



Quantitative methods to study *Phytophthora infestans* oospore ecology

P.J. van Bekkum & G.J.T. Kessel

Introduction

Nowadays, oospores are one of the *P. infestans* primary sources of inoculum. Oospore derived infections are reported in several European countries. Quantitative insight into oospore ecology, as related to soil type and abiotic conditions, may stimulate further refinement of potato late blight management strategies.

Four steps are necessary to study oospore ecology:

- A reliable production method for oospores
- A reliable extraction method to concentrate oospores for experimental purposes
- A simple incubation method in-soil
- A simple recovery method for (germinated) oospores from soil

Production of oospores

Potato leaflets are spray inoculated with a mixture of A1 and A2 isolates. Leaves are incubated for three weeks at 10°C in the dark during which time oospores are formed.

Extraction of oospores from plant tissue

Oospores containing leaves are blended using an Ultra Turrax mixer (Figure 1), treated with enzymes and sieved over a 20 µm sieve to concentrate oospores. Oospore containing leaf residue can be air-dried at room temperature for later use.



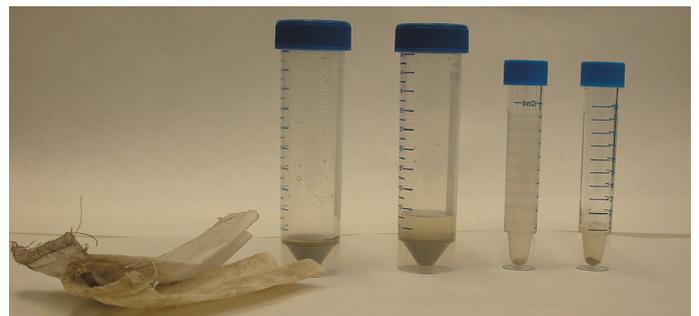
Incubation-in-soil

Oospore-containing leaf residue was added to quartz sand only containing particles > 125 µm. The oospore - sand mixture is transferred to small gauze bags (15µm mesh, Figure 2) and buried in soil under e.g. differential temperatures for experimental purposes.



Recovery of (germinated) oospores from soil

Oospore containing sand from the gauze bags is transferred to a centrifuge tube. Water is added, the tube inverted and supernatant is transferred to a 10 ml centrifuge tube. After centrifuging the supernatant is discarded until 1 ml of oospore containing debris was left for quantification (Figure 3).



Conclusions

- Depending on the combination of isolates, the oospore production method reliably produced large quantities of oospores.
- Average recovery of oospore extraction from plant tissue was 97%.
- The incubation method in-soil was found effective and reliable for laboratory and field studies.
- The recovery method for (germinated) oospores from soil was found to be effective and allowed quick processing of samples for further analysis.