Monitoring of CO_2 levels in stored wheat grains for early detection of *Fusarium graminearum* colonization and zearalenone (ZON) and derivatives accumulation

Esther Garcia-Cela[1], Elisavet Kiaitsi[1], Michael Sulyok[2], Angel Medina[1], Naresh Magan[1]

[1]Applied Mycology Group, Environment and AgriFood Theme, Cranfield University, Cranfield, Beds. MK43 AL5, U.K.
[2]Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad-Lorenz-Str. 20, Tulln 3430, Austria.

Introduction

- Cereals are commonly colonised by *Fusarium* species pre-harvest. Subsequently, damp harvesting conditions, inefficient drying and poor storage can result in increased contamination with mycotoxins such as zearalenone (ZON). Indeed, legislative limits exist for ZON in cereals for food and feed in Europe.
- Effective monitoring of grain respiration activity (CO_2) during storage could be used as a sensitive early indicator of the activity of mycotoxigenic moulds. This could be combined with measurement of Temperature (T) and Relative Humidity (RH) sensors for early and real time detection of the activity of moulds

Objectives

The aim of this study was to develop data sets for the development of a post-harvest real time Decision Support System (DSS) based on T, RH and CO_2 measurements for better post-harvest management of stored wheat by establishing:

(a) the boundary conditions for growth/ZON production and
(b) the relationship between dry matter loss (DML) and ZON accumulation due to colonisation by *F. graminearum.*

Methodology and Results

1) Fungal growth in wheat media agar

- Central inoculation of 5µL (10^5spores/mL) of *F. graminearum* Fg08.111.isolated from wheat on 2% milled wheat media.
- Incubation in the dark at water activity (0.88-0.995a_W) x T(6-35°C) for 30 days.
- Fungal colony measured daily during 30 days.

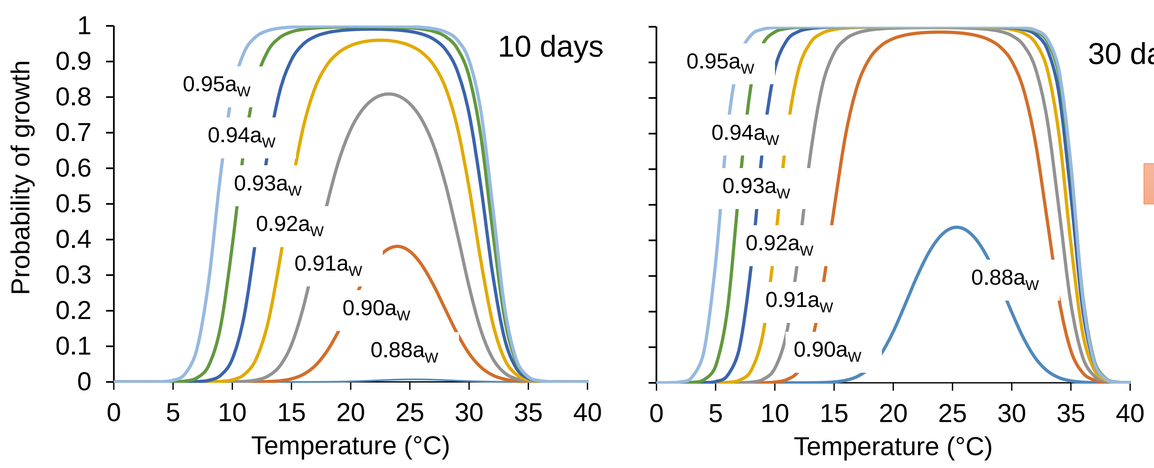


Fig. 1 Predicted effect of T and a_w on probability of *F. graminearum* growth

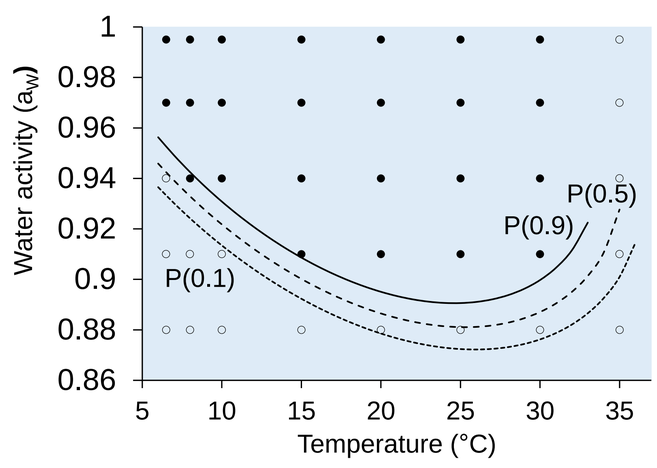


Fig. 2 The predicted growth /no growth boundaries for one month

Probability (P) of growth increase with the time. For a one month storage period, the probability of growth was always under 0.50 when water availability was under 0.88a_W. The model predicted correctly 95.84% of the cases, with 3.97% false positives and 4.37% false negatives (cut off=0.5)

$$Logit\ P = In\left(\frac{P}{1-P}\right) = b_0\pi + b_1 a_w + b_2 T + b_{11} a_w^2 + b_{22} T^2 + b_{12} a_w \mathrm{T} + \mathrm{time}$$

2) CO_2 production in wheat

- Inoculation with 4 plugs (0.5cm Ø) in 10 g of wheat.
- Incubated in the dark at a_w (0.90-0.95) x T (10-25°C), 15 days.
- One hour of respiration rate (mg CO_2 kg^{-1} h^{-1}) measured every 2 days by means of GC-TCD.
- Dry Matter Losses (DML) data determined from the respiration rate measurements.

