Impact of interacting abiotic factors on targeted metabolomic profiles in stored maize inoculated with *Aspergillus flavus*

Esther GARCIA-CELA[1], Benjamin INGRAM[2], Michael SULYOK[3], Elisavet KIAITSI[1], Carol VERHEECKE-VAESSEN[1], Angel MEDINA[1], Rudolf KRSKA[3], Naresh MAGAN[1]*

[1]Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, Beds. MK43 0AL, UK. [2]Facultad de Ingeniería, Universidad de Talca, Chile. [3]Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Konrad Lorenz str. 20, A-3430 Tulln, Austria. M.E.Garcia-Cela@cranfield.ac.uk

Introduction

- Maize is often harvested at moisture contents (m.c. =17-19% ~ 0.80-0.90 water activity, a_w) which are conducive to mould growth which can allow *Aspergillus* and other mycotoxigenic fungi to colonize and contaminate the grain with mycotoxins.
- The most important environmental factors which influence the ability for the naturally contaminated mycobiota to colonise the maize post-harvest are Temperature (T) and a_w.
- Fungi such as *A.flavus* produce a battery of secondary metabolites (SMs) in addition to the key toxigenic ones for which legislation exists including aflatoxins (Afs) and cyclopiazonic acid (CPA) and related compounds.

Aim

To explore the effect of a_w (0.80–0.99) x temperature (15–35°C) on SM production on naturally contaminated stored maize and that inoculated with *A.flavus*.

Methodology

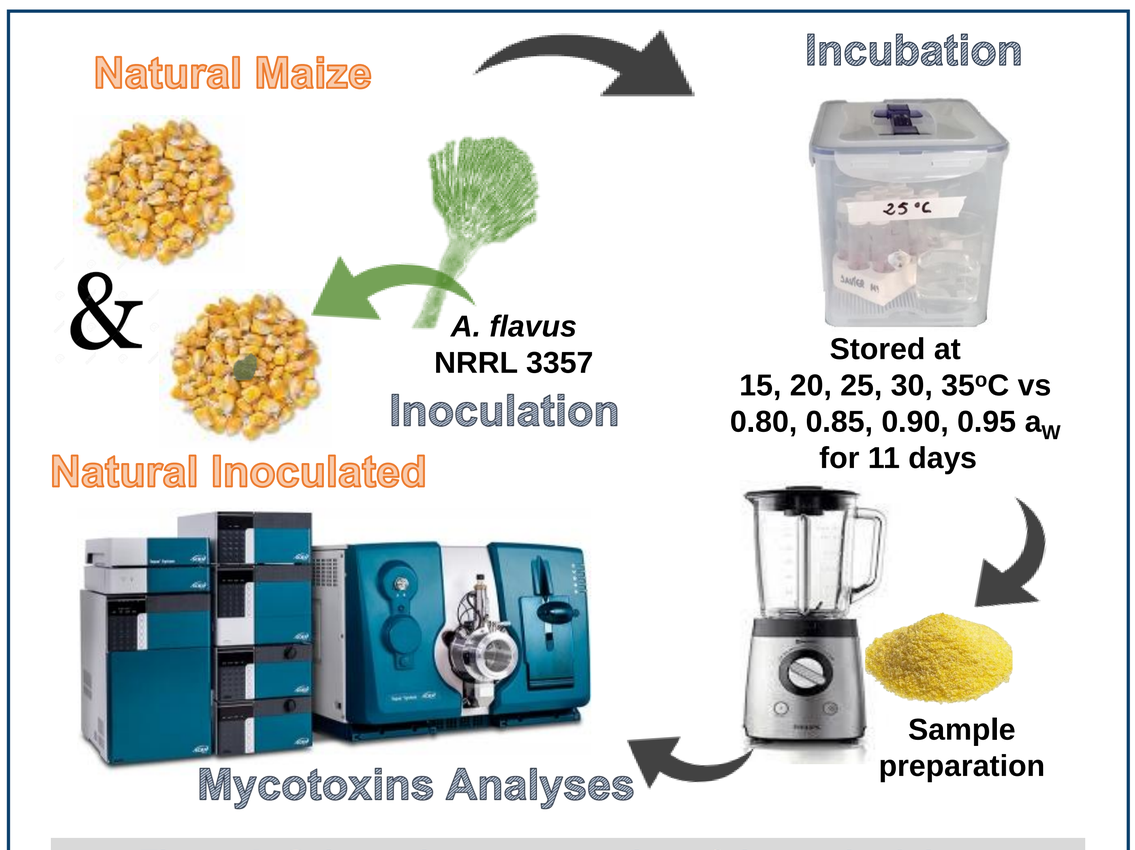

Figure 1: Schematic representation of the methodology.

