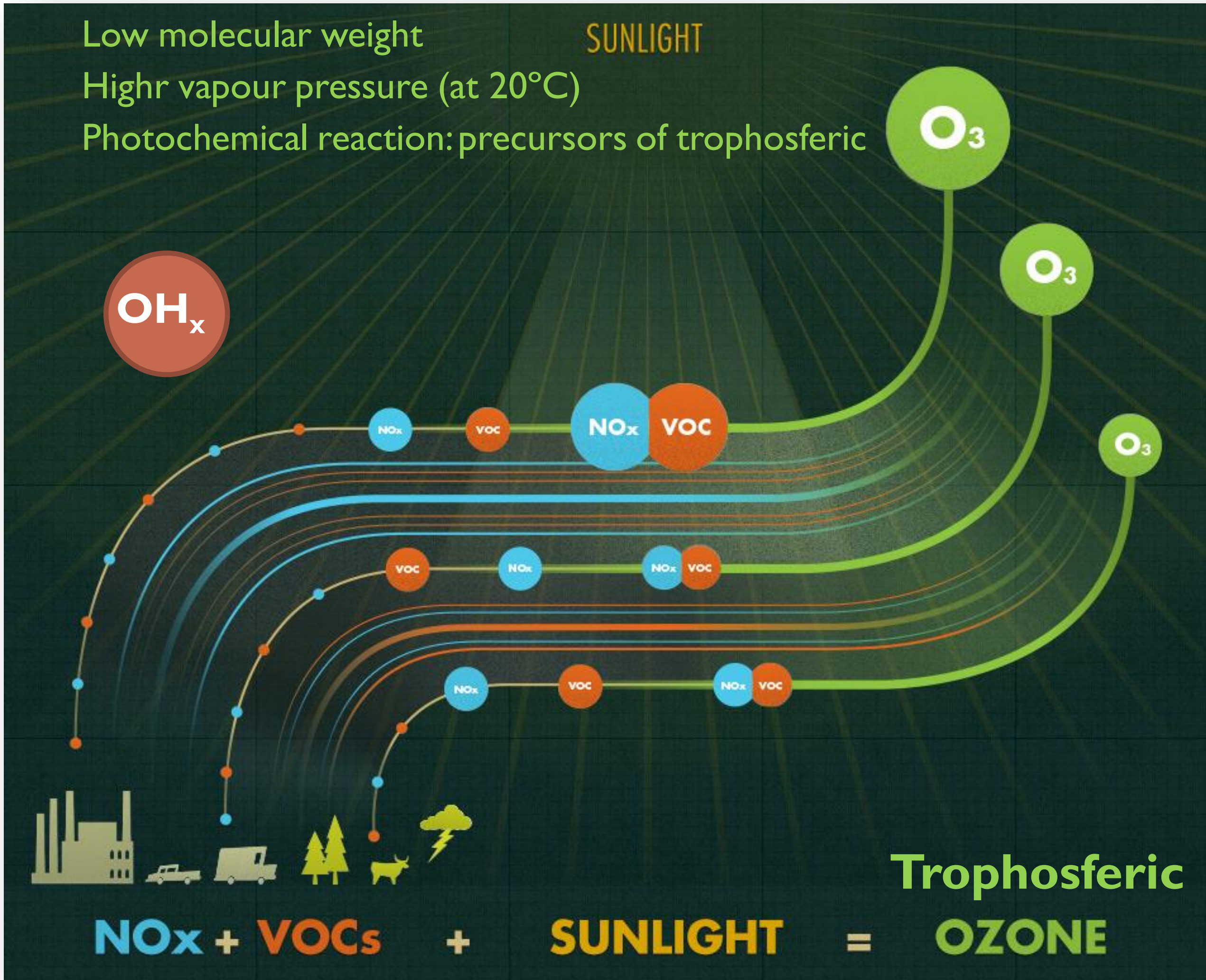
Objectives

- **Identify**
  - ✓ VOCs distribution among whole rice (*O. sativa* L. cv. Aríete) cycle growing phases under different treatments:
    1. soil textures (silty clay and loamy sand);
    2. increasing temperature;
    3. simultaneous temperature and CO_2 concentration enhancement.

- **Understand**
  - ✓ VOCs emission from rice field behaviour in climate change scenarios:
    1. increasing temperature;
    2. increasing CO_2 concentration.

Introduction

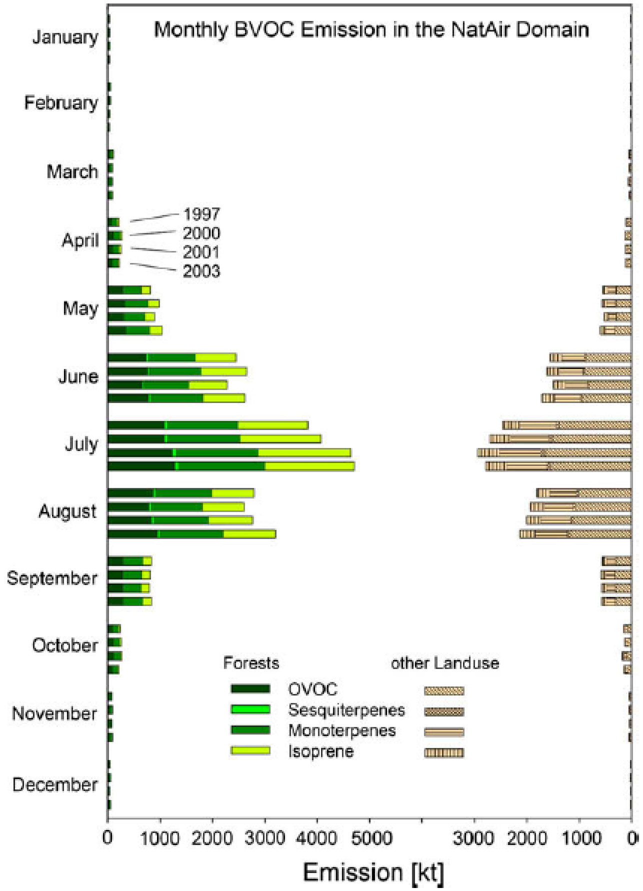
VOCs behavior on atmospheric chemistry



(Source: Sheng *et al.*, 2013)

