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Establishment of a Taqman-based approach to monitor *Fusarium* spp. airborne spores

IRRIGATED AGRICULTURE

H. Cardoso[1], M.D. Campos[1], B. Tomero[1], M.R. Felix[1], I. Velada[1], A. Peixe[1], C. Penedos[2], C. Nunes.[2,3], M. Morais-Almeida[2,4], E. Caeiro[1,2]

1- ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Formação e Investigação Avançada, Universidade de Évora. Pólo da Mitra, Ap. 94, 7006-554 Évora.
2- SPAIC – Sociedade Portuguesa de Alergologia e Imunologia Clínica, Lisboa
3- Centro de Imunoalergologia do Algarve, Portimão
4- Centro de Alergia, Hospitais CUF-Descobertas e CUF Infante Santo, Lisboa

INTRODUCTION

Aerobiological studies provide important information about the biological particles present in the air. Monitoring the presence of airborne fungal spores can help farmers to prevent the onset of fungal diseases that may affect both quantity and quality of crops. The fungi *Fusarium* spp. are among the most important phytopathogenic fungal communities with high impact at regional level by affecting important cultures such as almond, tomato, maize and cereals. The establishment of an approach that would enable farmers to early react upon the possibility of a *Fusarium* spp. infection will for sure lead to a better control of the diseases associated to those fungi. Currently, to monitor *Fusarium* spp. airborne spores it is followed the Hirst-type methodology, which is based on spore's identification and quantification by optical microscope; a hard and time consuming process due to the spore's small size and colorless wall (Fig. 1). In this context, the development of an alternative methodology that enable to get accurate and reliable results in a faster way will be for sure of high interest. A Taqman specific assay for *Fusarium* spp. detection and quantification was previously established for a different purpose [1] and was here applied as a molecular-based tool to detect *Fusarium* spp. spores in the airborn.

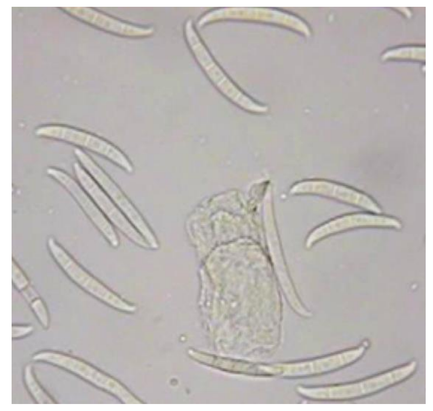

Fig. 1: *Fusarium* sp. spores observed at optical microscope.

METHODOLOGY

SAMPLE COLLECTION

Biological particles were collected from the atmosphere using a Burkard 7-Day Volumetric Spore Trap (Fig. 2). As proof-of-concept, the analysis was focused on samples weekly collect, from 1st October - 31st December 2018 (14 weeks) at the station of Portuguese Aerobiology Network (RPA – SPAIC) (38º34'N; 7º54'W).

Fig. 2:. *Burkard 7-Day Volumetric Spore-trap®* of Évora monitoring station RPA – SPAIC.

AEROBIOLOGICAL METHOD

Hirst associated methodology, was used as the methodology recommended by the European Aerobiology Society (EAS) and International Association for Aerobiology (IAA) [2].
Fusarium spp. spores were identified by appearance and morphological characteristics (colour, size and shape) using an optical microscope at a magnification of 400x along one longitudinal line at the center of the slide, and by comparison with bibliographic material [3, 4]. Spore counts were expressed as daily average number of spores per cubic meter of air (spores/m^3/day).

MOLECULAR APPROACH

GENOMIC DNA (gDNA) was extracted from collected biological particles, adhered to the melinex tape containing a silicon solution, by following the CTAB protocol [5] including some modifications (Fig. 3). The concentration and purity of the solutions were obtained through the NanoDrop 2000c Spectrophotometer (Thermo).

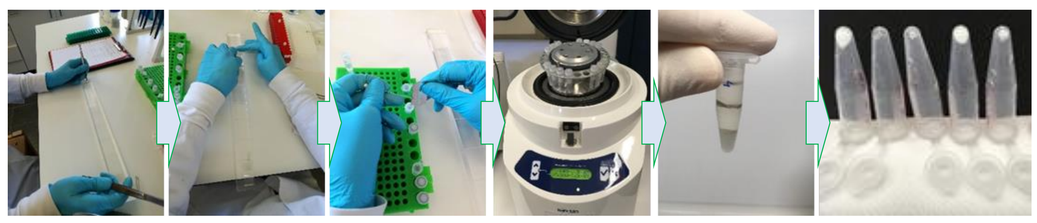

Fig. 3: Images showing several steps of the gDNA extraction procedure, going from removal of biological particles from the milenex tape till DNA precipitation.

SYBRGreen TECHNOLOGY was used. qPCR reactions were carried out using 2 µL of gDNA following the procedure prevously described [1]. gDNA from *Fusarium* spp. was included in the analysis as positive control.
As a measure of **SENSITIVITY** and the **QUANTITATIVE RANGE** of the developed qPCR procedure, the limit of detection was determined. Standards were prepared by a two-fold serial dilution of the gDNA from *Fusarium* spp.

