The impact of oospores of *Phytophthora infestans* on late blight epidemics

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The oomycete *Phytophthora infestans*, the cause of late blight in potatoes and tomatoes, is considered to be among the most important pathogens of potato crops worldwide (Hooker, 1981). The pathogen is feared by farmers around the globe due to its ability to quickly destroy entire fields of potatoes and tomatoes. The pathogen affects foliage and stems, reducing the photosynthetic capacity of the crop and therefore leading to tuber yield reduction. In addition, *P. infestans* can infect fruits and tubers, which adds to total losses in marketable yield.

Crop losses due to late blight have been estimated to account for 10 to 15 percent of the total global annual potato production (CIP, 1996). The economic value of the crop lost, plus the costs of crop protection amount to $US three billion annually (Duncan, 1999). In the developed world, control of potato late blight is heavily dependent on the use of fungicides. Despite frequent fungicide use, late blight epidemics have proven to be increasingly more difficult to control (Turkensteen et al., 1997; Schepers, 2000).

Resurgence of the late blight pathogen

The increased problem with controlling potato late blight coincides with the displacement of the US-1 clonal lineage by a new, more variable *P. infestans* population in many parts of the world (Spielman et al., 1991). New populations are marked by more aggressive genotypes of the pathogen (Day and Shattock, 1997; Lambert and Currier, 1997, Turkensteen et al., 1997). In regions where both mating types have been found, evidence is accumulating that sexual reproduction takes place (Drenth et al., 1994; Andersson et al., 1998). In sexual populations, both sexual (oospores) and asexual (i.e. mycelium in infected tubers) propagules serve as inoculum sources, whereas asexual populations are totally dependent on asexually produced inoculum.

Prior to the 1980s, a single A1 clonal lineage of *P. infestans,* designated US-1, was spread throughout the world, whilst the occurrence of the A2 mating type was confined to an area of the highlands of central Mexico (Niederhauser, 1956). Oospores in field crops (Gallegly and Galindo, 1958) were first reported from the Toluca Valley of central Mexico, and evidence indicates that the highlands of central Mexico are indeed the center of origin of *P. infestans* (Fry and Spielman, 1991; Goodwin et al., 1992). Populations of *P. infestans* outside central Mexico were restricted to asexual reproduction and survived during crop-free periods by existing as mycelium inside potato tubers.

During the 1980s, potato late blight became more difficult to control in Europe and. resistance to the fungicide Ridomil (active ingredients are metalaxyl and mancozeb) developed rapidly (Davidse et al., 1981). It is plausible to suggest that the displacement of the US 1 population by 'new' isolates may have been accelerated by the concomitant introduction of phenylamides in Europe, as tolerance to phenylamides (including metalaxyl) is more common amongst 'new' isolates.

The presence of A2 mating type strains in Europe was first reported in Switzerland (Hohl and Iselin, 1984), and was soon followed by a UK report on the presence of A2 mating type strains in imported ware potatoes from Egypt (Shaw et al., 1985). These observations led to a revival of late blight research in Western Europe. Population genetic studies using allozymes (Spielman et al., 1991) and DNA fingerprinting revealed the presence of a new, genetically variable population of *P. infestans* in Western Europe.

Impact of 'new' populations

Farmers' experience of controlling late blight suggests that the onset of late blight epidemics appears less predictable and control has become more difficult. It was found that 'new' isolates are more aggressive than 'old' isolates when infection frequency, latent period, sporulation and tuber infection were measured under controlled conditions (Day and Shattock, 1997; Flier et al., 1998; Flier and Turkensteen, 1999).

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It is conceivable that the presence of aggressive strains will lead to shorter infection cycles and a more rapid epidemic development of the disease. In monocyclic tests, the difference between the individual components for aggressiveness in the old and the new population are strikingly in favor of the new population. The combined effect of the components of increased aggressiveness on polycyclic late blight epidemics is dramatic. Comparison of infection efficiencies and sporulation capacity of isolates representing the old and the new population of *P. infestans* in the Netherlands shows that isolates of the newly established population are able to infect potatoes at temperatures ranging from 3 to 27 °C while old population isolates caused infections from 8 to 23 °C (Flier et al., unpublished). Recent results (Flier et al., unpublished) indicate that isolates of the new population are marked by more rapid spore germination and host penetration, leading to shorter critical leaf wetness-periods. Under normal field conditions, isolates need only a few hours of leaf wetness (approx. 4 hours at 15 °C) to penetrate potato leaves instead of the 8 hours that was widely considered to be the minimum time needed for germination and infection. In 1999, we successfully inoculated a field crop under extremely high temperatures (max/min: 34°C/27°C) and observed a latent period of approximately 2.5 days under field conditions. In 2000 and 2001, comparable latent periods were observed (Flier, unpublished).

