

**Sexual reproduction in**  
***Phytophthora infestans***

**– epidemiological consequences**

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## Abstract

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Potato late blight, caused by the oomycete *Phytophthora infestans*, is one of the most devastating plant diseases worldwide. It is becoming increasingly difficult to control the disease in Sweden, leading to an ever more intensified use of fungicides in the potato production. This unsustainable development could be caused by changes in the pathogen due to the introduction of sexual reproduction. These changes could result in an enhanced capability of the pathogen to adapt to different factors, e. g. weather, cultivar resistance, chemical control or other cultivation measures. This might manifest itself in earlier infections and a late blight that spreads faster in the potato fields requiring increasing efforts to control.

Different approaches were taken to clarify the role of sexual reproduction in *P. infestans* in the epidemiology of potato late blight. The formation of sexually formed oospores and their ability to serve as primary inoculum was studied, both as field observations and by determining within-field genotypic diversity. To further estimate the importance of reproduction on a large spatial scale a population study based on molecular and phenotypic markers was performed on *P. infestans* isolates from the whole Nordic region. The variation in the aggressiveness of Nordic populations of *P. infestans* was studied by determining different components of aggressiveness, e.g. sporulation capacity, lesion growth and infection efficacy. The aggressiveness study was combined with an analysis of the phenotypic structure of the Nordic population of *P. infestans*.

The results clearly indicate that the Swedish populations of *P. infestans* are influenced by sexual reproduction. The facts that both mating types are found all over the country in near 1:1 proportions and that oospores are commonly formed in field crops and serve as inoculum under field conditions support this. Studies of the genetic diversity also indicate that sexual reproduction has an effect on the population structure of *P. infestans* in Sweden and the Nordic region as a whole.

*Keywords:* Potato late blight, *Phytophthora infestans*, potato, epidemiology, oospores, sexual reproduction

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## Appendix

This thesis is based on the following papers, which will be referred to by their Roman numeral:

- I. Andersson, B., Sandström, M. & Strömberg, A. 1998. Indications of soil borne inoculum of *Phytophthora infestans*. *Potato Research* 41, 305-310.
- II. Widmark, A.-K., Andersson, B., Cassel-Lundhagen, A., Sandström, M. & Yuen, J.E. 2007. *Phytophthora infestans* in a single field in southwest Sweden early in spring: symptoms, spatial distribution and genotypic variation. *Plant Pathology* 56, 573-579.
- III. Andersson, B., Sandström, M., Flier W., Bødker, L. Hannukkala, A., Hermansen, A, Yuen, J.E. & Högberg, N. Analysis of population structure of *Phytophthora infestans* in the Nordic countries using AFLP markers (Manuscript)
- IV. A. Lehtinen, B. Andersson, A. Hannukkala, A. Hermansen, R. Naerstad, V. H. Le, M. B. Brurberg, B. J. Nielsen, J. G. Hansen, E. Ketoja & J. Yuen. Foliar aggressiveness of *Phytophthora infestans* in four Nordic countries (Manuscript)
- V. A. Lehtinen, A. Hannukkala, B. Andersson, A. Hermansen, V. H. Le, R. Naerstad, M. B. Brurberg, B. J. Nielsen, J. G. Hansen & J. Yuen. Phenotypic variation in Nordic potato late blight populations in year 2003. (Plant Pathology, accepted for publication)

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## Description of the thesis

The overall aim of this thesis is to study how the possibility of sexual reproduction in *Phytophthora infestans* (Mont.) de Bary has affected the epidemiology of potato late blight. The work was done in the context of improving the understanding of the reasons behind the increasing problems to control the disease in Swedish potato production.

The thesis includes a historical background describing the appearance of potato late blight in Europe and Sweden and the ensuing debate on the cause of the disease. Changes in *P. infestans* due to the introduction of sexual reproduction are discussed by comparing the “old” and the “new” late blight situation. The basis of the thesis is the work done in Sweden and the Nordic countries concerning different aspects of sexual reproduction in *P. infestans*. Both direct consequences in the form of a new inoculum source (oospores) and the more long term effect of sexual recombination and increased genetic variation is studied in the five papers included.

Different approaches were used to evaluate the impact of sexual reproduction in *P. infestans*. The importance of oospores as an inoculum source of *P. infestans* was studied by mapping potato late blight occurrence in relation to infections in a previous potato crop (Paper I) and by determining the within-field genotypic distribution (Paper II). The possible effect of sexual reproduction on the population structure of Nordic isolates of *P. infestans* was investigated using phenotypic and genotypic (AFLP) markers (Paper III). The variation in the aggressiveness of Nordic isolates of *P. infestans* (Paper IV) and the phenotypic makeup of Nordic populations of *P. infestans* was studied (Paper V).

## Introduction

### The biology of *Phytophthora infestans*

Potato late blight is one of the most devastating plant diseases worldwide. It is caused by *Phytophthora infestans*, a plant pathogen that infects members of the *Solanaceae* family and is economically significant on potato and tomato. Late blight is considered by most researchers as a fungal disease, but the *Phytophthora* genus 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 characterized by their capability of releasing heterokont asexual zoospores formed in sporangia. The cell walls of oomycetes contain cellulose unlike the true fungi which contain chitin. Oomycetes are diploid for the major part of their life cycle, and sexual reproduction occurs by the development of oospores that result from the joining of oogonia and antheridia. The oomycetes are a very varied group of microorganisms that include saprophytes as well as pathogens of most classes of organisms ranging from vertebrates and plants to other oomycetes.