Introduction

VOCs annual variation



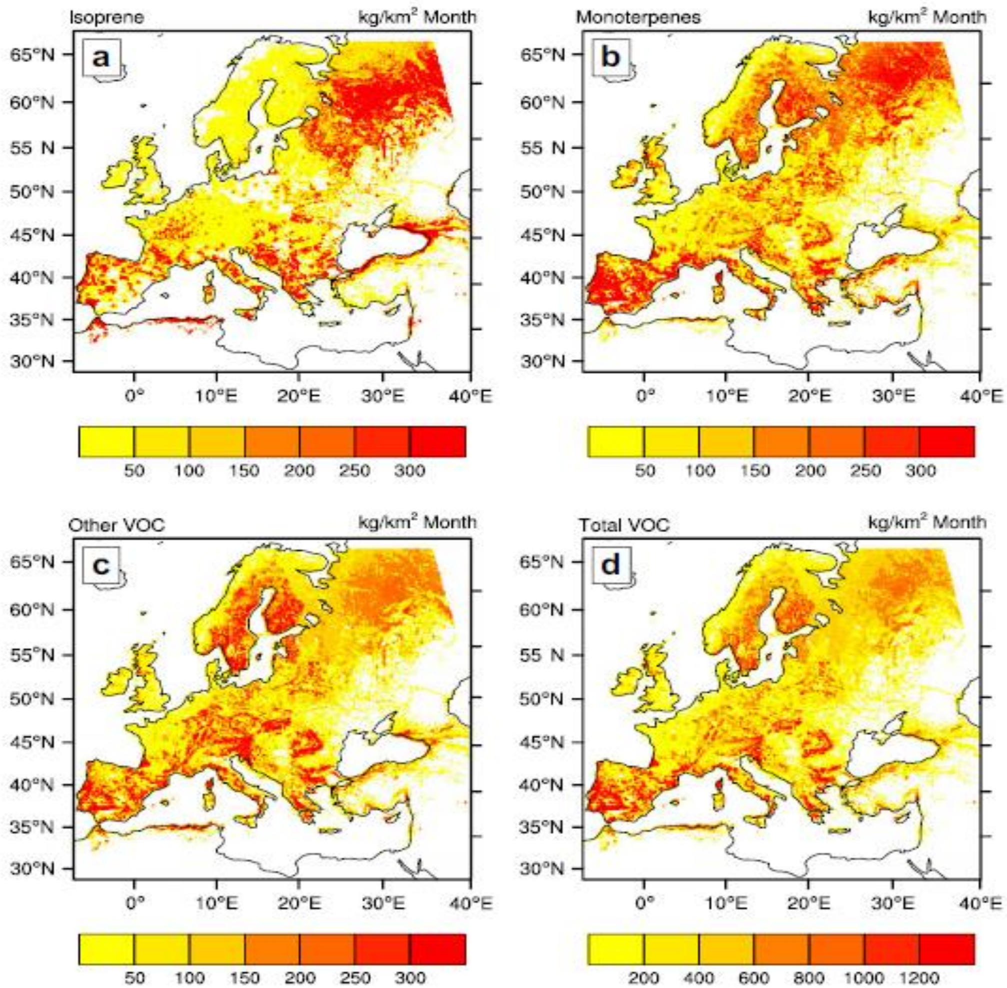
(Source: Steinberg *et al.*, 2009)

- **Emission dependence**
  - ✓ temperature (summer peak);
  - ✓ light (mid-day peak).
- **Synthesis dependence**
  - ✓ photosynthesis rate;
  - ✓ CO_2 (50% less).
- **Other factors**
  - ✓ N availability;
  - ✓ water availability;
  - ✓ O_3 exposure.

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Introduction

Europe scale

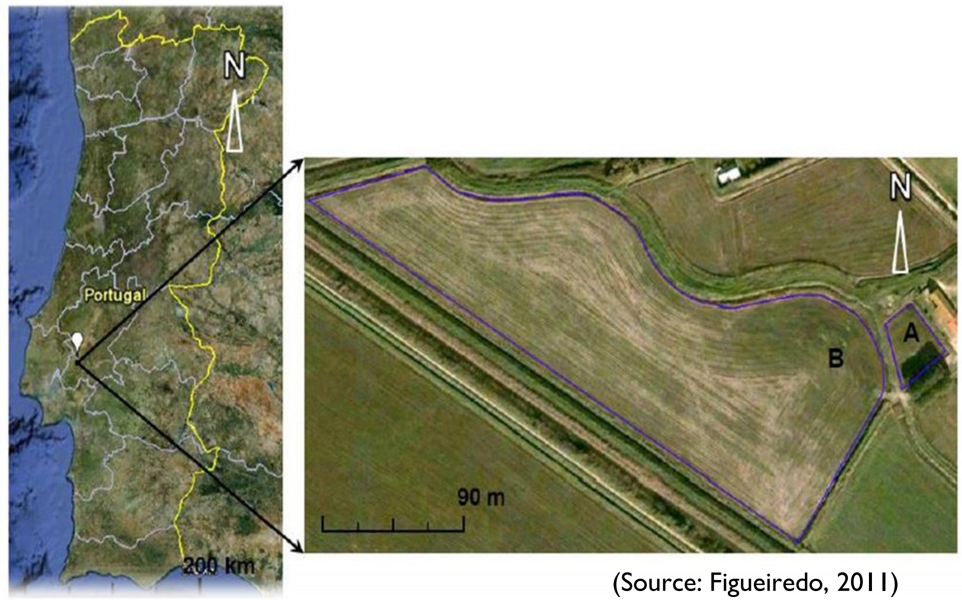


(Source: Steinbrecher *et al.*, 2009)

