Rapid detection and quantification of *Cercospora beticola* in soil using PCR and ELISA assays

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Cercospora leaf spot (CLS) is considered to be the most common and destructive foliar disease in sugar beets worldwide. It is caused by the fungus *Cercospora beticola* which survives as stromata on sugar beet leaf residues in soil. These stromata germinate under warm and moist conditions by producing conidia which are dispersed as primary inoculum to initiate infection of sugar beet leaves. The objective of this research was to develop effective methods for qualitative and quantitative detection of *C. beticola* in soil. The soil borne inoculum is suggested to be an important factor for disease prediction. Therefore, these methods are aimed to improve integrated pest management systems (IPM) of sugar beet. In this study two diagnostic methods, polymerase chain reaction (PCR) and ELISA technique were successfully applied for qualitative and quantitative detection of *C. beticola* in soil.

The primer set ITS3/ ITS4 was found highly specific since it amplified one single fragment only from *C. beticola* isolates but not from the other fungal pathogens tested. Also, it has a high level of detection since it could detect as little as 0.5 pg *C. beticola* genomic DNA. Therefore, it proved that it can be used for detection of *C. beticola* in soil. For the detection of *C. beticola* antigen, specific monoclonal antibodies (mAbs) were produced using the method of Köhler and Milstein (1975) at the Institute of Biochemistry, University of Kiel. Eighteen clones were obtained against *C. beticola* which gave positive results only with *C. beticola* and negative results with the other fungal species tested indicating that these antibodies are specific for *C. beticola*.

ELISA and real-time PCR were used to determine the degradation of *C. beticola* inoculum in soil during 6 months under controlled conditions in climate chamber. Both detection methods showed degradation of *C. beticola* inoculum over time.

After developing and proving the detection methods under controlled conditions at known inoculum amounts, these methods were applied to soil samples from natural fields of different locations. The soil samples were collected from two regions in Germany, Bavaria and Lower Saxony and in addition from one location in Montana (USA). These fields were under different crop rotations and soil maintenance. The samples were taken from three different layers: 0-5 cm, 5-15 cm and 15-30 cm. CLS could be detected in all different soil layers.

Both detection methods showed that the highest amounts of *C. beticola* inoculum were obtained from the fields which were cultivated with sugar beet 1 year before sampling date. In contrast, the lowest amounts of *C. beticola* inoculum were obtained from the fields which were cultivated with sugar beet 3 years before sampling date. Additionally, amplicons derived from PCR using the primer set ITS3/ ITS4 were sequenced and compared to DNA sequence from pure culture *C. beticola*. Alignment of sequences of the amplified products confirmed them to be those of *C. beticola*.