During the epidemic phase *P. infestans* is spread by airborne sporangia which are formed on infected plants. Under conditions with moderate temperature (~20°C) and high air humidity (>90% RH) massive numbers of sporangia can be formed in an infected crop. The sporangia are usually released in the mornings when the rising temperature causes a sharp decrease in the air humidity. The sporangia are then spread by air movements. When they are deposited on a susceptible host plant they will, under moist conditions (free water on the leaf surface), infect. The infection can take place either by direct infection by the sporangium itself or by indirect infection via the release of 5 to 10 motile zoospore per sporangium, each which in turn can infect the host plant. The breaking point between direct and indirect germination appears to be around 15°C, (Harrison, 1992; Judelson & Blanco, 2005) with indirect infection being most common below this temperature and direct infection dominating above 15°C. At germination the sporangia and zoospores form germ tubes and infect by the formation of appressoria and penetration pegs. After penetration an infection vesicle is formed and the hyphae will grow both inter- and intracellularly and develop haustoria to extract nutrients from within the cells of the host and thus destroy the plant tissue (Grenville-Briggs & van West, 2005). After a latent period as short as 3 days (Flier & Turkensteen, 1999; Carlisle *et al.*, 2002) new sporangia are formed and spread to infect new plants. The fast and efficient spread, infection and colonization of the host plant gives the potential of destroying all above ground parts of a crop within a week. Depending on when this happens in the crop development cycle, the result can be very serious quantitative yield losses. The potato tubers can also be infected if sporangia are rinsed off the haulm down into the soil (de Bary, 1876; Lacey, 1965; Andrivon, 1995). Tuber infection will reduce the quality of the harvest and if a large proportion of the tubers are infected it can result in total crop loss. Another very important aspect of this is that infected tubers can function as inoculum sources for late blight epidemics the following season.

*P. infestans* has two mating types designated A1 and A2, and can usually undergo sexual reproduction only if both mating types are present. Self fertile isolates of *P. infestans* can be found, but their role and importance is not fully understood (Smart *et al.*, 1998). In addition to the increased genetic variation by recombination, sexual reproduction also gives the pathogen the possibility of surviving between seasons in the soil in the form of oospores.

### **Origin of *P. infestans***

It is believed by most researchers that *P. infestans* originates from the Mexican highlands. Originally, it was only here that both mating types could be found and at a 1:1 ratio. The population of *P. infestans* in this area has been found to be very diverse, both phenotypically and genetically (Goodwin & Drenth, 1997; Grünwald *et al.*, 2001). Also, the numerous native *Solanaceae* species resistant to *P. infestans* found in Central Mexico indicates that this area is the region of origin of the pathogen (Niederhauser, 1991). However, based on studies of mitochondrial and nuclear loci it has been suggested that *P. infestans* has its origin in the Andean parts of South America (Gómez-Alpizar, Carbone & Ristaino, 2006).

## The “old late blight”

### The Irish potato famine

The potato was introduced in Europe in the 16<sup>th</sup> century. Although the potato had suffered some problems with diseases it was regarded as a reliable crop, but in 1845 a new unknown disease made its arrival, killing the haulm and destroying the tubers in the fields. The dramatic appearance of the “potato murrain” (as the disease was called) in Europe resulted in human despair in many countries such as The Netherlands, Switzerland and France, but the consequences of the disease in Ireland were by far the worst. The Irish cottagers were more or less completely dependent on the potato to produce a subsistence diet on their small plots of land. During 1845 – 1850 the Irish potato harvest shrunk to almost nothing due to direct damage and lack of seed caused by the blight. The loss of the potato crop resulted in famine and the death of more than 1 million persons. This in turn caused a wave of immigration of 1.2 million Irish, primarily to USA (Eriksson, 1916; Large, 1946; Salaman, 1949; Bourke, 1991; Turner, 2005). The Irish population is to this day not as big as it was before “The Potato Famine”.

It has been suggested that *P. infestans* reached Europe directly from Mexico (Andrison, 1996) or by way of the United States (Fry *et al.*, 1993; Goodwin, 1994). The *P. infestans* population in Europe was until recently characterized by low genetic variation caused by founder effects by the introduction of a few “individuals”. By seed trade these clonal lineages were then spread worldwide (Fry *et al.*, 1993).

### The cause of the potato murrain

The outbreak of the potato blight was followed by a long and heated discussion about the cause of the disease and how it could be controlled. The wet summer of 1845 and deterioration of the potato due to vegetative propagation were first considered as the reasons behind the disease. Other proposed explanations for the cause of the blight ranged from industrial pollution or gases from sulphur matches to contaminants from outer space. Interestingly, the true origin of the disease was proposed already in the autumn of 1845 in Belgian newspapers. It was reported by several authors that they had observed a fungus of the *Botrytis* family in diseased potato leaves, and they claimed this was the cause of the blight. Furthermore, some of these early publications also suggested removal of infected foliage as a mean of control (Bourke, 1991). Even though the fungal theory had strong advocates such as Miles J. Berkeley in England and Charles Morren in Belgium (Large, 1946) it had by the end of 1847 lost most of its supporters and it was believed that the observed fungus was a consequence of the disease and not the cause. A fungal origin of the disease received new and strong support when Anton de Bary published his findings in 1861 and 1863. In two papers he described the life cycle of *Peronospora infestans*, as the blight fungus now had been classified. He reported having infected healthy potato leaves with spores and followed the infection process and the ensuing production of new spores. By burying healthy

tubers in soil and shaking blighted potato haulm over them and watering he showed that the spores could be washed down into the soil and infect the tubers. He also put the blight fungus in a new genus, *Phytophthora* in the *Peronosporaceae* family (de Bary, 1876; Large 1946). In some ways, the work on potato late blight by pioneers such as Berkeley and de Bary laid the foundation for the modern science of plant pathology, where micro-organisms were recognized as the cause of disease in plants, and were not merely present as part of the decay process (Whetzel, 1918).

One thing that was still not resolved was the overwintering of the fungus. Three main hypotheses were put forward: (1) the mycelia survived as a saprophyte, (2) the mycelia survived in infected tubers; (3) oospores functioned as survival organs. de Bary argued in 1861 that *P. infestans* survived as living mycelia in tubers. These infected tubers would survive the winter in the field or in storage and give rise to infected plants the following season (de Bary, 1876). However, the matter of overwintering of *P. infestans* in tubers was under much debate for several decades. Even though de Bary (1876) showed that *P. infestans* could grow from diseased tubers into sprouts it proved difficult to find plants infected from tubers in the field (Hirst, 1955). By detailed investigations under field conditions it was shown by Hirst (1955; 1960) and van der Zaag, (1956) that the proportion of infected plants emerging from infected tubers was very low. They also showed that very few infected plants were required to start a late blight epidemic.

### **The “potato disease” in Sweden**

Potato was introduced in Sweden in the beginning of the 1700s. As in many European countries the potato crop was treated with much suspicion and used primarily for animal feed. The establishment of the potato as a human staple food was aided by Swedish soldiers returning from the Pomeranian War (1757-1762) during which they had learnt to grow and appreciate potato (Westrin, 1915). From the beginning of the 1800s the cultivation of potato in Sweden increased and reached a peak around the year 1900 with 150 000 ha. The current figure (2007) is around 30 000 ha.