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Key findings

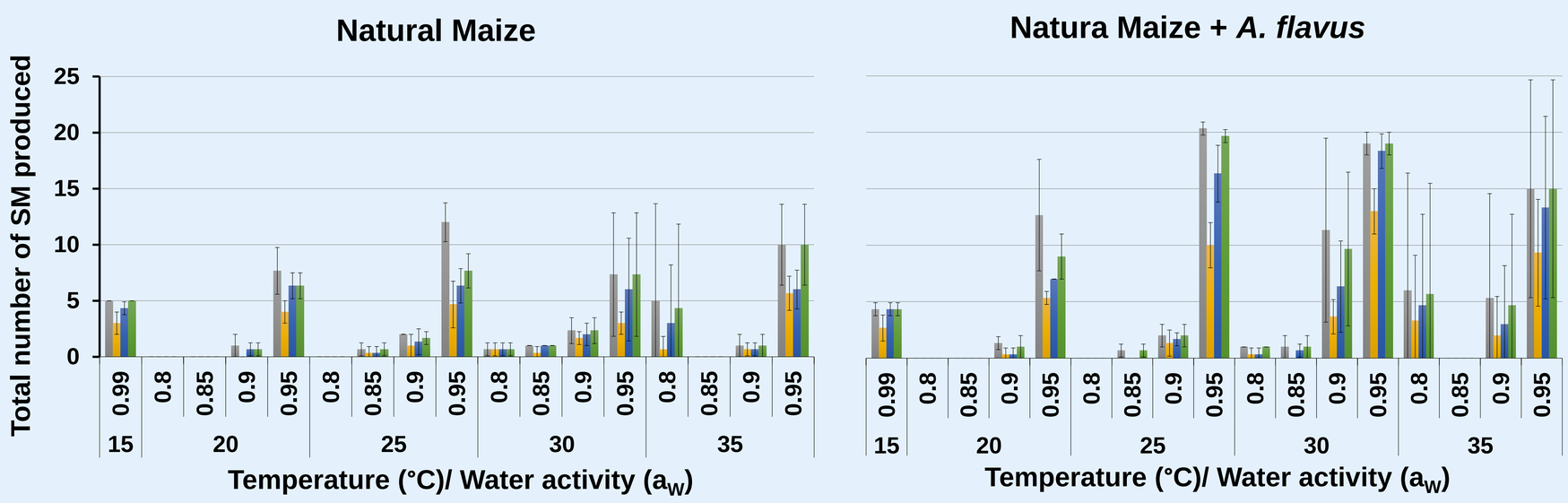

Figure 2 Number of SM (out of 27) produced in the two stored maize kernels under different interacting T x a_w conditions for 11 days. Data are for means ± SE

Table 1: Production of SMs in the stored maize kernels.

