The Role of Oospores in the Epidemiology of Potato Late Blight

B. Andersson, A.-K. Widmark and J.E. Yuen
Swedish University of Agricultural Sciences
PO Box 7026, SE-75007 Uppsala
Sweden

B. Nielsen and S. Ravnskov
Aarhus University
Research Centre Flakkebjerg
Forsøgsvej 1, DK-4200 Slagelse
Denmark

G.J.T. Kessel, A. Evenhuis and L.J. Turkensteen
Plant Research International
Postbus 16, 6700AA, Wageningen
The Netherlands

J.G. Hansen
Aarhus University, Research Centre Foulum
Blichers Allé, Postbox 50
DK-8830 Tjele
Denmark

A. Lehtinen
Agrifood Research Finland
Institute of Plant Protection
31600, Jokioinen
Finland

A. Hermansen, M.-B. Brurberg and B. Nordskog
Bioforsk Plant Health and Plant Protection Division
Hogskoleveien 7, 1432 Ås
Norway

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Abstract

Potato late blight *(Phytophthora infestans)* is a plant disease feared globally by farmers and the potato industry. *P. infestans* is a heterothallic oomycete with two mating types. Until recently the pathogen was limited to surviving between seasons as living mycelia in its host plant in most parts of the world. This was due to the fact that populations of *P. infestans* consisted of only one mating type (A1) in all parts of the world except Mexico, the putative centre of origin of the pathogen. Migration of new genotypes from Mexico, including genotypes of the second mating type (A2) has resulted in that now both mating types can be found worldwide. The formation of oospores is only possible if both mating types coexist. Oospores will give the pathogen the ability of surviving for extended periods of time outside its host, for example in the soil. There are reports of oospore formation under field conditions from many parts of the world. Also, in some places oospores are considered as a new, additional inoculum source and as a consequence the onset of late blight epidemics have become earlier. Oospores are formed through sexual recombination. If they act as a source of inoculum, this will increase the genotypic variation in populations of *P. infestans* leading to an enhanced adaptability of the pathogen. As a consequence, an earlier start of epidemics caused by oospores in the soil and a more aggressive behaviour of the pathogen due to new sexually formed genotypes could make potato late blight even more difficult to control in the future.

INTRODUCTION

Potato late blight is one of the most devastating plant diseases worldwide. It is caused by *Phytophthora infestans* (Mont.) de Bary, a plant pathogen that infects members of the Solanaceae family. Late blight is considered as a fungal disease, but the genus *Phytophthora* is not related to true fungi. It belongs to the oomycetes of the kingdom Straminopila that includes for example golden and brown algae. The oomycetes are a very diverse group of microorganisms that include saprophytes as well as pathogens of most classes of organisms ranging from vertebrates and plants to other oomycetes.

Late blight can have a very dramatic effect on a potato crop. Fast and efficient

spread, infection and colonization of the host plant give the pathogen the potential to destroy all above ground parts of a potato crop within a week. This can result in serious quantitative yield losses. If sporangia are rinsed off the haulm down into the soil by rain or irrigation the potato tubers can also be infected. Tuber infection will reduce the quality of the harvest and can ultimately result in total crop loss. Another very important aspect of this is that infected tubers can act as inoculum sources for late blight epidemics the following season. *P. infestans* is heterothallic with two mating types designated A1 and A2. The coexistence of both mating types enables sexual reproduction and the formation of oospores. These oospores can survive adverse conditions outside the host plant, e.g., in soil and can function as an additional source of infection.

MATING TYPE DISTRIBUTION

The heterothallic nature of *P. infestans* was resolved by Smoot et al. (1958) and Gallegly and Galindo (1958). They found two mating types which they designated A1 and A2. These were able to form oospores when grown together with isolates of the opposite mating type. Smoot et al. (1958) included isolates from the United States, Canada, Western Europe, South Africa, the West Indies and Mexico in their studies of mating types of *P. infestans*. They discovered that all isolates were of the A1 mating type, except for some isolates from Mexico which were of the A2 mating type. In a further study of Mexican *P. infestans* isolates the two mating types were found in an about 1:1 ratio. It was also shown that oospores were formed in potato leaves under field conditions in Mexico (Gallegly and Galindo, 1958). The heterothallic nature and the fact that only one mating type of *P. infestans* could be found outside Mexico gave the answer to the old question as to why oospores of *P. infestans* had only been sparsely found, mostly under artificial conditions by blight researchers in Europe and the United States .