The potato disease reached Sweden in the autumn of 1845 and caused damage particularly in the south and southwest parts of the country. Surveys were initiated already the same year by the Royal Academy of Agricultural Sciences. Renowned mycologists were sent out to different parts of the country to determine the distribution and damage of the disease. In addition to this a questionnaire, “Question on the Potato-disease” was sent to The Rural Economy and Agricultural Societies in important agriculture areas. The outcome of these efforts was collated and presented to The Royal Academy of Agricultural Sciences in a report (Wahlberg, 1847). In this report many international theories of the cause of the disease were presented. However, it was concluded that it was very difficult to ascertain the true cause of the disease. Factors such as wet weather, poor seed health and excessive use of non-composted manure were suggested to have contributed to the appearance of the disease. A fungal origin of the disease was

dismissed by the author. He did not believe that the diseased foliage and visible symptoms on the tubers had the same cause, and argued that the disease overwintered in seemingly healthy tubers. Just like in the rest of Europe fungi were believed to be a result rather than a cause of the potato disease. The degeneration of the potato crop by clonal propagation was put forward as the true cause of the potato plague by the most prominent mycologist in Sweden at that time, Professor E. Fries (Fries, 1845).

### **The search for oospores**

Observations made by de Bary on other *Peronosporales* species showed the ability of this family of fungi to produce sexual spores, so called oospores. de Bary and his followers tried to find oospores of *Phytophthora infestans* in leaves killed by blight and in infected tubers for decades without success (de Bary, 1876; Large 1946; Turner, 2005).

In the late 1800s several advances were made in the handling of microorganism under laboratory conditions. One important example of this is the development of artificial media suitable for fungal culturing (Large, 1946). These new media enabled new ways of maintaining and studying *P. infestans*. One of the results of this was reports of oospore formation in the beginning of the last century (Jones, 1909; Jones & Giddings, 1909; Clinton, 1911). Common for these reports is that the oospore formation was observed when *P. infestans* was cultured on specific media (oat or lima bean) and that it was more common in old cultures. Also, the number of formed oospores was usually very low. Clinton (1909) also reported that other *Phytophthora* species were able to induce oospore formation in *P. infestans*. These reports have been disputed by Goodwin & Drenth (1997) as observations of other oomycetes or not truly sexually formed oospores of *P. infestans*.

Male and female mycelial strains in *P. infestans* had been discussed (Clinton, 1911; Eriksson, 1916), but the heterothallic nature of the pathogen was resolved by Smooth *et al.* (1958) and Gallegly & 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. It was later suggested that hormones were responsible for inducing the formation of sexual organs (antheridia and oogonia) in *P. infestans* (Ko, 1988). However, self fertile isolates in *P. infestans* are not uncommon (Tantius *et al.*, 1986; Goodwin & Drenth, 1997, Smart *et al.*, 1998). In Swedish isolates of *P. infestans* the proportion of self fertility has been found to be around 2-3 % (ca 1200 isolates, Andersson & Sandström, unpublished data). The possible importance of oospores formed by self fertile isolates is not clear.

## The “new late blight”

### New migrations of *P. infestans* - population shifts

Smoot *et al.* (1958) included isolates from the United States, Canada, western Europe, South Africa, the West Indies (105 isolates) and Mexico (4 isolates) 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 95 Mexican *P. infestans* isolates it was found that the two mating types were found in an about 1:1 ratio (Gallegly & Galindo, 1958). It was also shown that oospores were formed in potato leaves under field conditions in Mexico. 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 & 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 & Umaerus, 1987; Mosa *et al.*, 1989). Like the epidemics of the 1840s, this worldwide appearance of the A2 mating type in the 1980s has been explained by migration. 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 worldwide 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 coincides with the appearance of new alleles at different loci (Goodwin & Drenth, 1994). Examples of this are studies of isozyme variation (Spielman *et al.*, 1991) and RLFP DNA fingerprinting (RG-57) (Drenth *et al.*, 1994) that showed a marked increase in variation when comparing isolates sampled before respectively after the early 1980s. This change was also evident in a shift in the frequency of virulence factors (Drenth *et al.*, 1994).

Griffith & Shaw (1998) showed that the mitochondrial haplotype Ib was associated with the A1 mating type, isozyme genotypes Gpi-1 86/100 and Pep-1 92/100 and the multilocus RG57 fingerprint of the clonal lineage US-1, all of which associated haplotype Ib with the old population of *P. infestans*. Later studies of herbarium material have suggested that Ib was not predominant in the European and US *P. infestans* populations in the 1800s. However, in isolates sampled from 1950 to 1970 the Ib haplotypes is common, implying that migrations could have taken place also between the 1840s and 1970s (May & Ristaino, 2004).

A Pan-European survey on *P. infestans* has been conducted in the EU Concerted Action “Eucablight” ([www.eucablight.org](http://www.eucablight.org)). In this project, data on different characteristics of *P. infestans* have been collated in a database covering most of

North and middle Europe (Cooke *et al.*, 2006). 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 are 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. Frozen soil during winter will also conserve the oospores and synchronize a germination peak with the potato planting in the spring (Widmark *et al.*, 2007). Sexual reproduction and oospores acting as primary inoculum should create a more even distribution of the mating types compared to more clonal populations (Smart *et al.*, 1998) and this could explain the differences in mating type distribution in Europe. 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).

### **Asexual vs. sexual reproduction in *P. infestans***

The coexistence of both mating types enables formation of oospores under field conditions. The formation of oospores turns *P. infestans* into a soil borne pathogen. An additional infection source giving early infections located low in the crop where they are difficult to control can have a definite epidemiological effect on late blight. There are a number of reports of oospore formation under field conditions from different European countries, Germany (Götz, 1991), The Netherlands (Drenth *et al.*, 1993), UK (Hanson & Shattock, 1998) and the Nordic countries (Andersson *et al.*, 1998; Dahlberg, 2001; Dahlberg *et al.*, 2002; Lehtinen & Hannukkala, 2004). The quantitative importance of oospores for initiating epidemics of *P. infestans* is not clear. It is not possible to distinguish infections originating from infected tubers from oospore derived infections by the disease symptoms. There are however, some characteristics of epidemics that indicate that oospores serve as primary inoculum of *P. infestans*. Such indications can be that the spatial distribution of infection foci in a field corresponds with infections in the previous potato crop (Andersson, Sandström & Strömberg, 1998a, 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 & Hannukkala, 2004).

