学位論文内容の要旨

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学位論文題名

The use of color as alternative to size measurements in *Fusarium graminearum* growth studies and prediction of deoxynivalenol synthesis

（*Fusarium graminearum*の生長評価における形態測定の代替としての色調変化の利用とデオキシニバレノール産生量の予測）

Fusarium graminearum Schwabe is a fungal plant parasite responsible for head blight (FHB) in cereals. This mold also synthesizes deoxynivalenol (DON), an emetic mycotoxin responsible for at least eight outbreaks since the beginning of the 20th century in Japan, including Hokkaido. The toxin is also a major problem all over the world.

DON is highly stable and resistant to common processing methods such as milling, heating, drying and fermentation, often making into the food if synthesized. Thus, prevention is still the best alternative to control this chemical, and for that it is a key step to understand how environmental factors affect growth of *F. graminearum* and DON synthesis. Temperature and water activity (a_w) have already been known to affect DON synthesis. The former factor seems to have an optimal range of minimal toxin production, while the latter is likely to be directly proportional to DON quantity. In any case, DON production seems associated to stress caused by suboptimal growth conditions.

Although size-based variables (e.g. radius, diameter) represent fungal growth in the recently used models, they do not provide important growth information such as metabolic activity or state of maturity, especially during the stationary phase. Changes in the color during fungal growth would be suitable for the purpose, because the changes in color are a product of the mold's metabolism, changing even when the fungus is no longer expanding in the substrate. The present study aimed to analyze if *F. graminearum* surface colors are suitable as alternative to size in studies of growth and DON synthesis.

1. *F. graminearum* surface color as predictor of DON production in yeast extract agar (YEA)

After a review on the principles underlying *F. graminearum* growth, toxin production and its color, the first step was to see if the mold's surface colors present a predictable pattern, fit for use as an estimator of metabolic changes and state of maturity. Three specimens were incubated in yeast extract agar (YEA) at 25 °C for 20 days, photographed daily and the proportion of pixels with red, green and blue (RGB) components in the mold's surface were analyzed using ImageJ software. All RGB components cubic polynomial growth patterns as function of incubation time, and all color components were highly correlated, especially red and green.

Some experiments aimed to analyze the relationship between RGB components and DON synthesis under different temperature and a_w. Specimens were incubated at 15 °C, 25 °C and 30 °C and a_w of 0.94, 0.97 and 0.99, and each 4 days photos were taken and RGB was analyzed, and toxin concentration was determined. DON concentration was determined using HPLC (UV = 220 nm, 35 °C). All RGB components presented cubic relationship with DON, regardless of the temperature. However, water activity below 0.99 did promoted mycelium growth, masking the mold's surface and making it impossible to visualize the color variation.

2. Analysis of color change in *F. graminearum* contaminated grains and the predictability of DON contamination

Reproduction of the same experiments in oat and rice grains showed similar trends: RGB variation was predictable and showed consistent relationship with DON concentration across temperatures. In this case, it was possible to verify that RGB is related to DON variation at different a_w level. The latter observation was feasible in grains but not in YEA likely because grains presented much wider surface for mold growth and their cellulose wall slowing down the rate of degradation and production of white mycelia. These observations suggest that RGB components can effectively be used to predict the extent and severity of DON contamination when *F. graminearum* colonizes oat and rice grains at different temperatures and a_w.

3. Conclusion

The present studies demonstrated that changes in the color of fungal metabolites will be appropriate as an alternative for size variables in *F. graminearum* growth studies and prediction of synthesis of DON quantity.