The appearance of the A2 mating type of *P. infestans* in Europe was reported in 1984 (Hohl and Iselin, 1984). This set off mating type surveys all over the world and was soon followed by reports showing that the A2 mating type had spread not only to Europe but worldwide (e.g., Malcolmson, 1985; Shaw et al., 1985; Tantius et al., 1986; Kadir and Umaerus, 1987; Mosa et al., 1989). Like the epidemics of the 1840s caused by the first introduction of *P. infestans* in Europe by import of infected seed, this worldwide emergence of the A2 mating type in the 1980s has been explained by migration (Fry et al., 1993). There are reports of shipping of large quantities potato in the mid 1970s to Europe from regions in Mexico where the A2 mating type is common (Niederhauser, 1991). Although alternative explanations to migration for the global appearance of the A2 mating type outside Mexico have been put forward (Ko, 1994) convincing support for the migration hypothesis has been supplied by genetic studies showing that the first detection of A2 outside Mexico coincides with the appearance of new alleles at different loci in the *P. infestans* genome, e.g., studies of isozyme variation (Spielman et al., 1991) and RLFP fingerprinting (Drenth et al., 1994). The population change was also evident in a shift in the frequency of virulence factors (Drenth et al., 1994).

A Pan-European survey on *P. infestans* has been conducted in the EU Concerted Action "Eucablight" (www.eucablight.org). In this project, data on different characteristics of *P. infestans* have been collated in a database covering most of Europe (Cooke et al., 2006, 2009). The database contains data on phenotypic and genotypic traits and at present especially data on mating type is very well represented. On a European scale it appears that high proportions of the A2 mating type were mainly found in the Nordic countries and in continental Europe, which are areas with cold winters. In a cold climate, dump piles and volunteer plants will be of less importance compared to areas with mild winters making oospore-derived inoculum relatively more important in the former. Frozen soil during winter will also conserve the oospores and synchronise a germination peak with the potato planting in the spring (Widmark et al., 2007). However, there is a recent trend toward a more even distribution of the mating types also in areas where the A2 mating type used to constitute a small proportion of the *P. infestans* population (Détourné et al., 2006; Cooke et al., 2009).

OOSPORE FORMATION UNDER FIELD CONDITIONS

Even though coexistence of both mating types is a prerequisite for oospore formation, skewed mating type ratios can result in a large production of oospores (Cohen et al., 1997). This means that there is a potential for oospore production even in areas where the population is strongly dominated by one mating type. The first observations of oospore formation under field conditions were made in the 1950s in Mexico (Gallegly and Galindo, 1958). In Europe the first reports came 33 or more years later from Germany (Götz, 1991), The Netherlands (Drenth et al., 1993), The United Kingdom (Hanson and Shattock, 1998) and the Nordic countries (Andersson et al., 1998). These were more sporadic observations of oospore formation. To see how common the formation of oospores was, a survey of untreated organic potato field in Sweden and Norway was done during the summer of 2001. Leaves with more than one lesion were collected 1-2 weeks after the first symptoms of late blight had been observed. About 40 fields in south Sweden were surveyed, and samples were frozen directly after sampling and later checked under a dissecting microscope. Formation of oospores was observed in 1/3 of the fields. In Norway the survey showed formation of oospores only after incubation of field leaf samples, giving an indication of the potential for oospore formation (Hermansen et al., 2001; Dahlberg et al., 2002). In 2002, leaf samples were collected in mid Sweden in the same way as in 2001. In this survey, sampling was done about 4 weeks after the first infections of late blight, and here almost all surveyed fields showed oospore formation (Hjelm, 2003). Later, similar surveys in Sweden have shown large differences in oospore formation between locations and years. In Finland infected leaves and stems were sampled in blight foci occurring early in fields where blight had been present in previous potato crops. After incubation, oospore formation was observed in the leaf samples. In some of the stem samples oospores were present without incubation showing that formation could take place also under field conditions (Lehtinen and Hannukkala, 2004). A Dutch field survey of oospore formation was done in 2001 in a similar way. Leaflets with two or more lesions were collected and checked for presence of oospores after incubation for three weeks. The proportion of leaflets with oospores varied between geographical regions from 15 to 78%. This variation was explained by differences in cultivar, soil type and crop rotation (Kessel et al., 2002). The regions with the highest proportion of oospore formation are starch regions with few years between the potato crops.