Whether observations like this should be regarded as rare incidents or to represent the current performance of *P. infestans* is still under debate, yet evidence supporting the hypothesis of increased levels of pathogenic fitness is accumulating.

The increased chance of infection at sub-optimal temperatures, in combination with shorter leaf wetness periods will increase the number of critical infection periods during the growing season, whilst shorter latent periods will boost the speed of the epidemic. The window of opportunity for action by the potato grower is narrowing and it has become extremely difficult to achieve proper fungicide application timing. This grim view of the negative impact of 'new blight' on late blight control is supported by recent figures on fungicide use in the Netherlands and other countries in Western Europe. To date, the number of fungicide applications to control late blight in potatoes range from an average of 7 to more than 20 applications per season (Schepers, 2000), which is approximately 40% higher than fungicide use in the late 1970s.

The roles of oospores in late blight epidemics. The presence of a mixed A1/A2 mating type pathogen population has been reported for a growing number of potato producing countries. Consequently, oospores may be produced in field crops during the growing season when field infections occur. The perfectly designed outer walls of oospores make them perfectly adapted for survival in soils for longer periods. Experimentally, it has been shown that oospores remained infectious for 4 years under Dutch climatological conditions (Turkensteen et al., 2000). Oospores may serve as an additional source of initial inoculum that can potentially infect field crops throughout the growing season under blight favorable conditions. Oospores may also play important roles in catalyzing genetic variation through meiotic recombination and conserving genetic variation by reducing genetic drift caused by extreme population reduction during the crop-free season (recurrent population bottle-necks).

In the Netherlands, a research program aimed to elucidate key aspects of oospore ecology was initiated in 1999, following reports on oospore formation in field crops (Drenth et al., 1995; Turkensteen et al., 1996). Some results obtained from observational and experimental work on oospore formation in the field and the interaction between oospore production and fungicidal activity will be discussed in the text below.

Oospore survey. In 2000, a survey was conducted in the northeastern (NE), central (CE), southeastern (SE) and southwestern (SW) potato production areas in the Netherlands. Sandy soils dominate the NE and SE production areas whereas clay soils dominate the CE and SW production areas. In each of these four areas, five unsprayed (volunteer) potato crops were sampled by collecting 25 *P. infestans* infected leaflets with two or more lesions. These leaflets were incubated in water agar Petri dishes at 15°C for three weeks. Following incubation the leaves were microscopically examined for the presence or absence of oospores. The density of oospores per leaf was estimated using a 0 – 9 density index (0 = no oospores, 9 = high densities of oospores throughout the entire leaflet). Thus the potential for oospore formation is measured. This was done to avoid the influence of local (micro) climate on oospore formation (Cohen et al., 1997; 2000).

Oospores were found in 78% of the leaflets from the NE production region and in 50%, 30% and 15% of the leaflets from the SE, CE and SW regions, respectively. The average number of lesions on the sampled leaves was 6, 14, 2.5 and 2.5 for the NE, SE, CE and SW areas, respectively. A2 isolates constituted 62%, 17%, 9% and 6% of the isolates collected in each of the regions, respectively. When oospores were found, the oospore density was usually high. Average densities for the NE and SE areas was 7 on a 0 – 9 scale. Oospore density in leaflets from the CE and SW production areas was not determined.

Effect of fungicides on oospore formation. Oospore formation in the presence of fungicides was studied in-vitro and in-vivo. For the in vitro experiments, crosses of A1 x A2 isolates were carried out in Rye A agar in the presence of sub-lethal concentrations of several fungicides. Fungicide concentrations were chosen so that the radial growth rate of *P. infestans* colonies on Rye A agar was reduced by 75%, as determined in a preliminary experiment. Oospores were quantified in the contact zone of the A1 and A2 colonies after four weeks of incubation. Oospores were extracted from the agar and viability was determined using tetrazolium bromide (Jiang and Erwin, 1990).

For the in-vivo experiments, fully grown potato plants of cultivar Bintje were inoculated with *P. infestans* A1 and A2 isolates and incubated at 10°C and high relative humidity. Eight days after inoculation the foliage was sprayed with fungicides at the recommended dose rate. Fourteen days after inoculation, 10 leaflets, with two lesions or more, were collected per plant and incubated in Petri dishes containing water agar at 11°C for three weeks, after which oospores were extracted and quantified.

Most of the tested fungicides have a pronounced effect on both the production and the viability of oospores of *P. infestans* (Figure 1). Protectant fungicides (except fluazinam) do not have specific activity against oospore formation. In general, currently available (semi) systemic or translaminar fungicides have excellent activity against oospore formation and reduce the viability of oospores considerably. Oospore formation is inhibited more than proportional with (semi) systemic and translaminar compounds as compared to mycelial growth, which was inhibited by 70%.