Despite being restricted to asexual reproduction, *P. infestans* was able to form different clonal lineages that could infect potato cultivars with race specific resistance and develop fungicide resistance. Asexual reproduction will maintain and multiply successful genotypes, and with an effective system of reproduction and spread like in *P. infestans* any genotype that has some kind of advantage in relation to other genotypes will be selected and might quickly come to dominate a population. Sexual recombination will result in an increased genetic variation, and 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 sexual recombination to separate harmful mutations from beneficial ones and to combine beneficial mutations from separate ancestries. The

combination of sexual recombination (new genotypes) and clonal propagation (maintain and spread of successful genotypes) will further enhance the evolutionary potential of *P. infestans* (McDonald & Linde, 2002).

## **Studies of *P. infestans* in Sweden and the Nordic countries**

In the late 1980's and early 1990 there were several observations of "unexplainable" late blight infections in both commercial and experimental fields in Sweden. These reports were characterized by observations of very early infections, first observation of late blight in the same location in field as infections in the previous potato crop and a spatial distribution of the disease foci within the field which could not be explained by tuber borne inoculum.

### **Mating type distribution**

The changed behaviour of late blight indicated that oospores were a new source of inoculum of *P. infestans*, and as a consequence surveys of the mating type incidence and distribution were initiated (Andersson, Sandström & Strömberg, 1998b; Sandström & Andersson, 1998a; Sandström & Andersson, 1998b; Andersson & Sandström, 1999; Andersson, Sandström & Yuen, 1999; Andersson & Sandström, 2000; Andersson & Strömberg, 2001). The results of these investigations showed that both mating types were present, that they were evenly distributed all over Sweden and that they could often be found within the same field (Figure 1). Overall about 1200 isolates were sampled in 1998-2003 showing a close to 1:1 ratio of the A1 and A2 mating types. Also in Finland and Norway surveys on mating type were done (Brurberg *et al.*, 1999; Hermansen *et al.*, 2000). These surveys showed a high proportion of the A2 mating type in some regions of these two countries. Contrary to the Swedish results with more or less even mating type distribution all over Sweden no isolates of the A2 mating type were sampled in the northern parts of Finland and Norway.

### **Oospore formation by *P. infestans***

During the summer of 2001 formation of oospores in untreated organic potato field was investigated in Sweden. About 40 fields in south Sweden were surveyed. Leaves with more than one lesion were collected 1 – 2 weeks after first symptoms of late blight were observed. The samples were frozen and later checked under the dissecting microscope. Formation of oospores was observed in 1/3 of the fields (Dahlberg, 2001). 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). This indicates that oospore formation is common in non-fungicide treated Swedish potato fields left standing with late blight for longer periods of time. However, later similar surveys have shown large differences in oospore formation between years.

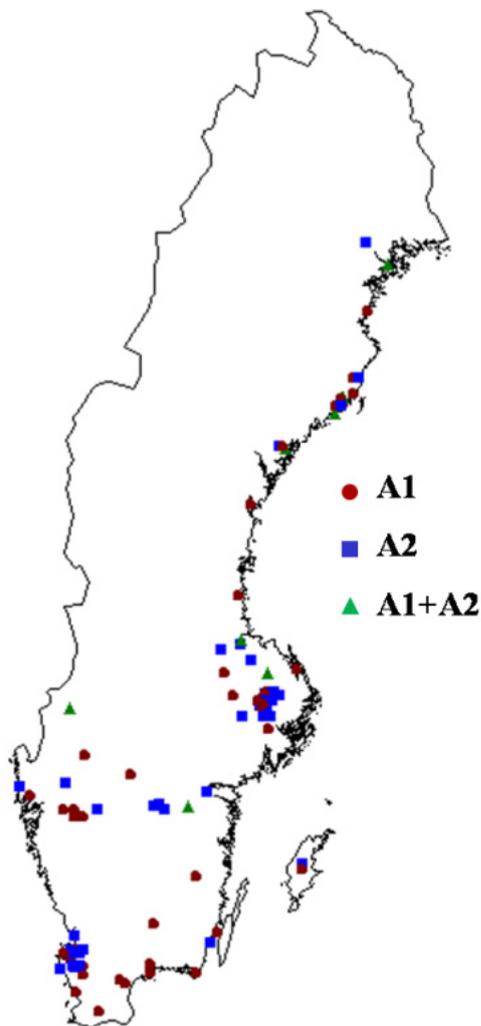


Figure 1. Distribution of mating types of *P. infestans* in Sweden 1998. Each point represents a single sampled field

Oospore surveys done in Norway have shown formation of oospores after incubation of field leaf samples. Only few samples showed oospores without incubation (Dahlberg *et al.*, 2002). 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 & Hannukkala, 2004).

### **Soil borne inoculum of *P. infestans***

Early potato has been produced in the Kullen and Bjäre regions in Southwest Sweden since the early 1900s. Some fields have been used for early potato production every year for 50 years or more. Late blight was previously not considered a problem since the potato was harvested in May and June before the blight appeared. However, since the early 1990s fields with late blight have been found. 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.

The first more solid indication of oospores functioning as primary inoculum of *P. infestans* in Sweden came from a potato trial located at Ultuna, Uppsala during 1996. Two years earlier, in 1994, a potato field trial was inoculated with *P. infestans* isolates of different origin. The resulting epidemic of late blight was very severe, and 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, see figure 2. Tuber samples taken in different plots in the trials showed 40 – 50% infected tubers in the plots with the most severe blight infection, and about 1 – 3% infected tubers in the plots with low levels of blight on the haulm. The haulm and the tubers were left in the field and ploughed under. 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 with plants in some areas completely killed by blight (Figure 2). The infection foci in this trial corresponded almost perfectly with the infections in the 1994 trial. This is a clear and strong indication of soil borne inoculum (Andersson *et al.*, 1998 [Paper I])

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 single field located in southwest Sweden (Widmark *et al.*, 2007 [Paper II]). 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 (Figure 3). This was interpreted as evidence that inoculum had come from both tubers and oospores within this field.



