Monitoring of the competitiveness of an aflatoxigenic and a non-aflatoxigenic *A. flavus* strain on maize by Droplet Digital PCR

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The contamination of cereals and nuts with the mycotoxin aflatoxin produced mainly by *Aspergillus flavus* and *A. parasiticus* poses a serious problem worldwide. Particularly in Sub-Saharan countries, aflatoxin endangers the food safety and leads to serious health risks of the population. For several years, non-aflatoxigenic *A. flavus* strains are used as biocontrol system in the field to reduce the aflatoxin biosynthesis of toxigenic strains. However, the complex interaction between toxigenic and non-aflatoxigenic *A. flavus* strains is not yet fully understood. The non-aflatoxigenic *A. flavus* strain AF36 was the first strain which was used as commercial biocontrol product and its application on maize fields was authorized in Texas and Arizona in 2012 [1]. To get a better understanding of the competitiveness of aflatoxigenic and non-aflatoxigenic *A. flavus* strains, a droplet digital PCR (ddPCR) system was developed, which enables the differentiation and quantification of AF36 and the toxigenic strain MRI19. A single nucleotide polymorphism in the *pks*A gene of the aflatoxin gene cluster, which is responsible for the inability of AF36 to produce aflatoxin [2], was chosen as binding site for two specific probes. The competitiveness of the two *A. flavus* strains was analyzed by mixing the spores in varying proportions and inoculating maize-based agar and maize kernels. The fungi were incubated at 25 °C for a period of 7 days on maize-based agar and up to 20 days on maize kernels. In contrary to the expectation, the aflatoxigenic strain seemed to be the more competitive strain at most inoculated spore ratios. Nevertheless, the aflatoxin B_1 biosynthesis of the aflatoxigenic strain was already remarkedly inhibited by the addition of 20 % of spores of the non-aflatoxigenic strain. On maize-based agar, the aflatoxin B_1 biosynthesis was inhibited at a spore ratio of 50:50 for the aflatoxigenic to the non-aflatoxigenic strain, whereas it was still not completely inhibited at a spore ratio of 20:80 on maize kernels. The study demonstrates that the ddPCR system can be applied for the monitoring of the competitiveness of the two strains and in further analysis it can be used to get a better understanding of the inhibiting effect of non-aflatoxigenic *A. flavus* strains in biocontrol products.

[1] Abbas, H.K., Accinelli, C. and Shier, W.T., 2017. Biological control of aflatoxin contamination in U.S. crops and the use of bioplastic formulations of *Aspergillus flavus* biocontrol strains to optimize application strategies. Journal of Agricultural and Food Chemistry 65: 7081-7087.

[2] Ehrlich, K. C. and Cotty, P. J., 2004. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. Applied Microbiology and Biotechnology 65: 473-478.