Development of a quantitative PCR method specific to *Claviceps purpurea*, ergot of cereals: applications to study of its dispersion and evaluation of its toxicity

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The rise of ergot in cereals in France becomes a major health issue. Indeed, the plant pathogenic fungus responsible for this disease, *Claviceps purpurea*, produces sclerotia containing toxic alkaloids for humans and animals. The aim of this study was to develop a quantification method specific to G1 group of *Claviceps purpurea* by real-time PCR. This molecular tool could allow increasing our understanding about this fungus by characterizing its dispersal means (primary and secondary inocula) and by predicting health risks.

Method development

Detection and quantification of *Claviceps purpurea* DNA (G1 group) by real-time PCR is based on TaqMan® technology in order to have optimum specificity.
For reliable use of this molecular tool on different samples, the method development had to check several tests (standard, specificity, statistics, limits, reproducibility) that were validated (Figure 1).

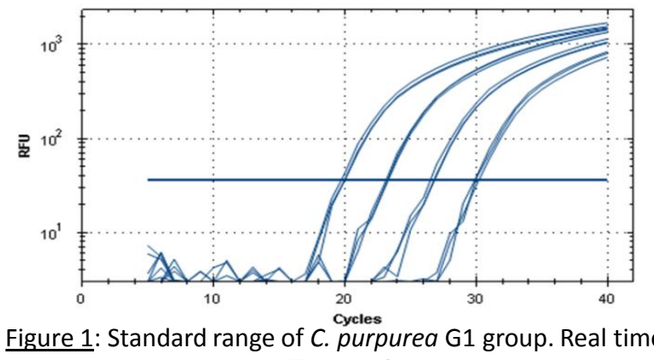

Figure 1: Standard range of *C. purpurea* G1 group. Real time PCR by TaqMan®probe

First use - Spread of primary inoculum by an abiotic factor : the wind

To follow the primary inoculum dispersal (ascospores) by wind, four spore traps were put at four different distances of a contaminated area (sclerotia on ground). Catches were done between May 10 and June 13, 2012.

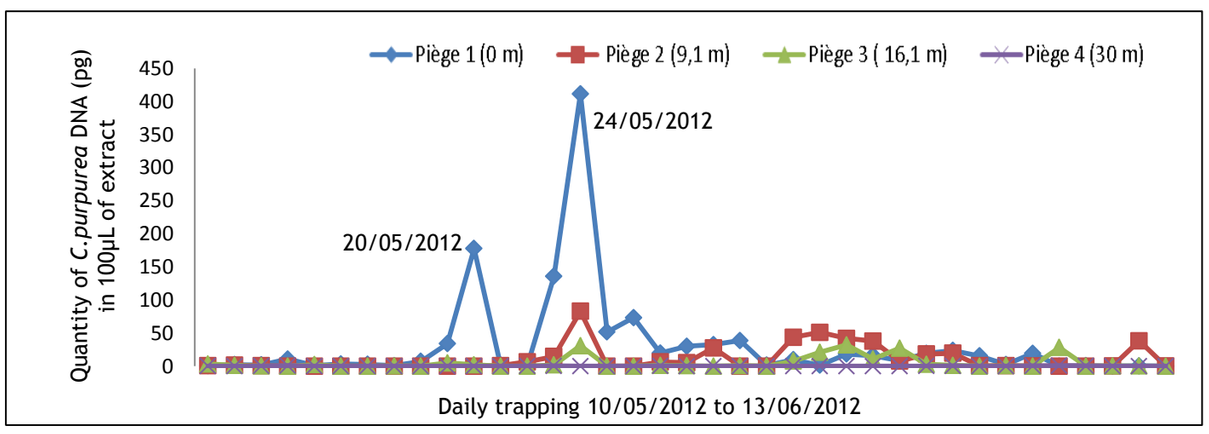

Figure 2: DNA quantification of *Claviceps purpurea* (pg/100µl of DNA extract) per day and according to distance from the inoculum source

DNA quantification of *Claviceps purpurea* performed on bands of spore traps (Figure 2) showed that:
-1st trap (0 m, blue) : the main catch of ascospores of *Claviceps purpurea* (two peaks the 20 and 24 may 2012)
-2nd trap (9.1 m, red) : few ascospores were catched (peak, 24/05/2012)
-3rd trap (16.1 m, green) : very few ascospores were catched (24/05/2012)
-4th trap (30 m, purple) : no detection

The increase of DNA quantities shows that there has been two periods of ascospore ejections detected. **The ascospores are disseminated at a small distance, within 20 meters. These results are consistent with those already obtained in 2011 by looking presence of sclerotia on wheat ears in a crop surrounding a known inoculum source** (unpublished data).

