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THE IMPACT OF OOSPORES OF *PHYTOPHTHORA INFESTANS* ON LATE BLIGHT EPIDEMICS

ABSTRACT

Several aspects of the ecology of oospores of *Phytophthora infestans* were studied in the Nether–lands using both observational and experimental methods. Following the introduction of a variable late blight population in Europe during the 1970s, *P. infestans* epidemics have become more severe, leading to an increase in fungicide use in many potato production areas in Northwestern Europe. In the Netherlands, oospores are readily produced in unsprayed crops and volunteer potatoes and their incidence varied from 78% to 15% of the sampled leaflets with two or more lesions, for the northeastern and southwestern region in 2000, respectively. A fungicide application following in–fection of plants with an A1 and A2 mating type strain significantly reduced the number of oospores produced as well as oospore viability. Several alternative hosts facilitating oospore formation have been identified for the Netherlands: *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium*. The impact of oospores on late blight epidemics is being discussed.

Key words: alternative hosts, fungicides, infection sources, integrated disease management

INTRODUCTION

The oomycete *Phytophthora infestans*, the cause of late blight in pota–toes and tomatoes, is considered to be among the most important patho–gens 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, reduc–ing 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 eco–nomic 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 po–

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tato late blight is heavily dependent on the use of fungicides. Despite the frequent fungicide use, late blight epidemics have proven to be increas–ingly 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 patho–gen (Day and Shattock 1997, Lambert and Currier 1997, Turkensteen *et al.* 1997, Peters *et al.* 1999). 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 ori–gin of *P. infestans* (Fry and Spielman 1991, Goodwin *et al.* 1992). Popula–tions of *P. infestans* outside central Mexico were restricted to asexual reproduction and survived during crop–free periods by existing as myce–lium 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).

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 favour 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, whereas old population isolates caused infections at 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. To

date, production of oospores in field crops have been reported from the following countries: Denmark, Netherlands, Norway, Sweden, Finland, Germany, Poland, Switzerland and Russia. In addition, it is to be expected that oospores are present in several other countries which include: the United Kingdom, Belgium, Luxembourg, Austria, Baltic States, Belarus and Ukraine.

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 climatic 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 favourable 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 (5–10 ha per field) were sampled by random collection of 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 leaflets were microscopically examined for the presence or absence of oospores (Turkensteen *et al.* 2000). The density of oospores per leaflet 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 leaflets 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 × 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* (Fig. 1). Protectant fungicides (except fluazinam) do not have specific activity

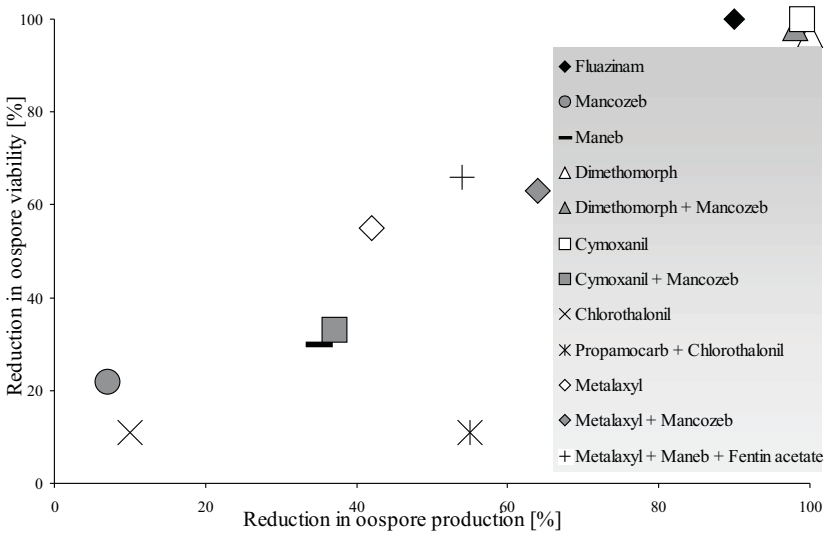

Fig. 1. Results from the *in vitro* experiment on the effect of fungicides on oospore formation. Crosses between *P. infestans* A1 and A2 isolates were carried out in Rye A agar in the presence of sub–lethal concentrations of fungicides. Concentrations were chosen such that the radial growth rate of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

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 was 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 were 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 was no clear separation between non–systemic and (semi) systemic or translaminar fungicides. Absorption of fungicides by necrotic tissue may be responsible for the 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.