OOSPORE SURVIVAL UNDER FIELD CONDITIONS

The number of years between susceptible crops in the crop rotation is very important when dealing with soil borne inoculum. Hence, the longevity of oospores in the soil is of fundamental importance for their role as inoculum. Field trial observations have shown that oospore can survive at least two winters under Swedish conditions (Andersson et al., 1998). In an oospore survival experiment, oospores of *P. infestans* from 6 crossings of different Nordic strains of the pathogen on potato leaf discs were buried in soil at 10 cm depth in Finland, Sweden, Denmark and Norway. The oospores were tested for viability after 18, 30, and 38 months in the soil by staining with tetrazolium bromide (Sutherland and Cohen, 1983). The viability tests indicated that oospores of *P. infestans* are capable of surviving at least four winters in the soil under Nordic weather conditions (Nordskog et al., unpublished data). In a Dutch experiment, oospores produced from a single crossing were mixed into the soil. The soil was put into pots, which were buried in a field. Survival of the oospores was checked with a leaf baiting method developed by Drenth et al. (1995) which showed a survival of up to 4 years (Turkensteen et al., 2000). Using the same method Fernandez-Pavia et al. (2004) reported a survival of two years in Mexico, but observed a decline in vitality and infectivity in the oospores over time. In an artificially inoculated field trial in Uppsala, Sweden, soil samples were collected 1, 7, 10 and 18 month after harvest. By the baiting the soil samples *P. infestans* could be isolated until 10 months after harvest. Samples taken after 18 month yielded no isolates (unpublished data). Lehtinen and Hannukkala (2004) used the baiting method to

determine oospore occurrence in Finnish soil samples. Of samples taken in 16 infection foci only 3 gave infection on the baiting leaves, but the results indicated that oospores could survive in the soil at least to the next growing season.

OOSPORES AS SOIL BORNE INOCULUM OF *P. INFESTANS*

By crossing isolates of opposite mating type large amount of oospores of *P. infestans* can be produced. However, the proportion of oospores that will germinate varies as reported by e.g., Smoot et al. (1958), Chang and Ko (1991), Pittis and Shattock (1994), Strömberg et al. (2001) and van Bekkum et al. (2007). In these studies the germination ratio in untreated oospores was found to be from below 1% to 25%. Different treatments had mostly limited effect on the germination in these studies. Higher soil temperatures seem to increase oospore germination (Pittis and Shattock, 1994; van Bekkum et al., 2007). Soil moisture is necessary for germination although water saturation of soil proved to decrease the ratio of germinating oospores (van Bekkum et al., 2007).

It is difficult, if at all possible to distinguish infections originating from infected tubers from oospore derived infections by the disease symptoms in the field. There are however, some characteristics of epidemics that indicate that oospores serve as primary inoculum of *P. infestans*. Such indications are that the spatial distribution of infection foci in a field corresponds with infections in the previous potato crop (Andersson et al., 1998; Turkensteen et al., 2000), both mating types are present within a field early in the epidemic (Drenth, 1995) or first lesions appear low in the crop, particularly on leaves touching the soil (Lehtinen and Hannukkala, 2004).

In the period 1999 till 2005, a project on monitoring early outbreaks of late blight in Dutch potato fields was performed. In the course of eight years, 184 fields with primary foci were surveyed and the farmers concerned were interviewed according to a question list, concerning amongst others crop history, spray schedules and crop rotation. In total 2075 isolates of *Phytophthora infestans* were collected and stored in liquid nitrogen. Mating type, haplotype and AFLP fingerprints were determined. Based on all information gathered, a classification concerning the origin of initial inoculum and timing of infections was made. In 41 fields oospores were considered the primary inoculum source (Evenhuis et al., 2007).

In southwest Sweden, some fields have been used for early potato production every year for 50 years or more. Late blight was earlier not considered a problem since the potato was harvested before the blight appeared. However, from the early 1990s, fields with late blight were found in this area. The early potato is grown under fleece and the symptoms were observed as soon as the cover was removed. The infections could be very severe with plants completely killed by blight. In some fields disease foci could be found in the same location from year to year indicating a soil borne inoculum source. A more solid indication of oospores functioning as primary inoculum of *P. infestans* came from a potato trial located in Uppsala, Sweden during 1996. In 1994 a potato field trial was inoculated with *P. infestans* isolates of different origin. In the trial different fungicide dosages were applied. At the last blight assessment the untreated plots in the trial were completely killed by blight. In the areas treated with the highest amounts of fungicides about 0-0.1% of the leaf area was infected by blight. In 1996 a similar trial was established in the same field, partly overlapping the trial from 1994. Just two weeks after emergence heavy infections of late blight were observed and the infection foci corresponded almost perfectly with the infections in the 1994 trial (Andersson et al., 1998).