RESULTS

AEROBIOLOGICAL ANALYSIS BY TRADITIONAL METHODOLOGY

- ✓ The maximum daily concentration was recorded on 13th November 2018, with 177 spores/m^3 of air (Fig. 4).
- ✓ A total of 3449 spores of *Fusarium* spp. were recorded (identified and quantified) in the atmosphere samples of autumn of 2018 of Évora, and they were identified throughout all the period of study (Fig. 5).

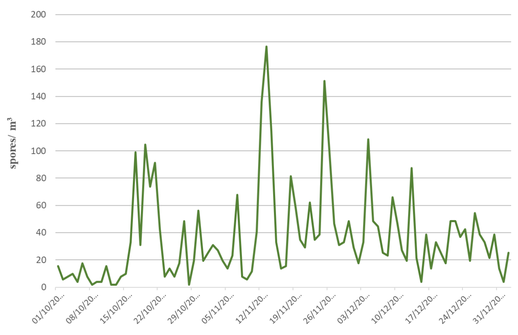

Fig. 4*: Fusarium* spp. spores concentration in the atmosphere.

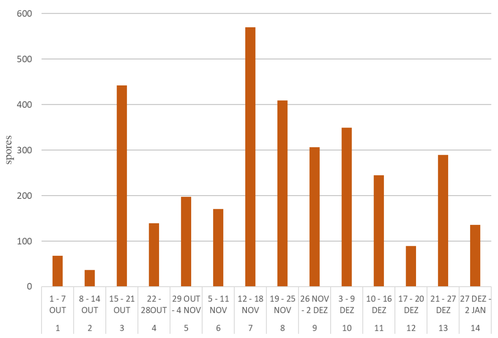

Fig. 5 *Fusarium* spp. spores index per week during the period of study.

AEROBIOLOGICAL ANALYSIS BY MOLECULAR APPROACH

Establishment of the method

- ✓ Detection limit of the *Fusarium* spp. qPCR assay was determined from the quantification cycle (Cq) of the lowest plasmid dilution that fell within the linear standard curve. qPCR assay presented an efficiency of 106 % (slope=-3.181 and R2=0.99), with all parameters felling within the acceptance criteria, allowing the detection of 7.60 x 10-4 ng of *Fusarium* spp. (Table 1).

Table 1 Sensitivity of the TaqMan qPCR assay

Dilution	gDNA in PCR (ng)	*Fusarium* spp. Cq value (±SD)
P.C.	100.00	20.86 (± 0.23)
2^{-1}	50.00	21.76 (± 0.15)
2^{-2}	25.00	23.06 (±0.10)
2^{-3}	12.50	23.46 (± 0.20)
2^{-4}	6.25	24.68 (± 0.14)
2^{-5}	3.13	25.92 (± 0.38)
2^{-6}	1.56	27.00 (± 0.09)
2^{-7}	7.81×10^{-1}	27.68 (± 0.23)
2^{-8}	3.91×10^{-1}	28.60 (± 0.11)
2^{-9}	1.95×10^{-1}	29.72 (± 0.11)
2^{-10}	9.77×10^{-2}	30.81 (± 0.20)
2^{-11}	4.88×10^{-2}	31.28 (± 0.10)
2^{-12}	2.44×10^{-2}	32.50 (± 0.03)
2^{-13}	1.22×10^{-2}	33.46 (± 0.15)
2^{-14}	6.10×10^{-3}	34.24 (± 0.34)
2^{-15}	3.05×10^{-3}	35.12 (± 0.10)
2^{-16}	1.53×10^{-3}	36.20 (± 0.27)
2^{-17}	7.60×10^{-4}	37.27 (± 0.54)

P.C.: *Fusarium* spp. positive control

Evaluation of samples during the period of study

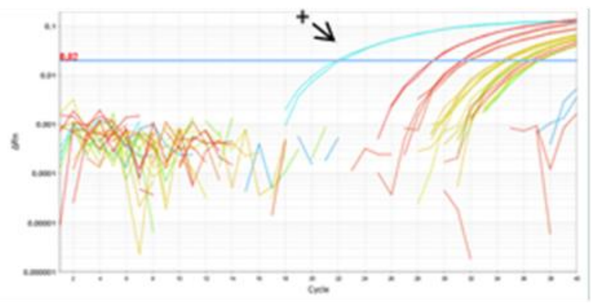

Fig. 6: Amplification plot of the samples collected during the 14 weeks for detection of Fusarium spp. by qPCR. +: positive control.

- ✓ Amplification plot (Fig. 6) reveled amplification of the samples throughout all the period of study. There were observed Cq values that vary from 29,10 to 37,02, all in the range detectable by the method.

CONCLUSIONS

- ✓ Both approach allow the identification of *Fusarium* spp. in the samples collected from 1st October - 31st December 2018.
- ✓ Given the morphological characteristics of this spore type (mostly colorless, very small in size and the different forms it presents), its monitoring by the standardized methodology (*Hirst* methodology) is a process that consume very time. Therefore, the use qPCR combined with TaqMan technology arises as a alternative methodology that enable to get accurate and reliable results in a faster way.
- ✓ Although with results still preliminary, we consider the Taqman-specific assay as an promising methodology for monitoring *Fusarium* spp airbone biological spores.

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