Figure 2. Aerial photograph of the potato trial at Uppsala 1996 and close up of areas heavily affected by late blight. Rectangle in black shows the location of a late blight trial in 1994.

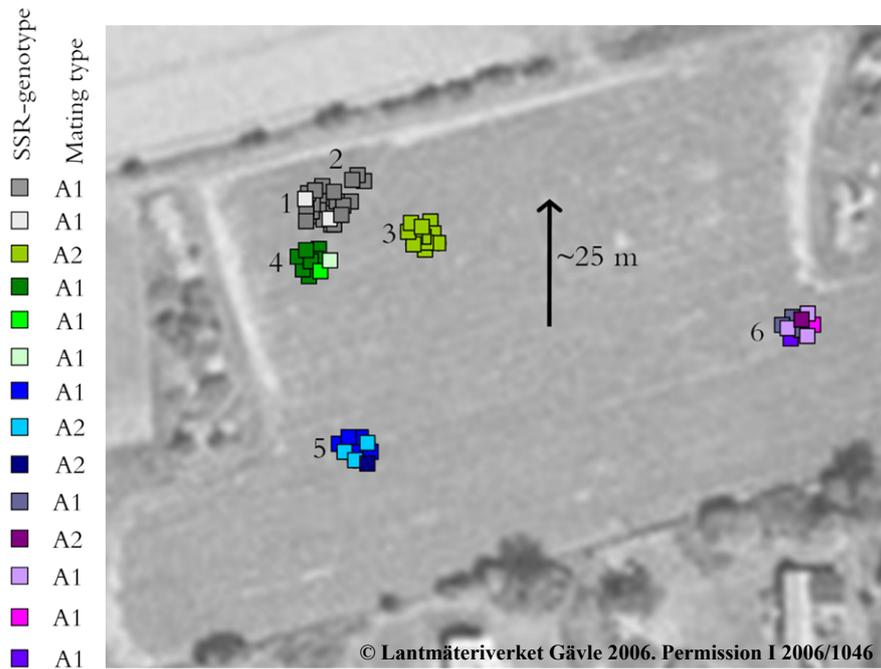


Figure 3. Aerial photograph of the sampled field in southwest Sweden in 2001 with SSR-genotypes of *P. infestans* in the different foci indicated by colours.

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. The observations from the trial in Ultuna 1996 showed that oospore can survive at least two winters under Swedish conditions. Dutch results showed a survival under field conditions of up to 4 years (Turkensteen *et al.*, 2000). Fernandez-Pavia *et al.* (2004) reported a survival of two years in Mexico, but observed a decline in vitality and infectivity in the oospores. In an artificially inoculated field trial in Uppsala soil samples were collected 1, 7, 10 and 18 month after harvest. After up to 10 months *P. infestans* could be isolated with a baiting method developed by Drenth *et al.* (1995). Samples taken after 18 month yielded no new isolates (Sandström & Andersson, unpublished data). Lehtinen & Hannukkala (2004) used the same baiting method to determine oospore occurrence in soil. From soil samples taken in 16 infection foci only 3 were infective, but the results indicated that oospores could survive in the soil to the next growing season. However, results from the baiting method used here have proven hard to reproduce, probably due to low sensitivity (Lehtinen & Hannukkala, 2004). There are several suggested ways of improving the sensitivity – freezing/thawing, treatment with  $\text{KMnO}_4$  (Chang & Ko, 1991), different incubation temperatures and incubation under different light regimes. None of these have in our experience resulted in any marked improvement of the performance of the method (Hjelm, 2003).

To compare different inoculum sources of *P. infestans* under natural conditions field trials were established in 1999 and 2001. 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 function as inoculum and that they gave earlier infections than inoculum coming from infected seed tubers or from sources outside the field (Yuen & Andersson, unpublished data).

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 in Finland showed that the epidemic onset was 9 days earlier in fields where potato was grown after potato as compared to fields with alternate crops between the potato crops (Hannukkala *et al.*, 2007).

### **Genetic diversity**

Drenth *et al.* (1994) showed in the beginning of the 1990's that sexual reproduction of *P. infestans* took place in the Netherlands and that the genetic diversity had increased after 1979. Studies based on mating type and RFLP (RG-57) of Norwegian and Finnish isolates collected in 1992-96 revealed a large

genetic diversity, and this was interpreted as an indication that sexual reproduction was contributing to the genetic variation of *P. infestans* in the Nordic countries (Brurberg *et al.*, 1999).

A population study based on mating types, mitochondrial DNA haplotype and AFLP-genotype conducted on isolates sampled in 1999 from Denmark, Sweden, Finland and Norway confirmed that the genetic and genotypic diversity was high within the four countries with small differences between the countries. The isolates in the investigation were taken from fields where oospore derived infections could be suspected. The absence of any real population structure and the large variation among and within disease foci in the same field all points towards oospores functioning as a inoculum source in Nordic potato fields (Andersson *et al.*, [Paper III]). A within field study was done in an early potato field in southwest Sweden. The genotypic diversity was determined in isolates sampled from discrete late blight foci ranging in size from 3-4 m<sup>2</sup> (Widmark *et al.*, 2007 [Paper II]). In total 61 sampled isolates yielded 14 multilocus SSR genotypes. The genotypic variation varied between the disease foci. As a measure of the genotypic variation the normalized Shannon index was calculated. This index gives a value of 0 if all isolates are of the same genotype and a value of 1 if all isolates are unique genotypes. Within this single field the Shannon index varied from 0 to 0.67 in the individual disease foci displaying high variation in diversity even at this spatial scale.

### **Epidemiological studies**

The control of late blight demands frequent, repeated fungicides applications, and as a direct consequence of this the use of pesticides is high in potato compared to other agricultural crops. Despite the introduction of more effective fungicides and improvements in the application technique the problems of controlling late blight are growing. This is reflected in an increase of the number of fungicide applications used against late blight. In south Sweden this number has more than doubled during the last 20 – 30 years.