- **Portugal**, **Spain** and **Greece** (oaks, eucalyptus and aromatic plants);
- **Boreal forest** (taiga) coniferous forests (pines, spruces and larches);
- **Feedback interactions**
  - ✓ temperature;
  - ✓ CO_2;
  - ✓ biotic.

Materials and methods

COTArroz location



(Source: Figueiredo, 2011)

Materials and methods

Sampling layout

- TN – Open field soil plot A (loamy sand texture)
- TE – Open field soil plot B (silty clay texture)
- TE_C – Open Top Chamber with induced temperature
- TE_{CC} – Open Top Chamber with induced temperature and CO_2 concentration

Materials and methods

Sampling layout

Treatments	Rice cycle (days)	Sampling (days)	Average and standard deviation temperature (°C)	Average and standard deviation $[CO_2]$ (ppm)
TN and TE	158	6	20,1±2,1	375,4±38,5
TE_C		5	22,8±2,3	398,1±33,4
TE_{CC}		5	22,0±2,2	547,3±65,7

+ 1 sampling **+ 3 °C** **+ 172 ppm**

Sampling date	Rice cycle phase	Samples chosen for analyses
4th July	Vegetative	-
24th July	Vegetative	✓
16th August	Reproductive	✓
4th September	Ripening	-
19th September	Ripening	✓
26th September	Ripening	-

Materials and methods

VOCs extraction techniques

Solid phase micro extraction (SPME)

- Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber;
- Fiber conditioning into hot GC injection port at 250 ^{0}C for 20 min;
- 0,3 g of fresh cut rice leaves were placed in a 15 mL vial. The fiber was exposed to the vial headspace for 45 min at room temperature.

Materials and methods

VOCs extraction techniques

Steam distillation extraction (SDE)

- 7 g of fresh cut rice leaves were placed into 250 mL round bottomed flask with twice-distilled water;
- Solvent: diethyl ether – pentane 2:1 (v/v);
- 2h extraction;
- Extracts were stored at -20 ^{0}C;
- Extracts were concentrated to final volume of 1 mL for analysis.

Materials and methods

VOCs analysis method

Gas Chromatography coupled with Mass Spectrometry (GC/MS)

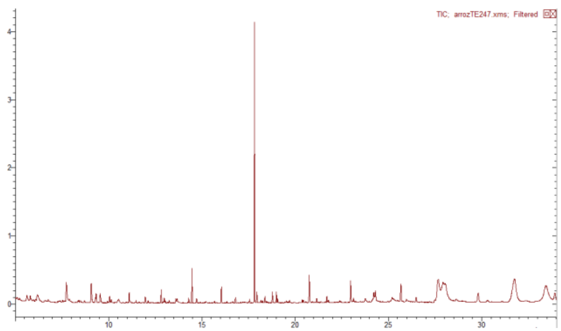
Two stationary phases with different polarities:

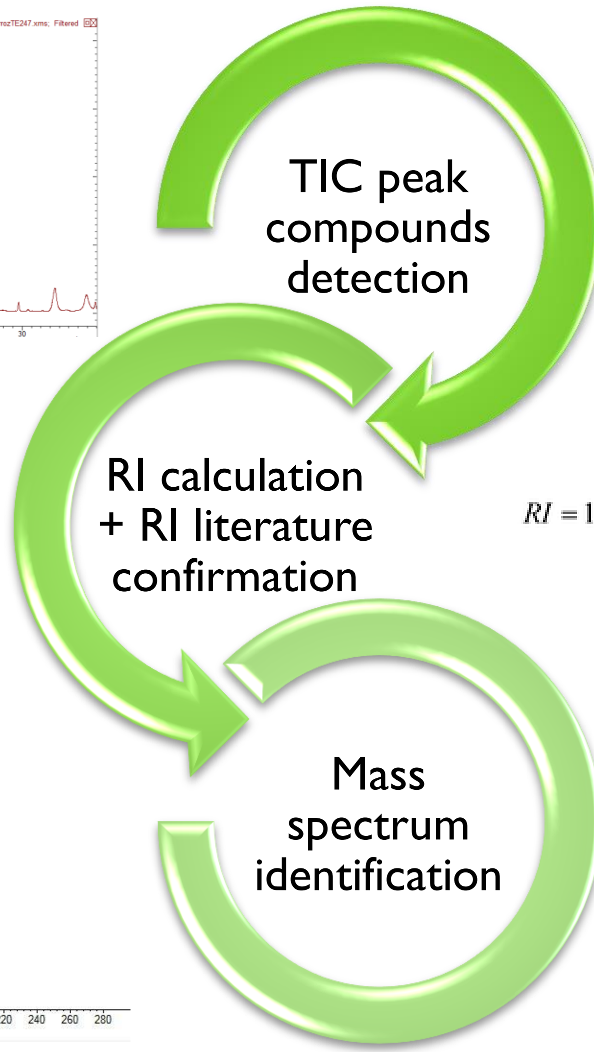
- (gas samples) SPME → DB-5 (non polar column)
- (liquid samples) SDE → DB-WAX (polar column)