Table 1

Influence of fungicides on oospore formation *

Fungicide	Active ingredients	Dose [per ha]	Oospores [per 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

* (oospore density in leaves of potato plants cv. 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 a LSD test at $\alpha = 0.05$)

ALTERNATIVE HOSTS

Oospore formation in plants was first observed in cultivated potatoes in Mexico (Gallegly and Galindo 1958). Later, oospores have been reported from other Solanaceous hosts like tomato (Cohen *et al.* 1997) and the *S. demissum*, one of the native host plant species in the Toluca Valley of central Mexico (Flier *et al.* 2001). Recently, four more host plant

species facilitating oospore formation have been identified in Europe. In Southern Sweden, *Solanum physalifolium,* an annual weed abundantly present in potato fields was reported as a host facilitating oospore formation (Andersson and Johansson 2003). In the Netherlands, lesions of *P. infestans* were found on woody nightshade (*S. dulcamara*), black nightshade (*S. nigrum*) and *S. sisymbriifolium* (Flier *et al.* 2003). In the collected blighted leaf samples, oospores of *P. infestans* were observed (Fig. 2). Oospores were observed in diseased leaves of both *S. nigrum* (46 oospores per cm^2) and *S. dulcamara* (178 oospores per cm^2), collected from roadsides and fields in 2000 and 2001. In *S. sisymbriifolium*, abundant oospore formation was found in diseased leaves taken from a blighted experimental crop in 2000 (297 oospores per cm^2) and after inoculation of detached leaves with A1 and A2 strains (147 oospores per cm^2). Oospore densities were higher in naturally infected leaves as compared to inoculated tissues for all host plant species tested, with average densities of 179 and 102 oospores per cm^2, respectively.

It appears that alternative hosts like *S. physalifolium*, *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* are able to produce oospores in significant quantities. It is therefore conceivable that alternative hosts generate a considerable potential source of (auto) infections in following years.

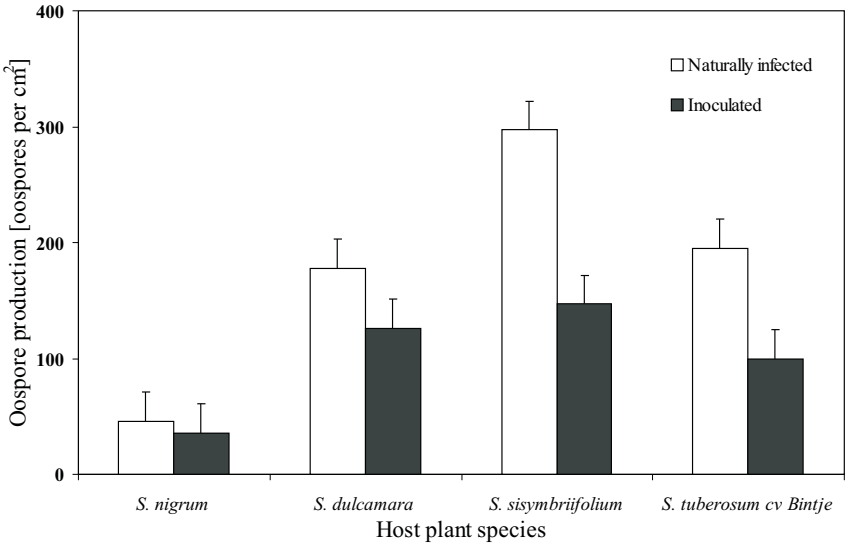

Fig. 2. Oospore production in leaf tissue of four host plant species following either inoculation with compatible A1 (IPO98014) and A2 (IPO428–2) mating type strains

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 both the aggressiveness and potential adaptation ability of the pathogen increase. 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.

REFERENCES

Andersson B., Johansson M. 2003. First report of *Solanum physalifolium* as a host plant for *Phytophthora infestans*. Plant Dis. 87: 1538.

Andersson B., Sandström M., Strömberg A. 1998. Indications of soil borne inoculum of *Phytophthora infestans*. Potato Res. 41: 305–310.

CIP. 1996. Research needed to halt rapidly spreading late blight strains. (In:) CIP in 1995. The International Potato Center Annual Report. Lima, Peru: 10–13.

Cohen Y., Farkash S., Reshit Z. Baider A. 1997. Oospore production of *Phytophthora infestans* in potato and tomato leaves. Phytopathology 87: 191–196.

