# Which factors trigger *Phytophthora infestans* to oospore production and germination?

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Fig. 1. Early *P. infestans* infection in a potato plant in the field

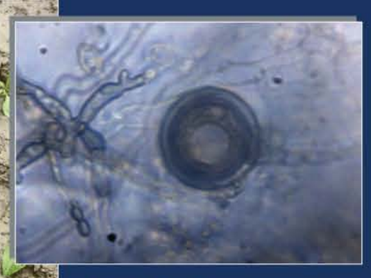

Fig. 2. *P. infestans* oospore

## Introduction

During the last decade still earlier outbreaks of potato late blight epidemics have been observed in Denmark (Fig. 1). The inoculum source for these earlier *P. infestans* infections in potato plants could be oospores in the soil (Fig. 2). However, the inoculum potential of *P. infestans* oospores in Danish fields and the reason for the rather high variation in the numbers of early infections by *P. infestans* from year to year are still unknown. In 2005, a markedly high number of early *P. infestans* infections in potato plants were registered and this initiated a project with the aim of studying the effect of different spraying and desiccation techniques on the production and survival of *P. infestans* oospores in the soil. Apart from the field experiments, laboratory experiments were conducted to study the effect of temperature, soil water contents, pre-crops, microbial background and oospore density on oospore germination and infection of plants.

## Materials and Methods (field experiments)

A total of three field experiments were conducted in 2006 and 2007 in cultivars Bintje (2006+2007) and Kuras (2007).

*P. infestans inoculum*
Approximately 50 crosses of *P. infestans* A1 and A2 isolates were tested for growth and oospore productivity on rye agar and in potato leaves. The most oospore productive A1 and A2 isolates were selected for the experiments. Eight *P. infestans* (4xA1+4xA2) isolates were grown on surface-sterilised tuber slices and a sporangia suspension of 1000 A1 sporangia and 1000 A2 sporangia $ml^{-1}$ was used for inoculation in the field.

*Experimental design in the field*
Treatments
1. Untreated
2. Fungicide (2006: Shirlan 0.4 l/ha and 2007: Dithane 2.0 kg/ha)
3. Mechanical desiccation: Top cutting
4. Chemical desiccation: Reglone
5. Thermal desiccation: Gas burning

Treatments were placed in a randomised block design with four replicates.

*Inoculation*
Plants were inoculated at the beginning of July by spraying with the inoculum suspension at sunset (Fig. 3) and then covering the plants with a nylon mesh until next morning (Fig. 4).

*Analyses of field experiments*
- Detection of late blight infection of plants during the growing season
- Detection of oospores in leaves and stems
- Detection of inoculum potential of *P. infestans* in the soil before, immediately after and a month after desiccation ([1]Lacey´s test)
- Detection of inoculum potential of *P. infestans* oospores in the soil one and three months after desiccation ([2]Drenth test)

*Detection of inoculum potential of P. infestans oospores in the soil in laboratory experiments*
A modification of [2]Drenth test was used to detect germination and infection from oospores in soil. Microtiter plates with twelve wells (Fig. 5) were used to separate leaf discs. Each well was amended with 1 g sterile soil and 1 ml oospore suspension in sterile water. A leaf disc (diameter 15 mm) was placed upside down on the surface. The soil in two of the wells remained uninoculated as controls for cross contamination (Fig. 6). The microtiter plates were placed at 15°C with 16 h whitelight/8 h darkness for at least 3 months. Leaf discs were analysed daily for *P. infestans* infections and replaced with new leaf discs every 10-14 days. Each treatment had 5 replicate microtiter plates in the different experiments.

*Factors tested for influence on oospore germination and infection in [2]Drenth test*
- Temperatures: -20°C followed by 15°C and 5°C, 15°C and 20°C.
- Soil water content ($g^{-1}$ soil): 350 µl, 700 µl and 1000 µl
- Pre-crops: Oat, rye, wheat, red clover, white clover, marigold, tagetes, white mustard, radish, oil-seed rape and turnip
- Oospore densities in soil (g soil): 50, 100, 500, 1000 and 2000 oospores
- Microbial background: Sterile/un-sterile field soil

Fig. 3. Inoculation with *P. infestans* in field experiments



Fig. 5. Microtiter plates used for [2]Drenth test

Fig. 4. Cover of field experiment overnight after *P. infestans* inoculation

Fig. 6. *P. infestans*-infected leaf disc and uninfected control leaf disc in [2]Drenth test

## Results

- In all three field experiments, the attack of late blight was 40-60% at the time of desiccation.
- In the three field experiments 720 leaf samples and 360 stem samples - all with at least two infection sites that had grown together - were analysed and oospores were found in one stem sample (Bintje 2007).
- [1]Lacey´s test was performed on 240 soil samples in each experiment and showed inoculum potential of *P. infestans* in the Bintje 2007 experiment only. There was no correlation between inoculum potential and desiccation treatment, but the inoculum potential was as expected significantly lower in the fungicide-sprayed plots.
- No inoculum potential of *P. infestans* oospores was found in any of the 240 soil samples from each experiment by [2]Drenth test.
- In the laboratory experiments, the oospores did only germinate and infect leaf discs in very few cases. Temperature, soil water contents, pre-crops, microbial background and oospore density did not seem to affect oospore germination and the plant infection level.

## Conclusion

In three field experiments over two years, with two different potato cultivars and superficially optimised conditions for *P. infestans* oospore production, oospores were only found in one stem sample out of 720 leaf samples, 360 stem samples and 720 soil samples. This result indicates either that *P. infestans* rarely produces oospores under Danish field conditions or that there are one or more biological, chemical or physical factor(s) not present under the experimental conditions that trigger oospore production.

Concerning the "key" to which factors that trigger oospores to germinate and infect plants, the experiments showed no effect of temperature, water contents, pre-crops, microbial background and oospore density on oospore germination and infection of plants. So, these results have not answered the question "which factors trigger *P. infestans* to oospore production and germination?" Maybe the obvious difficulties with oospore production in the field and with triggering oospore germination ought to be included in the ongoing discussion about the role of oospores in early outbreaks of potato late blight epidemics.

**Literature**
[1]**Lacey J.** 1965. Ann. Appl. Biol. Vol. 56:363-380
[2]**Drenth A., Janssen E.M., Govers F.** 1995. Plant Pathology 44:86-94.