GC programmed method	
Injection type	manual
Injection mode	splitless
Carrier gas (constant flow)	helium (1,2 mL/min)
$T_{injection}$	250 °C
T_{ramp}	4 °C/min
$T_{initial}$	40 °C
T_{final}	200 °C

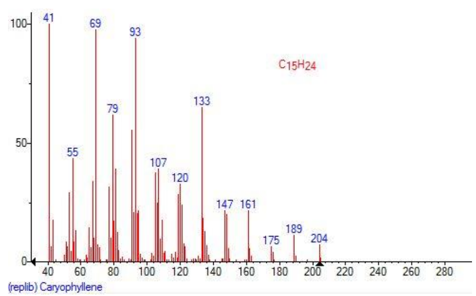
Materials and methods

VOCs identification process





$$RI = 100n + 100\left(\frac{t_x - t_n}{t_{n+1} - t_n}\right)$$



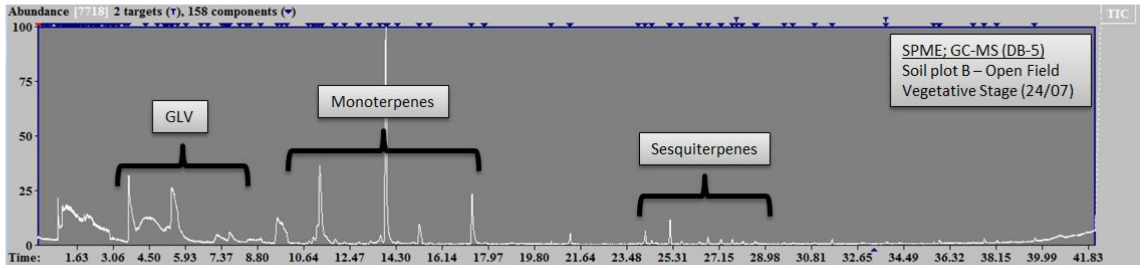
Results and discussion

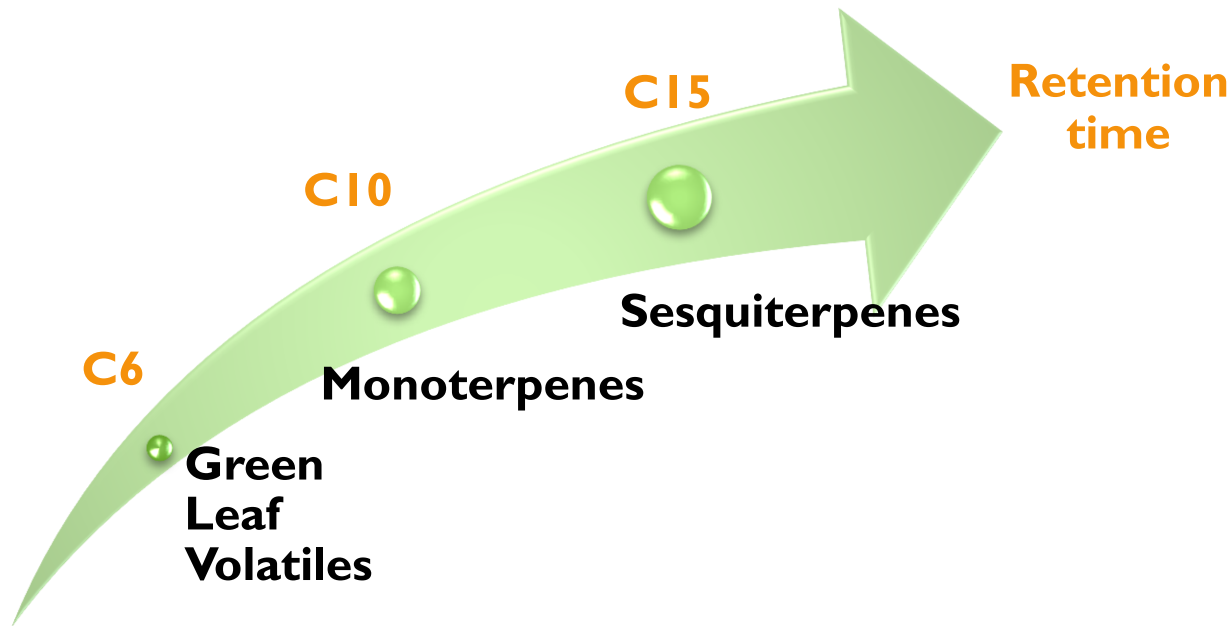
GC/MS DB-5

Group	Peaks	Chemical formula	RI $_{Lit}$	RI $_{Calc}$		Vegetative T_N	Vegetative T_E	Vegetative T_{ECC}	Vegetative T_{EC}		Reproductive T_N	Reproductive T_E	Reproductive T_{ECC}	Reproductive T_{EC}		Ripening T_N	Ripening T_E	Ripening T_{ECC}	Ripening T_{EC}
Green Leaf Volatiles	4-pentanal 2-methyl	$C_6H_{10}O$	776	784	.		X			.					.	X	X		
	3-hexenal	$C_6H_{10}O$	796	807	.	X	X		X	.	X	X		X	.				
	2-hexenal	$C_6H_{10}O$	850	854	.	X	X	X	X	.	X	X		X	.	X		X	X
	3-hexenol	$C_6H_{12}O$	853	868	.	X	X	X	X	.	X	X	X	X	.		X		X
	2,4-hexadienal	C_6H_8O	925	928	.	X	X	X		.	X	X		X	.	X	X		
Monoterpenes	α-pinene	$C_{10}H_{16}$	939	943	.	X	X		X	.					.		X	X	X
	myrcene	$C_{10}H_{16}$	989	1001	.					.					.				X
	phellandrene	$C_{10}H_{16}$	1004	1014	.				X	.			X		.				
	cymene	$C_{10}H_{16}$	1026	1032	.	X	X	X	X	.					.	X	X		X
	1-hexenol 2-ethyl	$C_8H_{18}O$	1028	1035	.		X		X	.		X		X	.	X	X		X
	limonene	$C_{10}H_{16}$	1035	1038	.	X	X	X	X	.			X	X	.	X	X		X
	1,8-cineol	$C_{10}H_{18}O$	1039	1047	.	X	X	X	X	.			X	X	.	X	X	X	X
	ocymene	$C_{10}H_{16}$	1050	1056	.		X			.					.				X
	α-terpinolene	$C_{10}H_{16}$	1089	1100	.	X			X	.					.				X
	benzoic acid	$C_8H_8O_2$	1102	1110	.	X	X	X		.			X		.			X	
	n-nonanal	$C_9H_{14}O$	1104	1111	.					.					.		X	X	
	linalool	$C_{10}H_{18}O$	1106	1117	.	X	X			.					.				
	2,6 nonadienal	$C_9H_{14}O$	1180	1191	.					.				X	.			X	
	methyl salicylate	$C_8H_8O_3$	1201	1207	.	X	X			.					.				
	n-decanal	$C_{10}H_{20}O$	1212	1221	.					.	X				.	X	X	X	
	β-cyclocitral	$C_{10}H_{16}O$	1219	1229	.					.					.			X	
Sesquiterpenes	coparene	$C_{15}H_{24}$	1386	1400	.	X				.					.				
	elemene	$C_{15}H_{24}$	1406	1418	.	X	X			.					.	X			X
	β-caryophyllene	$C_{15}H_{24}$	1425	1440	.	X	X			.					.				
	α-farnesene	$C_{15}H_{24}$	1430	1442	.	X	X			.		X			.				