		Natural maize																	Natural maize + *A. flavus*																
	T(°C)	15	20				25				30				35				15	20				25				30				35			
	A_w	0.99	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95	0.99	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95	0.80	0.85	0.90	0.95
Secondary Metabolites	1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	30.5	13.5	< LOD	1.7	< LOD	1717.2	19732.1	< LOD	< LOD	559.2	< LOD	< LOD	< LOD	< LOD	114.1	222.3	< LOD	< LOD	20196.8	< LOD	50893.1	24980.6	88661.5	86590.8	< LOD	16227.1	22382.8
	2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.7	< LOD	< LOD	< LOD	< LOD	48.5	216.9	< LOD	< LOD	22.5	< LOD	< LOD	< LOD	< LOD	12	12	< LOD	< LOD	1291	< LOD	2822.8	786.5	5725	3012.9	< LOD	515.1	1354.6
	3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	45.5	80.6	< LOD	< LOD	18.3	< LOD	< LOD	< LOD	< LOD	3.6	3.6	< LOD	< LOD	347.8	< LOD	750.2	190.1	1771.4	1007.1	< LOD	173.1	422.1
	5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	7.9	< LOD	12.3	4.1	72.4	7.1	< LOD	1.7	57.7
	6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	143.9	40	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	113.6	< LOD	219.7	15.7	1545.9	44.3	< LOD	30.9	472
	7	< LOD	< LOD	< LOD	< LOD	0.3	< LOD	< LOD	< LOD	1.1	< LOD	0.7	< LOD	18.4	1.1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.2	0.2	< LOD	< LOD	32.7	< LOD	90	1.8	111.1	22.2	< LOD	3.6	9.3
	8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.5	< LOD	< LOD	< LOD	54.7	5.9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.4	1.9	< LOD	< LOD	140.7	< LOD	383.6	9.4	625.5	83.8	< LOD	22.9	61.4
	9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.2	< LOD	0.2	< LOD	39.3	10.5	< LOD	< LOD	27.3	< LOD	< LOD	< LOD	< LOD	1.3	< LOD	< LOD	< LOD	299.6	< LOD	802.8	12.9	297.7	185.5	< LOD	46.8	58.5
	10	< LOD	< LOD	< LOD	< LOD	1.1	< LOD	< LOD	< LOD	3.9	< LOD	2.2	< LOD	116	79.2	< LOD	< LOD	28.7	< LOD	< LOD	< LOD	< LOD	7.5	10.6	< LOD	< LOD	357.1	< LOD	773.3	82.9	1184.8	1104.8	< LOD	290.5	588.8
	11	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4.1	< LOD	2.6	< LOD	108.2	15.1	< LOD	< LOD	25.1	< LOD	< LOD	< LOD	< LOD	4.3	3.6	< LOD	< LOD	150.9	< LOD	369.4	11.8	536.2	286.4	< LOD	36	267.1
	12	< LOD	< LOD	< LOD	< LOD	2.4	< LOD	< LOD	< LOD	5.7	< LOD	2	< LOD	158.7	277	< LOD	< LOD	68.3	< LOD	< LOD	< LOD	< LOD	11.3	18.2	< LOD	< LOD	440.7	< LOD	1011.1	292.4	2210	4483.1	< LOD	772.8	1129.2
	13	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.3	< LOD	0.3	< LOD	95.8	< LOD	< LOD	< LOD	47.9	< LOD	< LOD	< LOD	< LOD	1	1.2	< LOD	< LOD	64	< LOD	159.3	5.2	681.7	77.9	< LOD	16.9	476.7
	14	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3	< LOD	3	< LOD	< LOD	61.4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	7.1	9.7	< LOD	< LOD	208.7	< LOD	416.6	37	323	1122.6	< LOD	219.5	105.3
	15	116.9	< LOD	< LOD	209.4	199.6	< LOD	19.5	1823.1	142174.7	608.1	13536.5	20854.9	3057523	47332.3	< LOD	25061.8	3335509.7	155.9	< LOD	< LOD	1376.1	35421.8	13516.8	20.4	1030.4	1352856.8	202.7	934218.1	30531.2	5026014.5	120771.6	< LOD	76389.8	2056693.2
	16	7652.9	< LOD	< LOD	158.6	499	< LOD	< LOD	< LOD	264	< LOD	182.9	56.4	13279.1	118.1	< LOD	30.1	14677.4	10104.2	< LOD	< LOD	17.2	1889.1	1993.3	< LOD	6.4	16590.7	< LOD	26970.5	1192.1	59761	7257.8	< LOD	517.1	9010.8
	17	177.1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	137.7	552	344.8	< LOD	< LOD	< LOD	< LOD	< LOD	9244.8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4022.6	< LOD	10058	138.2	12747.2	< LOD	< LOD	< LOD	7016
	18	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	152.4	1212.1	< LOD	< LOD	< LOD	< LOD	389.9	< LOD	< LOD	70155.5	219.5	< LOD	< LOD	< LOD	665.4	449.7	< LOD	< LOD	13111.3	< LOD	31123.8	< LOD	90864.4	8041.3	< LOD	1190.2	135235.2
	19	327.5	< LOD	< LOD	0.5	244.2	< LOD	< LOD	< LOD	490.2	< LOD	355.7	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	423.5	< LOD	< LOD	0.9	185.5	213.4	< LOD	< LOD	703.7	< LOD	292.7	< LOD	< LOD	0.3	< LOD	< LOD	1043.9

Secondary Metabolites: 1) Aflatoxin B_1, 2) Aflatoxin B_2, 3) Aflatoxin G_1, 4) Aflatoxin M_1, 5) Aflatoxin P_1, 6) Aflatoxicol, 7) Sterigmatocystin, 8) O-Methylsterigmatocystin, 9) Averantin, 10) Averufin, 11) Versicolorin A, 12) Versicolorin C, 13) Nidurufin, 14) Norsolorinic acid, 15) Kojic acid, 16) 3-Nitropropionic acid, 17) Cyclopiazonic acid, 18) Asperfuran qnd 19) Aspinolid B.
Red indicates maximum production levels (ng/g) while green and shades of yellow/orange represents intermediate production levels. Shading is per column. LOD: Limit of Detection.

Conclusions

- Numbers of SMs increased with increasing water availability at 25°C, with more SMs present in both naturally contaminated maize and that inoculated with *A.flavus*.
- The maximum number of SMs were observed at 25°C although highest concentrations were found at 30°C (significant shift from 25 to 30°C).
- Higher SMs concentrations were produced under water stress conditions in the range of 25-35°C.
- Consumption by consumers of poor quality stored maize may result in exposure to toxigenic mycotoxins and well as a mixture of other SMs which may impact on health.