Results of the in vivo experiment are summarized in Table 1. Oospore formation is very significantly inhibited by all fungicide applications, despite the fact that fungicides were only applied eight days after inoculation when late blight symptoms were well developed. Differences between fungicides are relatively small when compared to the overall difference between the control treatment and fungicide applications. Viability of the oospores formed was not determined. Surprisingly, there is no clear separation between non-systemic and (semi) systemic or translaminar fungicides. Absorption of fungicides by necrotic tissue may be responsible for this observed effect. Oospores are only formed after contact between *P. infestans* "A1 and A2 lesions". Oospore formation itself is a relatively slow process. Thus, fungicides absorbed by necrotic tissue in the lesion may not prevent infection of the plant, but they may still inhibit the mycelium present in necrotic tissue and thus prevent or reduce oospore formation.
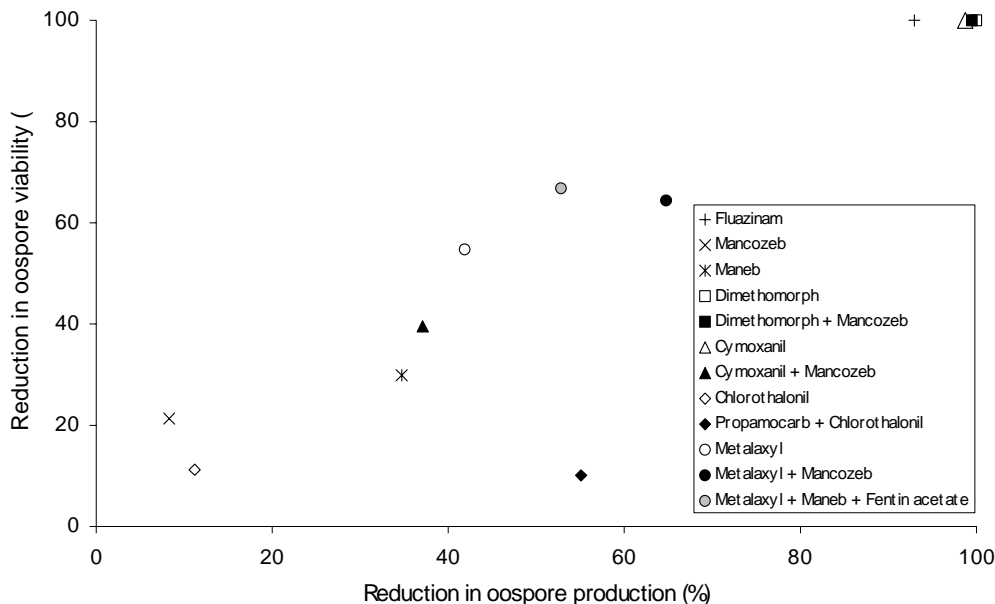

Figure 1. Results from the in vitro experiment on the effect of fungicides on oospore formation. Crosses between *P. infestans* A1 and A2 isolates were carried out in Rye A agar in the presence of sub-lethal concentrations of fungicides. Concentrations were chosen such that the radial growth rate of the colonies was reduced by 70%.

Table 1. Influence of fungicides on oospore formation. Oospore density in leaves of potato plans cultivar Bintje after inoculation with *P. infestans* A1 + A2 isolates. Fungicides were applied eight days after inoculation. Prior to quantification of oospores, leaflets were incubated in water agar Petri dishes at 10°C for three weeks. Average oospore densities followed by a common letter do not differ significantly according to analysis of variance followed by an LSD test at *P = 0.05*.

Fungicide	Active ingredients	Dose[1]	Oospores / cm^2 leaf area
Untreated	none	-	171.6 d
Acrobat	Dimethomorph + Mancozeb	2.0 kg/ha	0.4 a
Tattoo C	Propamocarb + Chlorothalonil	2.7 l/ha	0.4 a
Daconil 500	Chlorothalonil	3.5 l/ha	0.6 a
Penncozeb	Mancozeb	4.0 kg/ha	1.1 ab
Shirlan	Fluazinam	0.4 l/ha	5.8 abc
Ridomil Gold	Metalaxyl + Mancozeb	2.5 kg/ha	9.9 bc
Curzate M	Cymoxanil + Mancozeb	2.5 kg/ha	26.6 c

[1] ha = hectare

Conclusions

It is likely that in the years to come, more countries will face highly variable, sexually reproducing *P. infestans* populations. Consequently, late blight management will become more complicated as the aggressiveness and potential adaptation ability of the pathogen increases. A better understanding of oospore ecology may provide important clues that will enable us to design control strategies specifically targeted at oospores. For the time being, a proper prevention of late blight infections throughout the growing season offers the best control measure available to date. In addition, fungicides may be used to reduce oospore formation and viability, despite the fact that a late blight epidemic is well underway.

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