	bergamotene	$C_{15}H_{24}$	1435	1450	.	X	X			.					.				
	humulene	$C_{15}H_{24}$	1455	1470	.		X			.					.				
	aromadendrene	$C_{15}H_{24}$	1462	1479	.	X				.	X				.				
	α-curcumene	$C_{15}H_{24}$	1481	1501	.		X			.					.				
	β-ionene	$C_{13}H_{18}O$	1493	1504	.					.					.	X	X	X	X
	ziginberene	$C_{15}H_{24}$	1500	1514	.	X	X			.					.				
	bisabolene	$C_{15}H_{24}$	1511	1527	.					.					.				X
	β-sesquipheladrene	$C_{15}H_{24}$	1523	1537	.	X	X			.					.	X	X		
	Total					20	22	7	10	.	6	6	5	8	.	11	12	9	13

Results and discussion

VOCs main zone classes





Results and discussion

DB-5 *vs* DB-WAX

- Column affinity
  - ✓ DB-5 **non polar**
  - ✓ DB-WAX **polar**
- Extraction methods
  - ✓ headspace **SPME (gas)**
  - ✓ solvent **SDE (liquid)**
- SDE samples
  - ✓ volume extraction
  - ✓ concentration
  - ✓ storage condition
  - ✓ samples number
- Results from SPME GC/MS using DB-5 non-polar column allowed the whole rice cycle study.

Peaks	Chemical formula	Columns DB-5	Columns DB-WAX
2-pentanol	$C_5H_{12}O$		X
4-pentanal 2-methyl	$C_6H_{10}O$	X	
2-hexanone	$C_6H_{12}O$		X
n-hexanal	$C_6H_{12}O$		X
3-hexenal	$C_6H_{10}O$	X	
2-hexenal	$C_6H_{10}O$	X	X
3-hexenol	$C_6H_{12}O$	X	X
2,4-hexadienal	C_6H_8O	X	
n-heptanal	$C_7H_{14}O$		X
n-heptenol	$C_7H_{14}O$		X
1,6 hexanediol	$C_6H_{14}O_2$		X
n-octanal	$C_8H_{16}O$		X
α-pinene	$C_{10}H_{16}$	X	
myrcene	$C_{10}H_{16}$	X	
phellandrene	$C_{10}H_{16}$	X	
cymene	$C_{10}H_{16}$	X	X
1-hexenol 2-ethyl	$C_8H_{18}O$	X	
limonene	$C_{10}H_{16}$	X	
1,8-cineol	$C_{10}H_{18}O$	X	
ocymene	$C_{10}H_{16}$	X	
α-terpinolene	$C_{10}H_{16}$	X	
benzoic acid	$C_8H_8O_2$	X	
n-nonanal	$C_9H_{14}O$	X	X
linalool	$C_{10}H_{18}O$	X	
1,3 nonadienol	$C_9H_{14}O$		X
2,6 nonadienal	$C_9H_{14}O$	X	X
methyl salicylate	$C_8H_8O_3$	X	
n-decanal	$C_{10}H_{20}O$	X	X
decanol	$C_{10}H_{20}O$		X
β-cyclocitral	$C_{10}H_{16}O$	X	X
coparene	$C_{15}H_{24}$	X	
elemene	$C_{15}H_{24}$	X	
β-caryophyllene	$C_{15}H_{24}$	X	X
α-farnesene	$C_{15}H_{24}$	X	
bergamotene	$C_{15}H_{24}$	X	
cetene	$C_{15}H_{24}$		X
humulene	$C_{15}H_{24}$	X	
aromadendrene	$C_{15}H_{24}$	X	X
α-curcumene	$C_{15}H_{24}$	X	X
β-ionene	$C_{13}H_{18}O$	X	X
ziginberene	$C_{15}H_{24}$	X	
bisabolene	$C_{15}H_{24}$	X	
β-sesquipheladrene	$C_{15}H_{24}$	X	
2-pentadecanone	$C_{18}H_{36}O$		X
Total		33	22