Cohen Y., Farkash S., Baider A., Shaw D.S. 2000. Sprinkling irrigation enhances production of oospores of *Phytophthora infestans* in field–grown crops of potato. Phytopathology 90: 1105–1111.

Davidse L.C., Looijen D., Turkensteen L.J., Wal D. van der. 1981. Occurrence of metalaxyl–resistant strains of *Phytophthora infestans* in the Netherlands. EPPO Bull. 15: 403–409.

Day J.P., Shattock R.C. 1997. Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. Eur. J. Plant Pathol. 103: 379–391.

Drenth A., Janssen E.M., Govers F. 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. Plant Pathol. 44: 86–94.

Drenth A., Tas I.C.Q., Govers F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. Eur. J. Plant Pathol. 100: 97–107.

Duncan J.M. 1999. *Phytophthora* – an abiding threat to our crops. Microbiology Today 26: 114–116.

Flier W.G., Bosch G.B.M. van den, Turkensteen L.J. 2003. Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*. Plant Pathol. 52: 595–603.

Flier W.G., Grünwald N.J., Fry W.E., Turkensteen L.J. 2001. Oospore production and viability of *Phytophthora infestans* isolates from commercial potato fields and *Solanum demissum* in the Toluca Valley. Mycol. Res. 105: 998–1006.

Flier W.G., Turkensteen L.J. 1999. Foliar aggressiveness of *Phytophthora infestans* in three potato growing regions in the Netherlands. Eur. J. Plant Pathol. 105: 381–388.

Flier W.G., Turkensteen L.J., Mulder A. 1998. Variation in tuber pathogenicity of *Phytophthora infestans* in the Netherlands. Potato Res. 41: 345–354.

Fry W.E., Spielman L.J. 1991. Population biology. (In:) Advances in Plant Pathology; *Phytophthora infestans*, the cause of late blight of potato. Ingram D.S., Williams P.H. (eds.), Vol. 7. Academic Press, London, UK: 171–192.

Gallegly M.E., Galindo J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48: 274–277.

Goodwin S.B., Spielman L.J., Matuszak J.M., Bergeron S.N., Fry W.E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82: 955–961.

Hohl H.R., Iselin K. 1984. Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. Transactions of the British Mycological Society 83: 529–530.

Hooker W.J. 1981. Compendium of potato diseases. American Phytopathological Society, St. Paul, MN, USA.

Jiang J., Erwin D.C. 1990. Morphology, plasmolysis and tetrazolium stain as criteria for determining viability of *Phytophthora infestans*. Mycologia 82: 107–113.

Lambert D.H., Currier A.I. 1997. Differences in tuber rot development for North American clones of *Phytophthora infestans*. Am. Potato J. 74: 39–43.
Niederhauser J.S. 1956. The blight, the blighter, and the blighted. Transactions of the New York Academy of Sciences 19: 55–63.
Peters R.D., Platt W., Hall R., Medina M. 1999. Variation in aggressiveness of Canadian isolates of *Phytophthora infestans* as indicated by their relative abilities to cause potato tuber rot. Plant Dis. 83: 652–661.
Schepers H.T.A.M. 2000. The development and control of *Phytophthora infestans* in Europe in 1999. (In:) Proc. 4th workshop of an European network for development of an integrated control strategy of potato late blight. Oostende, Belgium, 29 September – 2 October 1999: 10–18.
Shaw D.S., Fyfe A.M., Hibberd P.G., Abdel Sattar M.A. 1985. Occurrence of the rare A2 mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with A1 mating types. Plant Pathol. 34: 552–556.
Spielman L.J., Drenth A., Davidse L.C., Sujkowski L.J., Gu W.K., Tooley P.W., Fry W.E. 1991. A second world–wide migration and population displacement of *Phytophthora infestans*? Plant Pathol. 40: 422–430.
Turkensteen L.J., Flier W.G., Baarlen P. van. 1996. Evidence for sexual reproduction of *Phytophthora infestans* in an allotment garden complex in the Netherlands. (In:) Abstracts of conference papers, posters and demonstrations. 13th Trien. Conf. EAPR. Veldhoven, Netherlands, 14–19 July 1996: 356–357.
Turkensteen L.J., Flier W.G., Mulder A., Roosjen Js. 1997. Toegenomen agressiviteit van de aardappelziekte en de MJP–G doelstellingen. Aardappelwereld 51: 16–20.
Turkensteen L.J., Flier W.G., Wanningen R., Mulder A. 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. Plant Pathol. 49: 688–696.