Nonetheless, late blight is considered to be more aggressive; the epidemics start earlier, the disease develop faster in the potato fields and the resistance to late blight in the cultivars is not as effective as it used to be. *P. infestans* has also been able to infect alternative hosts. There are reports of infection on several *Solanum* species, eg. black nightshade (*Solanum nigrum*) (Flier, van der Bosch & Turkensteen, 2003) bittersweet (*S. dulcamara*) (Cooke *et al.*, 2002). In Sweden hairy (green) night shade (*S. physalifolium*) has proven to be very susceptible to late blight and able to sustain production of oospores (Andersson *et al.*, 2003) (Figure 4).



Figure 4. Infection of *P. infestans* and oospore formed in *Solanum physalifolium*

The strong weather dependency of late blight has been used as a basis for different types of models to determine how to control the disease with fungicides as effectively as possible. These models or systems calculate how favourable the conditions for disease development are based mainly on observations of temperature, relative humidity and precipitation. There are many such examples of such late blight management systems (e.g. Smith, 1956; Ullrich & Schrödter, 1966; Krause *et al.*, 1975; Hansen *et al.*, 1995; 1996). Such so called decision support systems (DSS) have the potential of reducing the input of fungicides while maintaining an acceptable control level. The Negfry-system (Hansen *et al.*, 1995; 1996) has been tested in the Nordic countries with good results (Figure 5).

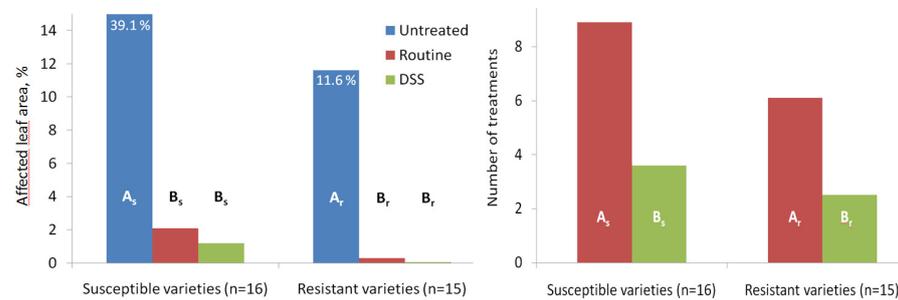


Figure 5. Leaf area affected by late blight and corresponding number of treatments using different treatment regimes in Swedish DSS validation trials of the Negfry-system. Treatments designated with the same letter are not significantly different ( $p=0.05$ ) within variety category.

However, these systems were all designed and validated against the old population of *P. infestans*. Changes in the pathogen biology with regards to critical factors such as temperature optima or infection efficiency can have significant effects on the validity of the output from these management systems. To maintain an acceptable system performance it is necessary to have both qualitative and quantitative knowledge about changes in pathogen behaviour.

In 2003, a joint Nordic project (NorPhyt, [www.planteinfo.dk/lbnordic/](http://www.planteinfo.dk/lbnordic/)) was initiated to study the epidemiology of the Nordic *P. infestans* populations. The aim was to collect data of the variability of the behaviour of late blight in Denmark, Finland, Norway and Sweden. In the project about 900 isolates were sampled to obtain as good geographical coverage of the participating countries as possible. To achieve this, a small number of isolates per field were collected from a large number of fields.

Of the collected isolates 25 were chosen per country. These 100 isolates were used in an aggressiveness study. The isolates were compared for infection efficiency, lesion growth, sporulation and latency period at 100 % relative humidity and 15 °C. All parameters were determined on two cultivars with different levels of late blight resistance, Bintje (susceptible) and Matilda (moderately resistant) (Lehtinen *et al.* MS [Paper IV]). The use of two cultivars with different levels of resistance to *P. infestans* was meant to improve the differentiation of the results. However, little differences in aggressiveness were found between the countries. As a whole the Nordic population of *P. infestans* tested in this investigations proved to be moderately aggressive compared to other similar European studies (Flier & Turkensteen, 1999; Carlisle *et al.*, 2002; Lebreton *et al.*, 1999).

Based on the laboratory results three isolates with different levels of aggressiveness were selected. These isolates were used to inoculate small plot field trials to see how well the laboratory results correlate to the behaviour of the isolates under field conditions. The results from the field trials demonstrated that there are big differences in the aggressiveness of different isolates under field conditions (Figure 6), and that the level of aggressiveness determined under controlled conditions correlated to this.



Figure 6. Plots of cv. Bintje within the same field inoculated with an aggressive isolate (A) and a “weak” isolate (B) of *P. infestans* 10 days after inoculation.

The same three isolates have also been used for climate chamber experiments where the parameters mentioned above have been determined at several different temperatures and humidity levels. These experiments have demonstrated large differences in sporulation and lesion growth at lower temperatures (Hansen *et al.*, 2006).

In the Norphyt collection of isolates a selection was tested for mating type, virulence (the ability to infect cultivars with different race specific resistance, R-genes), fungicide resistance (ability to infect at different dosages of fungicide). Both mating type ratio (near 1:1) and the common occurrence of both mating type within the same field indicates sexual reproduction. Resistance to fungicides (metalaxyl-M and propamocarb) was found in a relatively small proportion of the isolate, which could be expected considering that the isolates were collected early in the season. The virulence tests showed a large complexity in the virulence spectra in most tested isolates. Virulence to all R-genes except R9 was found (Lehtinen *et al.* [Paper IV]).

## Concluding remarks

The Swedish potato growers are experiencing increasing difficulties to control late blight in their fields. The increase in aggressiveness of late blight has been going on for the last decades and there are no indications of this trend levelling off. As a result of this the already intense use of pesticide in potato is increasing even further. This is a threat to consumer opinion of potato as a healthy staple food and could result in a decrease in potato consumption. What then is the cause of the growing problems with late blight? The interaction between *P. infestans*, host plants and abiotic factors such as weather is very complex. By studying different aspects of the late blight complex we have tried to get a better understanding of why late blight is an increasing problem for the potato growers.

Our results indicate that sexual reproduction is common in *P. infestans* in Sweden. The facts that both mating types are found all over the country in near 1:1 proportions and that oospores are commonly formed in field crops and serve as inoculum under field conditions support this. Also, studies of the genetic diversity imply that sexual reproduction influences the population structure of *P. infestans* in Sweden and the Nordic region as a whole. Sexual reproduction in *P. infestans* could result in an enhanced capability of the pathogen to adapt to different factors, e. g. weather, cultivar resistance, chemical control or other cultivation measures. This might manifest itself in earlier infections and a disease that spreads faster in the field.