GLV

Monoterpenes

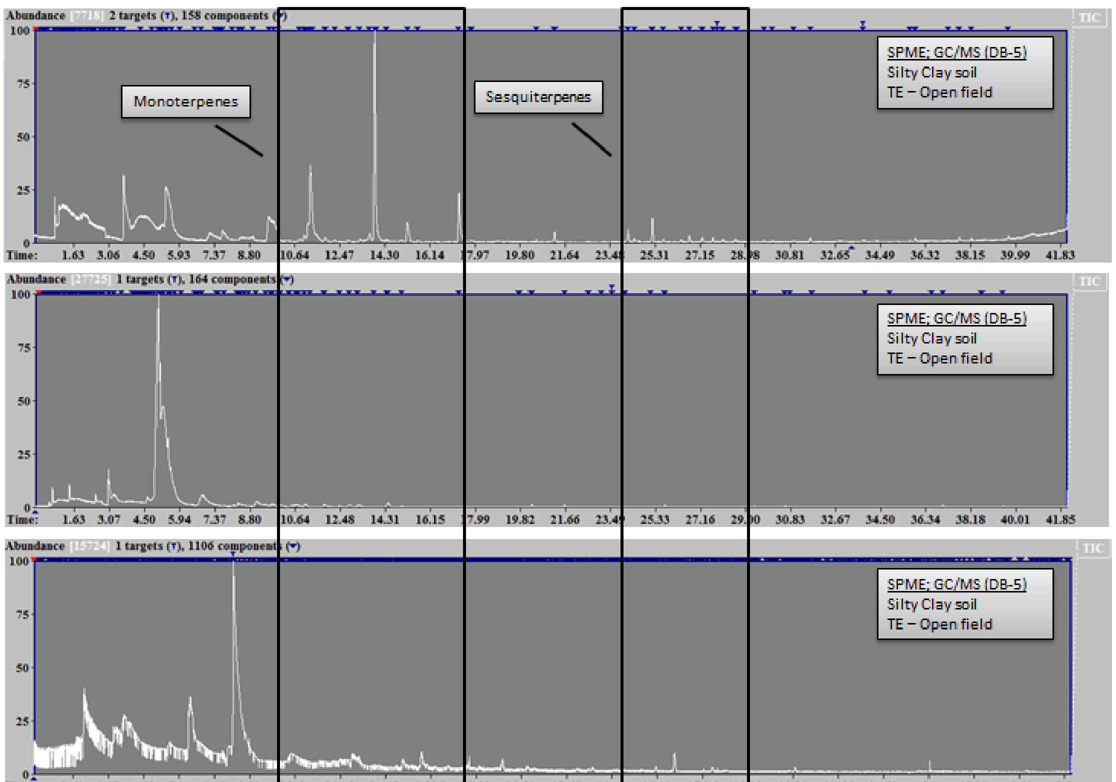
Sesquiterpenes

Results and discussion

Rice cycle stages (same T°, CO_2 and soil texture)

- More VOCs released at **vegetative stage**
  - ✓ greater rice plant activity on early developing stages;
  - ✓ same trend in 2011 rice cycle.

Vegetative					Reproductive					Ripening			
T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
20	22	7	10	.	6	6	5	8	.	11	12	9	13



