Optimization of a protocol to embed *Hypericum perforatum* L. seeds

Lana-Sophie Kreth and Monika Götz

Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Braunschweig

E-mail of corresponding author: lana-sophie.kreth@julius-kuehn.de

St John´s wort (*Hypericum perforatum*) is a widely known as medical plant for its biological properties. It has been cultivated and used for medical for thousands of years. *H. perforatum* is cultivated in a small area in Germany. Fungal infections are a major threat in cultivation of *H. perforatum* and can cause severe yield losses. One of the most important fungal pathogens is *Colletotrichum gloeosporioides,* which causes anthracnose. The fungus can be lethal for the plant by infecting the stem base. It spreads over splashing water between the plants. Investigations revealed that the fungus is a seed borne pathogen and infected seeds are an important inoculum source for first infections and for distributing the fungus. Previously studies showed that the fungus was localized in the seed coat. However, it is unclear that the fungus spreads into the embryo of the seeds. Therefore, localization of the fungus in the seeds is important to develop seed treatments to control the fungus as well as to prevent the early stages of plants infection. In this study, we optimized the protocol to embed *H. perforatum* seeds for preparation of cuttings with the microtome for their localization by light microscopy.

For the analysis, the seeds were embedded in resin (Technovit 7100). We found that, conventional embedding protocols are not suitable for small and hard *H. perforatum* seeds, because the embedding solution did not infiltrate the whole seed and many embryos of the seeds are lost after cutting with the microtome. The base of the adjustments was an embedding protocol for Melastomataceae seeds. We extended the time of fixation, infiltration of the embedding solution as well as added a step between fixation and dehydration. The steps were increased the porosity of the seed coat and rising the infiltration of the embedding solution into the seed. We incubated the embedded samples at 4 °C, resulting in an extension of the resin polymerization time. Our study shows that they are more successful in an increasing number of infected seeds from 10% to 40 %. The new embedding protocol was developed and optimized which can be improve for the studies on localization of pathogens in *H. perforatum* seeds. Currently, we work on our protocol for testing with other small and hard seeds.