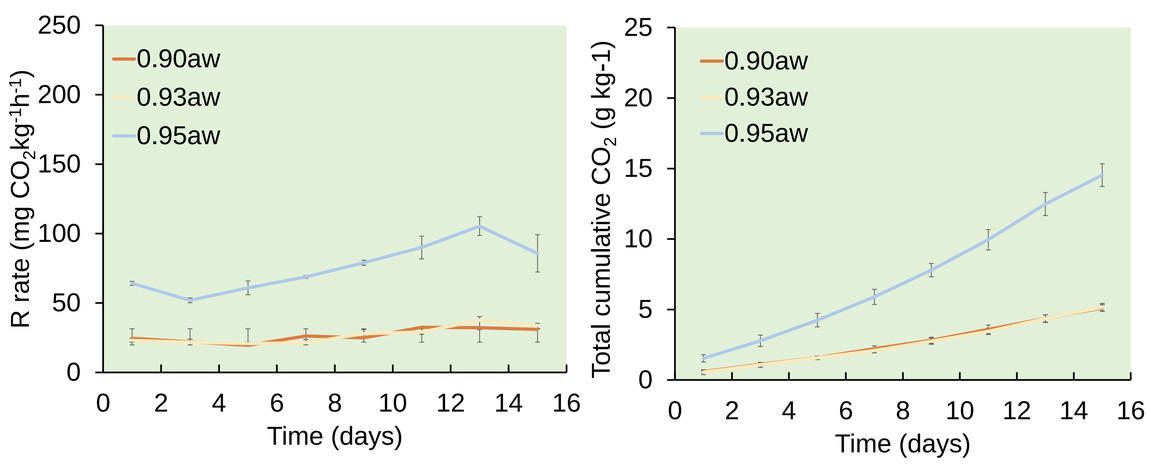


Fig. 3 Temporal and accumulated respiration rate of *F. graminearum* on natural wheat of different a_W levels at 20°C

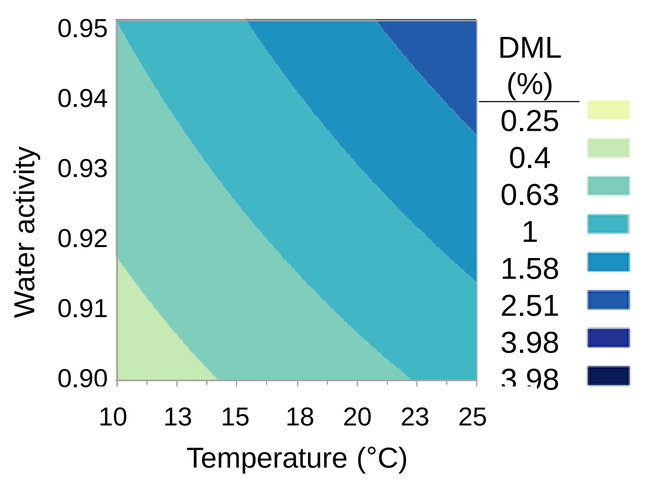


Fig. 4 Contour maps describing the Dry Matter Losses (DMLs) in natural grain + *F. graminearum*

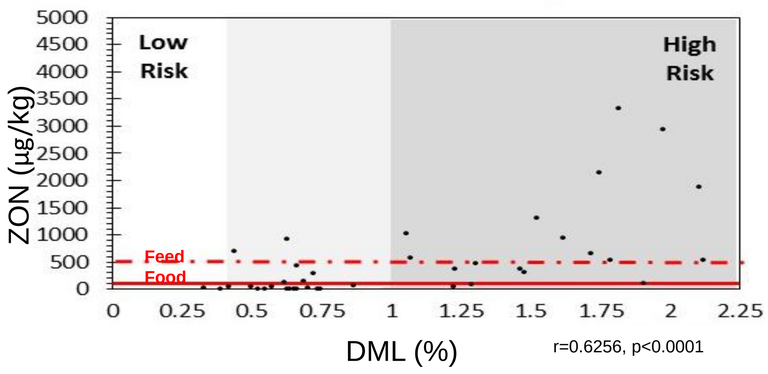
DMLs increased in the same environmental combination in natural wheat due to *F. graminearum* colonization. DMLs > 1% occurred with T > 14 °C in natural wheat, however; the same DMLs in the presence of *F. graminearum* occurred at lower Ts (10 °C/0.95 a_w).

3) Toxin production in wheat

Mycotoxin analysed by LC-MS/MS at day 15.

Table 1.ZON and its metabolites mean of 4 replicates (µk/kg)

T(°C)	a_w	ZON	alpha-Zearalenol	βeta-Zearalenol
10	0.9	0.06	0.40	0.40
10	0.93	6.43	0.40	0.40
10	0.95	0.77	0.40	0.40
15	0.9	195.87	0.95	6.18
15	0.93	551.80	1.17	7.44
15	0.95	241.88	1.65	8.15
20	0.9	110.87	0.40	1.10
20	0.93	11.42	0.40	0.40
20	0.95	810.24	6.94	35.69
25	0.9	382.60	2.42	16.41
25	0.93	1489.72	13.26	81.52
25	0.95	1461.44	11.40	78.25



DML>0.5% and >1.5% represent a medium and high level of ZON risk relative the EU limit.

Fig. 5. Relationship between Dry matter Loss and relative ZON production and associated level of risk. The lines indicate the legislative limits

Conclusions

- **Fungal growth and CO_2 production information could be used to predict the risk of mycotoxin contamination.**
- **This could be developed into a real time Decision Support System**

M.E.Garcia-Cela@Cranfield.ac.uk

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