Vegetative

Reproductive

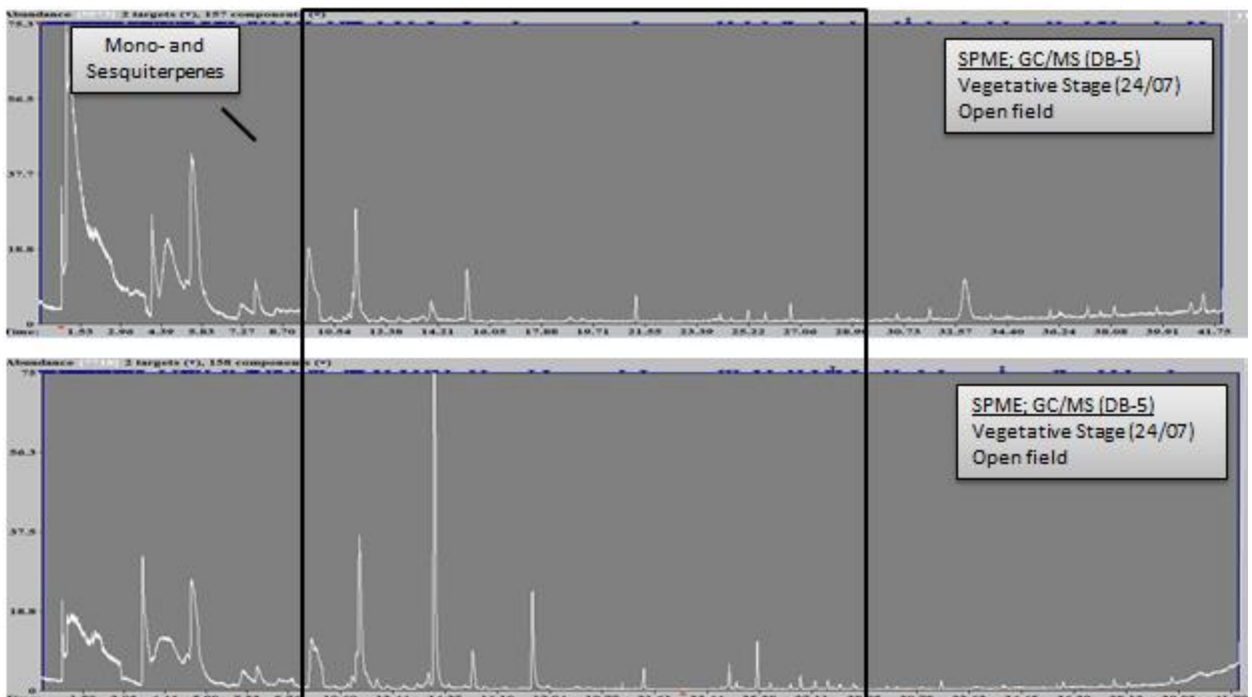
Ripening

Results and discussion

Soil textures (same T°, CO_2 and cycle phase)

- More VOCs released from **silty clay soil** texture at vegetative and ripening stages
  - ✓ soil proprieties and other factors

Vegetative					Reproductive					Ripening			
T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
20	22	7	10	.	6	6	5	8	.	11	12	9	13



Loamy sand

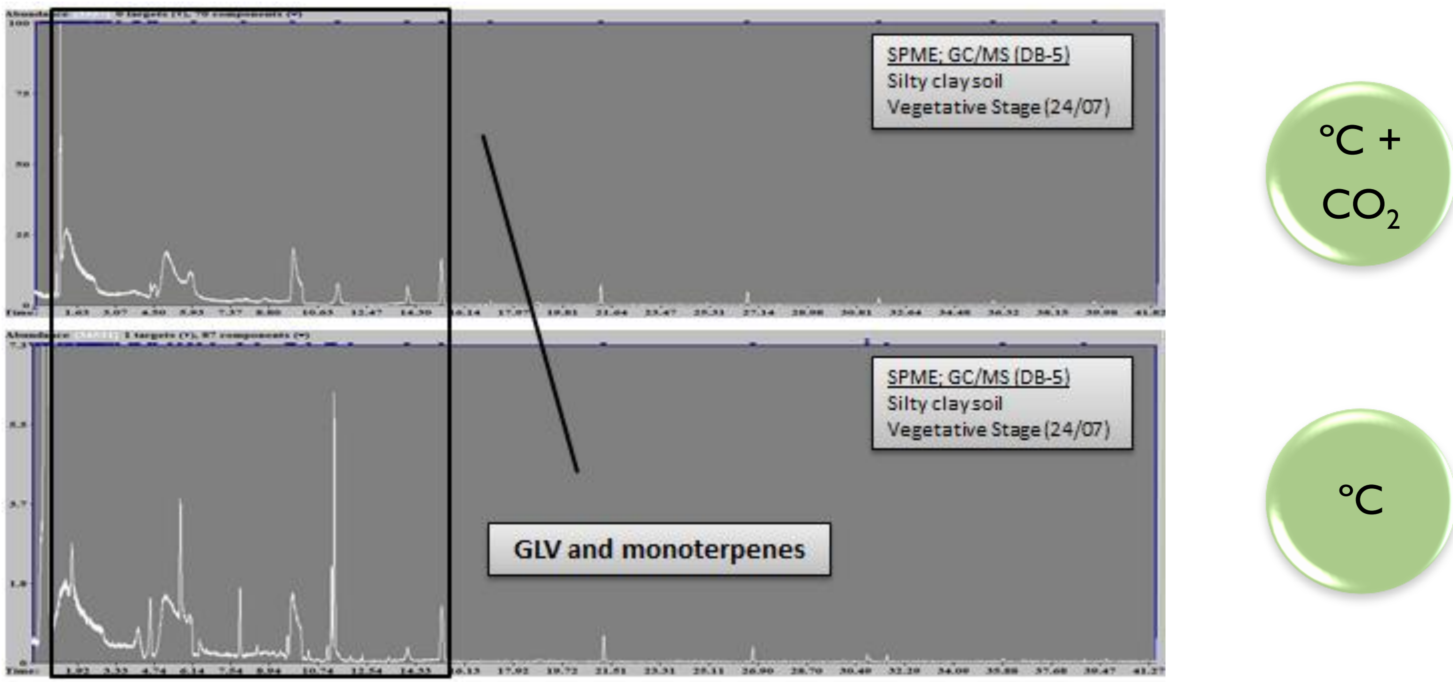
Silty clay

Results and discussion

Temperature and CO_2 (same cycle stage and soil textures)

- More VOCs released under **higher temperature** and **less** under simultaneous temperature and **CO_2** enhancement
  - ✓ thermotolerance protection function
  - ✓ photosynthesis rate changes

Vegetative					Reproductive					Ripening			
T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
20	22	7	10	.	6	6	5	8	.	11	12	9	13



For better peaks comparison this figure presents differences on chromatogram scales.

Results and discussion

Student's t-test

One sample t-test mean (Confidence Interval)

- Small population size *n*=3; data follows normal distribution; standard deviation is unknown and is replaced by an estimation parameter.
  - ✓ At 95% of confidence in each stage, at least one treatment do not belong to CI.
  - ✓ At 99% of confidence alltreatments in all stages are within the CI.

