Quantification of leaf stripe, *Pyrenophora graminea*, in barley seed by real-time PCR.

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In some years a large amount of organic seed lots is discarded due to the presence of high levels of seed-borne diseases. In barley it is especially the presence of the fungal species *Pyrenophora teres* and *P. graminea*, which causes rejection of seed lots. In Denmark, the threshold for the presence of *Pyrenophora* species is at present 5% infected seeds. This is a combined threshold for both *P. teres* and *P. graminea* due to the fact that the conventional seed health test currently used cannot distinguish between these two species. The threshold for *P. teres* alone is 15%. Only an additional test in the greenhouse for leaf symptoms can tell how much *P. graminea* is present. Most seed lots are tested without differentiation of the two species and consequently the 5% threshold shall apply. Since *P. graminea* is less frequent than *P. teres* in barley there is no doubt that seed lots are unnecessarily rejected.
The aim has been to develop a real-time PCR method for detection and quantification of *P. graminea* in seed lots. TaqMan primers and probe were designed for the specific detection and quantification of *P. graminea*. The dynamic range of the TaqMan assay was at least four orders of magnitude. Seed infection levels as low as 0.5%, which is the detection limit for the conventional test and the greenhouse test, could be detected. Seed samples harvested from a field trial infected with *P. graminea* were tested. The amount of *P. graminea* DNA in a sample of 200 seeds were significantly correlated with the number of infected seeds tested by the blotter method and with the number of infected plants tested in greenhouse despite of some large variations among samples. The infection level of individual seeds may vary considerably causing variation in the amount of DNA among seeds within a sample and this is a problem when trying to establish a relationship between the PCR assay and the number of infected plants or seeds. So far the correlation has been established for one single variety. We are in the process of testing varieties with different levels of resistance towards *P. graminea* in order to see if different DNA-thresholds apply for different varieties depending on the resistance level. Currently, the PCR method allows to make use of individual thresholds for the two diseases caused by *P. teres* and *P. graminea* and thereby preventing unnecessary rejection of seed lots having < 15% infection with *P. teres*.