Formation of oospores under field conditions is common in Sweden, at least in untreated potato crops. Despite this, all experience suggests that oospores are of less quantitative importance compared to infected seed under Swedish conditions. Early occurrence of late blight as a direct consequence of oospores in the soil in commercial potato production in Sweden seems to be rather rare. Apart from fields with more or less monoculture of potato and field trials the reports of oospore derived late blight have until now been few. It has proven difficult to study the longevity of oospores of *P. infestans* under natural conditions, but the collective results points to a relatively short survival. A crop rotation with 3- 4 years between the potato crops as recommended to manage other diseases and

pests in potato appears to be sufficient to keep soil borne inoculum of *P. infestans* under control.

However, the genetic diversity in the *P. infestans* population implies that sexual recombination plays an important role at all spatial scales from the entire Nordic region down to single fields. It is possible that oospores can either infect crops all during the season or germinate in leaves and stems directly after formation and in this way continuously supply the population with new genotypes. This would mean that soil borne inoculum (oospores) surviving the winter would not be the only way of introducing new genotypes and could be an explanation for a genetically diverse population of *P. infestans* despite the low number of fields with suspected oospore derived epidemics.

Another possible explanation for potato late blight becoming a plant disease ever more difficult to control is that the ongoing climate change has made the weather more favourable for late blight. Even small differences in temperature and humidity have effects on how the disease behaves in the field. The large variation between different years and different locations is a demonstration of this. A long term study of late blight in Finland has shown that changes in the climate towards more rain and higher temperatures during the growing season have had an effect on the occurrence of the disease (Hannukkala *et al.*, 2007). Computer models to describe occurrence and development of plant diseases are excellent tools when studying how biological changes in the pathogen/host or in abiotic factors as climate will affect the behaviour of a plant disease. Such a model for simulation of development of late blight has been developed by Bruhn & Fry (1981). This model has later been further developed (Andrade-Piedra *et al.* 2005a, 2005b) to the so called LB2004 model. This model is driven by data on temperature and relative humidity and based on this describes the development of late blight in the crop. Examples of model output are apparent infection rate ( $r$ ) and the amount of late blight in the crop at a given time (AUDPC, area under the disease progress curve). Based on the results from the NorPhyt-project validation of the LB2004 model has been carried out with promising results. A thoroughly validated and relevant model can be used to test different scenarios to make both quantitative and qualitative estimates of the cause behind the epidemiological changes in potato late blight.

Even though potato late blight is the most extensively studied plant disease there is still much to learn. I believe that the way to further extend our knowledge of this elusive and fascinating plant pathogen is to take a multidisciplinary approach. Combining molecular genetics with classic epidemiological studies and simulation modelling, and to work parallel in the laboratory, with the computer and in the field is the only way to really get to grips with the “Potato Murrain”.

## References

- Andersson, B., Sandström, M. & Strömberg, A. 1998a. Indications of soil borne inoculum of *Phytophthora infestans*. *Potato Research*, 305-310.
- Andersson, B., Sandström, M. & Strömberg, A. 1998b. *Sexual reproduction of Phytophthora infestans on potato in Sweden*. In: Proceedings of the workshop on the European network for development of an integrated control strategy of potato late blight. (Eds. Schepers, H. & Bouma, E.) *PAV-Special report no. 3 January 1998*. 92-96
- Andersson, B. & Sandström, M. 1999. *Late blight in Sweden, new problems with an old disease*. In: Proceedings of the European Association for Potato Research 14th triennial conference, May 2-7, 1999 Sorrento, Italy. 62-63.
- Andersson, B., Sandström, M. & Yuen, J. E. 1999. Evidence for soil-borne inoculum of *P. infestans* in Sweden. Late Blight: A threat to global food security. *GILB-meeting March 16-19, 1999 Quito Ecuador*, poster.
- Andersson, B. & Sandström, M. 2000. Ändrade förutsättningar för bekämpning av bladmögel. *Potatisodlaren*, 1.
- Andersson, B. & Strömberg, A. 2001. Vad har hänt med bladmögel i potatis? *Växtskyddsnotiser*, 2.
- Andersson, B., Johansson, M. & Jönsson, B. 2003. First report of *Solanum physalifolium* as a host plant for *Phytophthora infestans*. *Plant Disease*. 87, 1538
- Andrade-Piedra J. L., Hijmans R. J., Forbes G. A., Fry W. E., & Nelson R. J.. 2005a. Simulation of Potato Late Blight in the Andes. I: Modification and Parameterization of the LATEBLIGHT Model. *Phytopathology* 95, 1191-1199.
- Andrade-Piedra J. L., Hijmans R. J., Juárez H. S., Forbes G. A., Shtienberg D. & Fry W. E. 2005b. Simulation of Potato Late Blight in the Andes. II: Validation of the LATEBLIGHT Model. *Phytopathology* 95, 1200-1218
- Andrison, D. 1995. Biology, ecology and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology* 85. 1053-1056.
- Andrison, D. 1996. The origin of *Phytophthora infestans* populations present in Europe in the 1840s: A critical review of historical and scientific evidence. *Plant Pathology* 45, 1027-1035
- de Bary, H.A. 1876. Researches into the nature of the potato-fungus- *Phytophthora infestans*. *Royal Agricultural Society of England. Journal*, 12, 239-269
- Bourke, A. 1991. *Potato blight in Europe in 1845: the scientific controversy*. In: *Phytophthora*. Symposium of the British Mycological Society for Plant Pathology and the society of Irish Plant Pathologists held at Trinity College, Dublin, September 1989. (Eds. Lucas, J.A., Shattock, R.S., Shaw, D.S & Cooke, L.R.) Cambridge University Press, Cambridge
- Bruhn, J.A & Fry, W.E. 1981. Analysis of potato late blight epidemiology by simulation modelling. *Phytopathology*, 71, 612-616.
- Brurberg, M.B, Hannukkala, A. & Hermansen, A. 1999. Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research* 103, 1609-1615.
- Bødker, L., Pedersen, H., Kristensen, K., Møller, L., Lehtinen, A & Hannukkala, A. 2006. *Influence of crop history of potato on early occurrence and disease severity of potato late blight caused by Phytophthora infestans*. In: Proceedings of the Ninth Workshop of a European network for development of an Integrated Control Strategy of potato late blight, 2006. Tallinn, Estonia. (Eds. Westerdijk, C.E. and Schepers H.T.A.M). PPO Special report no. 11. 143-150. 53-56.
- Carlisle, D.J., Cooke L.R., Watson, S. & Brown, A.E. 2002. Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. *Plant Pathology* 51, 424-434
- Chang, T.T. & Koh, W.H. 1991. Factors affecting the germination of oospores of *Phytophthora infestans*. *Journal of Phytopathology*. 133. 1. 29-35.