Vegetative					Reproductive					Ripening			
T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
20	22	7	10	.	6	6	5	8	.	11	12	9	13

95%

Rice cycle phases	TN	TE	TE_C	TE_{CC}
Vegetative	$CI_{\mu 1}$ = 11,3±14,4	$CI_{\mu 4}$ = 12,7±9,56	$CI_{\mu 7}$ = 5,67±4,19	$CI_{\mu 10}$ = 3,00±2,03
Reproductive	$CI_{\mu 2}$ = 2,67±3,67	$CI_{\mu 5}$ = 3,67±3,08	$CI_{\mu 8}$ = 5,00±2,33	$CI_{\mu 11}$ = 4,03±2,36
Ripening	$CI_{\mu 3}$ = 6,67±1,17	$CI_{\mu 6}$ = 5,00±6,08	$CI_{\mu 9}$ = 8,67±6,51	$CI_{\mu 12}$ = 6,00±7,30

99%

Rice cycle phases	TN	TE	TE_C	TE_{CC}
Vegetative	$CI_{\mu 1}$ = 11,3±33,2	$CI_{\mu 4}$ = 12,7±19,3	$CI_{\mu 7}$ = 5,67±9,68	$CI_{\mu 10}$ = 3,00±4,70
Reproductive	$CI_{\mu 2}$ = 2,67±5,38	$CI_{\mu 5}$ = 3,67±10,1	$CI_{\mu 8}$ = 5,00±9,36	$CI_{\mu 11}$ = 4,03±5,38
Ripening	$CI_{\mu 3}$ = 6,67±2,69	$CI_{\mu 6}$ = 5,00±14,0	$CI_{\mu 9}$ = 8,67±15,0	$CI_{\mu 12}$ = 6,00±16.8

Results and discussion

Student's t-test

Independent two sample t-test (Difference between means)

- Both samples size *n*=3; data follows normal distribution; both distributions have same variance;
  - ✓ At 99% and 95% confidence no difference between treartments means
  - ✓ At 90% of confidence, at vegetative stage, silty clay open field treatment has diference from both open top chambers

$T_{tab99\%}=4,60$ **$T_{tab95\%}=2,78$** **$T_{tab90\%}=2,13$**

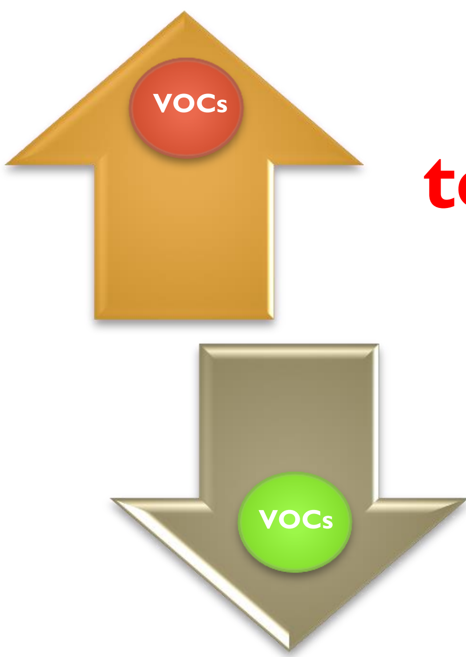
Vegetative					Reproductive					Ripening			
T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}		T_N	T_E	T_{ECC}	T_{EC}
20	22	7	10	.	6	6	5	8	.	11	12	9	13

Rice cycle phases	TN and TE	TE_C and TE_{CC}	TN and TE_{CC}	TN and TE_C	TE and TE_{CC}	TE and TE_C
Vegetative	$T_{calc1}=0,28$	$T_{calc4}=2,01$	$T_{calc7}=2,00$	$T_{calc10}=1,32$	$T_{calc13}=2,51$	$T_{calc16}=2,35$
Reproductive	$T_{calc2}=0,90$	$T_{calc5}=0,52$	$T_{calc8}=1,73$	$T_{calc11}=1,20$	$T_{calc14}=0,57$	$T_{calc17}=1,75$
Ripening	$T_{calc3}=0,94$	$T_{calc6}=2,00$	$T_{calc9}=0,32$	$T_{calc12}=1,07$	$T_{calc15}=0,37$	$T_{calc18}=1,07$

Results and discussion

Rice VOCs behavior under climate change scenarios

- ✓ Vestigial emission from rice plant, however the great rice area distribution cause a potential impact on air chemistry.
- ✓ According to the data on actual experimental conditions, higher temperature may increased VOCs emission and CO_2 may caused the reverse.



Higher temperature

Higher CO_2 concentration

Conclusions I

Based on experimental data observations

Rice VOCs emission	Vegetative stage released more emission. (H: Greater activity in earlier plant stages?)
	Silty clay soil texture produced more emissions than loamy sand. (H: Influence of other factors?)
	Temperature increased emission. (H: Thermotolerance VOCs function?)
	CO_2 concentration reduced emission. (H: Changes on photosynthesis rate?)

Conclusions II

Climate change scenario prediction: rise temperature and CO_2 atmospheric concentration

Rice VOCs emission	
	Higher temperature may increase emission
	Higher CO_2 concentration may reduce emission
	Data suggests that temperature and CO_2 have influence on qualitative emission, supporting further studies on the topic

Further research

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

- **Influence of other abiotic stress factors**
  - ✓ VOCs emission on *O. sativa* L. cycle, such as:
    1. photoperiod;
    2. soil moisture;
    3. O_3 exposure;
    4. N availability;
    5. water availability.

- **Identify**
  - ✓ VOCs profile among the other rice cycle varieties.

- **Emergent research field**
  - ✓ Study of VOCs from plant-plant and insect-plant interactions

Akcnowledgments

- To Ms. Nuno Figueiredo for field support and for informative academic material;
- To Dr. Corina Carranca for conferences invitation and for sharing information about field data;
- To other project partners from COTArroz for the great reception and premission for plant material collection.

Thank you!