A method of assessing *Spongospora subterranea* spore viability using RTqPCR technology

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The powdery scab pathogen *Spongospora subterrranea* persists in soil for up to 20 years. This is due to the dormant nature of the multi-celled cystosori that contain resting spores and their uneven release of infective zoospores over time. The obligate nature of the pathogen makes it difficult to quantify the amount of viable inoculum present in soil to determine its infective potential. The aim of this study was to develop molecular methods based on gene expression using RNA to measure the viability of spores present in cystosori. Primers and probe were designed for 3 genes (actin, polyubiquitin and 18S rRNA) specific to *S. subterranea*. RNA was extracted from 1) cystosori that were collected from infected Shepody potato tubers (Ballarat, 2009), 2) from cystosori collected in 1982 and stored at 4^{o}C at DPI Knoxfield and 3) from fresh cystosori collected from infected Kennebec potato tubers in 2010. Real-time PCR analysis demonstrated that only the 18S rRNA primers successfully amplified RNA samples extracted from cystosori. 18S rRNA gene codes for ribosomes that are the site of protein synthesis in all living cells so we would expect this gene to be expressed in viable spores. No PCR products amplified using actin and polyubiquitin primers. The function of the actin gene is to form microfilaments which give structure to cells and support signal transduction and the polyubiquitin gene codes for a protein involved in controlling a variety of biological functions ranging from proteolysis to DNA damage tolerance. These functions suggest that these genes are expressed during active growth of the pathogen and it is possible that these two genes are not expressed in the dormant state of this pathogen. Gene expression of the actin and polyubiquitin genes of *S. subterrranea* was confirmed in infected tomato roots using the real-time RT-qPCR assays. Further studies will explore the use of this technique to measure pathogen viability in response to potential control measures.