To compare different inoculum sources of *P. infestans* under natural conditions, field trials were established in Sweden in 1999. Oospores produced artificially in potato leaves were used to infest the soil in plots which were planted with healthy seed tubers. In other plots artificially infected seed tubers and healthy seed tubers were planted in soil free of oospores. The trials were monitored for first occurrence of late blight. The results indicated that oospores could act as inoculum and that they gave earlier infections than inoculum coming from infected seed tubers or from sources outside the field (unpublished

data).

Another approach to determine the source of the primary inoculum was taken by a genotype distribution study done of isolates collected in discrete infection foci in a single potato field located in southwest Sweden (Widmark et al., 2007). Mitochondrial DNA haplotype, mating type and SSR-genotype were determined and it was shown that some foci were monomorphic for all markers, while other foci displayed a large proportion of unique genotypes. This was interpreted as evidence that inoculum had come from both tubers and oospores within this field.

The importance of the crop rotation for the date of first incidence of late blight has been demonstrated in a Danish field survey. This survey showed that a short interval (1-2 years) between the potato crops in a field resulted in earlier occurrence of late blight compared to fields where potato was grown with longer intervals (Bødker et al., 2006). A more elaborate long term study of Finnish late blight epidemics showed that the epidemic onset was 9 days earlier in a field were potato was grown after potato as compared to a field with alternate crops between the potato crops in data from the period 1998-2002, while no difference could not be observed in data from 1992-1997 (Hannukkala et al., 2007). This indicates that oospores have had an impact on the start of recent potato late blight epidemics in Finland. Interestingly, the Finnish study also showed that although the blight epidemics had become generally earlier there had been no change in the rate of epidemic development.

Results point to the fact that oospores in soil can germinate at a slow rate whenever the conditions are favourable, i.e., wet soil with a temperature about 10°C. This means that inoculum will be at hand for most of the growing season if oospores are present in the soil. The sporangia produced by the oospores can infect the growing potato crop by leaves touching the soil or by soil splashing up on the leaves. In this way oospores can function as a source of inoculum not only at the time of crop emergence but all through the growing season continuously supplying the pathogen population with new genotypes. This could be an explanation for a genetically diverse population of *P. infestans* despite low number of fields with suspected oospore derived first infections. Another aspect of this is that it would not be impossible for tubers being directly infected from oospores, i.e., tuber infection could occur without any late blight on the haulm (van Bekkum et al., 2007).

CONCLUSIONS

Sexual reproduction is common in *P. infestans* in regions like the Netherlands and the Nordic countries. In these regions both mating types coexist and oospores are formed in field crops and serve as inoculum.

The ability of different parent isolates to produce oospores varies and some A1-A2 combination will fail to produce oospores altogether (Cohen et al., 2000; Flier et al., 2001; van Bekkum et al., 2007). Also some specific combinations of parent isolates will produce more oospores than others (Flier, 2001; van Bekkum et al., 2007). It is also likely that both individual isolates and the combination of parental isolates will influence the ability of the formed oospores to germinate and infect.

Oospores of *P. infestans* can have both direct and indirect effects on potato late blight epidemics. The direct effect is caused by oospores functioning as a soil borne source of inoculum. This will mean that the pathogen will change from being confined to overwintering only as living mycelia in tubers to being able to survive between seasons independent of a host. For practical control of potato late blight this means that oospores have to be considered as an additional soil borne source of inoculum. The indirect epidemiological effect of oospores comes from the increased genetic variation in populations of *P. infestans* as a result of sexual recombination. Sexual reproduction will allow for selection to act on individual genetic traits, unlike asexual reproduction that only can act upon the entire genetic makeup of an organism. This enables separation of harmful mutations from beneficial ones and the combination of beneficial mutations from separate ancestries. The coexistence of sexual recombination (new genotypes) and clonal

propagation (maintain and spread of successful genotypes) gives a high evolutionary potential in *P. infestans* (McDonald and Linde, 2002). This might manifest itself in a late blight which will spread faster in the potato fields, is more tolerant to fungicides and that is able to infect cultivars with higher levels of resistance resulting in a disease even harder to control than at present.

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