Second use - Secondary inoculum dispersal by biotic factor: arthropods

The secondary inoculum consists of conidia stuck in honeydew (sweet substance secreted by the host). This substance attracts arthropods during their foraging.
In order to follow the biotic dispersal of secondary inoculum, arthropods were caught in an experimental field at different dates.

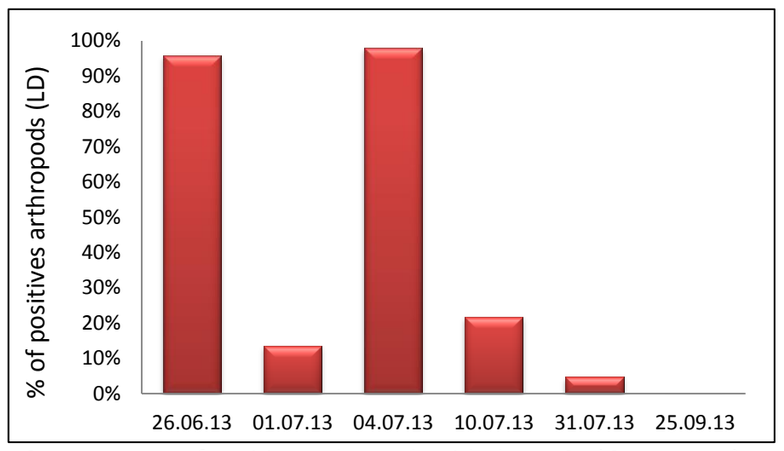

Figure 3: percentage of positive arthropods with the qPCR (*C. purpurea*) at each dates of catches

The DNA quantification on caught individuals (Figure 3) allowed demonstrating:
- Presence of *C. purpurea* spores on arthropods during the honeydew secretion
- The number of positive arthropods decreases when the honeydew production decreases
- No spores on trapped arthropods after harvest

The qPCR method has been efficient to confirm secondary inoculum dispersal by arthropods and could be useful to understand this secondary dispersal of ergot

Third use - Predict the health risk

The alkaloid contents, sclerotia weight and DNA quantification (qPCR) were analyzed on 171 samples harvested in fields in 2013.

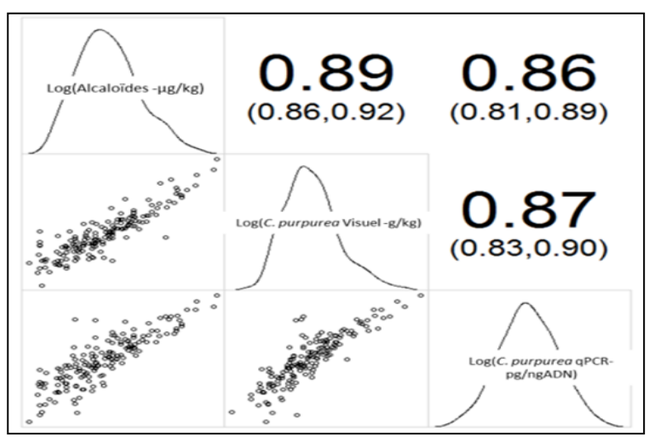

Figure 4: Correlogram between log (alkaloids-µg/kg), log (visual *C. purpurea* g/kg) and log (*C. purpurea* qPCR-pg/ng DNA)

A strong correlation between these three analytical criteria log(alkaloids), log(visual *C. purpurea*), log(qPCR *C. purpurea*) was demonstrated (Figure 4), **meaning that the method could be a good alternative as sanitary indicator by comparison with visual counting or before doing an expensive analysis of alkaloids content.**

A mixed linear model has also defined that 73% of alkaloids content is explained by DNA quantity (qPCR).

Conclusion

This work led to elaborate a detection and quantification method of *Claviceps purpurea* - G1 group specific - by real time PCR. By applying it to different samples, data were acquired and interpreted. Firstly, the capacity of dispersion of primary and secondary inocula by biotic and abiotic factors was evaluated. In both cases, **our works confirmed that dispersal of *C. purpurea* occurred at a small distance**. Secondly, *C. purpurea* DNA was quantified within batches of infected cultivated Poaceae in order to evaluate the method as a sanitary indicator. Through this method, **a strong correlation between DNA quantity, ergot concentration and alkaloid content was demonstrated** in these batches.
Thereby, our tool is useful for many applications and could facilitate research on *Claviceps purpurea* G1 group. **It could help the scientific community to acquire data on the dissemination of spores**. **It could also be useful for the food industry as an indicator to assess the sanitary quality of cereals.**

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