Near infrared hyperspectral imaging of *Fusarium*-damaged oats (Avena sativa L.)

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Contact: Selamawit Tekle, Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway.

Summary:

Fusarium infection results in shriveled and lightweight kernels contaminated with mycotoxins such as deoxynivalenol (DON). The feasibility of using near infrared hyperspectral imaging (NIR HSI) for detecting *Fusarium* damage and DON contamination in whole oat kernels was investigated. The use of NIR HSI as a screening tool for FHB resistance is an attractive option compared to labor-intensive field evaluations and conventional wet chemistry for toxin analysis.

Kernels of the susceptible oat, cv. 'Bessin', from a *Fusarium*-inoculated nursery were visually categorized as 'severely damaged', 'mildly damaged', or 'asymptomatic'. Uninoculated kernels of the same cultivar were used as a control. Hyperspectral images of 31 calibration kernels and 14 validation kernels from each kernel category were taken. Deoxynivalenol content and weight of each kernel were determined following imaging. Surfaces of hulled and de-hulled kernels as well as cross sections from each kernel category were studied under the scanning electron microscope. A calibration model was developed using partial least squares regression by pairing the average spectrum of each kernel with DON*= [log (DON)] ^3. The model was optimized by cross-validation, and the prediction performance was validated by predicting DON* values of the validation kernels. Linear discriminant analysis was used to classify kernels.

The uninoculated and asymptomatic kernels were plump and free of any fungal mycelia, while the severely damaged kernels were shriveled and heavily colonized with *Fusarium*. Dense mycelia were observed on the crease of the severely damaged kernels. The cross sections of uninoculated kernels revealed a well-formed aleurone layer and intact endosperm structure, while the severely damaged kernels showed a collapsed and highly colonized aleurone layer with partially digested endosperm structure. Damage to the seed coat and the aleurone layer was observed in the mildly damaged kernels but the inner endosperm structure was intact. The asymptomatic kernels had the highest mean kernel weight and the lowest mean DON content, while the severely damaged kernels had the lowest mean kernel weight and the highest DON content. However, there were a few kernels with very low DON in the severely damaged kernels category and a few kernels with substantial DON in the asymptomatic kernels category, indicating that visual symptoms do not necessarily match the DON content of kernels.



Fig: A pixel-wise map of *Fusarium* infection on single oat kernels was constructed using data from hyperspectral imaging and single kernel DON analysis. Uninoculated and asymptomatic kernels are on the left side, while severely damaged kernels are on the right side.

The model detected clear differences between kernel categories and the first component separated the uninoculated/ asymptomatic kernels from the severely damaged kernels. Infected kernels showed higher intensities at 1920, 2070, and 2140 nm, while non-infected kernels were dominated by signals at 1420, 1620, and 1850 nm. The DON* values for the validation kernels were estimated using the average spectrum, and the correlation between predicted and measured DON* was 0.82, showing a valid prediction model. These results indicate that NIR HSI can be used to detect and remove *Fusarium*-damaged kernels, thereby reducing toxin contamination and increasing industrial quality of oat seed lots.