- Clinton, G.P. 1911. Oospores of potato late blight, *Phytophthora infestans*. *Connecticut Experimental Station Biennial Report, 1909-1910*, 753-774.
- Cooke, L.R., Carlisle, D.J., Wilson, D.G., Deahl, K.L. 2002. Natural occurrence of *Phytophthora infestans* on woody nightshade (*Solanum dulcamara*) in Ireland. *Plant Pathology* 51, 392.
- Cooke, D.E.L., Lees, A.K., Hansen, J.G., Lassen, P., Andersson, B. & Balkony, J. 2006. *EUCABLIGHT: progress in characterising European P. infestans populations*. In: Proceedings of the Ninth Workshop of a European network for development of an Integrated Control Strategy of potato late blight, 2006. Tallinn, Estonia. (Eds. Westerdijk, C.E. and Schepers H.T.A.M). PPO Special report no. 11. 143-150.
- Dahlberg, J. 2001: Fältinventering av oosporbildning hos *P. infestans*. *Examensarbeten 2001:43 Inst för ekologi och växtproduktionslära*.
- Dahlberg, J., Andersson, B., Nordskog, B. & Hermansen, A. 2002. *Field survey of oospore formation by Phytophthora infestans*. In: Late Blight: Managing the global threat. GILB-meeting July 14-19, 2002 Hamburg, Germany. (Ed. Lizárra, C.) 134.
- Détourné, D., Duvauchelle, S. & Dubois, L. 2006. *The evolution of the population of Phytophthora infestans in France (Epidemiology and phenotypic markers)* In: Proceedings of the Ninth Workshop of a European network for development of an Integrated Control Strategy of potato late blight, 2006. Tallinn, Estonia. (Eds. Westerdijk, C.E. and Schepers H.T.A.M). PPO Special report no. 11. 151-155.
- Drenth, A., Turkensteen, L.J. & Govers, F. 1993. The occurrence of the A2 mating type of *Phytophthora infestans* in The Netherlands; significance and consequences. *Netherlands Journal of Plant Pathology*. 99, (3), 57-67.
- Drenth, A., Tas, I.C.Q. & Govers, F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* 100, 97-107.
- Drenth, A., Janssen, E.M. & Govers, F. 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. *Plant Pathology* 44, 86-94.
- Eriksson, J. 1916. Det primära utbrottet av bladmögel (*Phytophthora infestans*) på potatisplantan. In: Kungliga Lantbruks-Akademiens Handlingar och tidskrift för år 1916. Ivar Hæggströms boktryckeri aktiebolag 1916. 538-590.
- Fernández-Pavía, S.P., Grünwald, N.J., Díaz-Valasis, M., Cadena-Hinojosa, M. & Fry, W.E. 2004. Soilborne oospores of *Phytophthora infestans* in central Mexico survive winter fallow and infect potato plants in the field. *Plant Disease* 88, 29-33.
- Flier W.G. & Turkensteen L.J. 1999. Foliar aggressiveness of *Phytophthora infestans* in three potato growing districts in the Netherlands. *European Journal of Plant Pathology* 105, 381-388.
- Flier, W.G., van den Bosch, G.B.M. & Turkensteen, L.J. 2003. Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*. *Plant Pathology* 52, 595-603.
- Fries, E. 1845. Öfver Potates-Farsotens orsaker. Tidskrift för Landtmanna- och Kommunal-Ekonomien Nr 4
- Fry, W.E., Goodwin, S.B., Dyer, A.T., Matuszak, J.M., Drenth, A., Tooley, P.W., Sujkowski, L.S., Koh, Y.J., Cohen, B.A., Spielman, L.J., Deahl, K.L., Inglis, D.A. & Sandian, K.P. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, Pathways and Implications. *Plant Disease*, 77, 653-661.
- Gallego, M.E. & Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. *Phytopathology*, 48, 274-277
- Gómez- Alpizar, L., Carbone, I. & Ristaino, J.B. 2006. An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proceedings of the National Academy of Sciences. USA*, 104. 3306-3311.
- Goodwin, S.B., Cohen, B.A. & Fry, W.E. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences. USA*, 91, 11591-11595.
- Goodwin, S.B. and Drenth A. 1997. Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology*, 87, 992-999.

- Grenville-Briggs, L.J. & van West, P. 2005. The biotrophic stages of oomycete-plant interactions. *Advances in applied microbiology*, 57, 217-242.
- Griffith, G.W & Shaw, D.S. 1998. Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology*, 64, 4007-4014.
- Grünwald, N.J., Flier, W.G., Sturbaum, A.K., Garay- Serrano, E., van der Bosch, T.B.N., Smart, C.D., Matuzak, J.M., Lozoya-Saldaña, H., Turkensteen, L.J. & Fry, W.E. 2001. Population structure of *Phytophthora infestans* in the Toluca valley region of central Mexico. *Phytopathology* 91, 882-890.
- Götz E, 1991. Untersuchungen zum Auftreten des A2-Paarungstyps bei *Phytophthora infestans* (Mont.) de Bary in Ostdeutschland. *Potato Research* 34, 233-7.
- Hannukkala, A.O., Kaukoranta, T., Lehtinen, A. & Rahkonen A. 2007. Late-blight epidemics on potato in Finland, 1933-2002; increased and earlier occurrence of epidemics associated with climate change and lack of rotation. *Plant Pathology* 56, 167-176.
- Hansen, J. G., Andersson, B. & Hermansen, A. 1995. *NEGFY - A system for scheduling chemical control of late blight in potatoes*. In: "Phytophthora 150". EAPR conference proceedings, 9-16 sept 1995. (Eds. Dowley, L.J., Bannon, E., Cooke, L.R., Keane, T. & O'Sullivan, E.) Boole Press Ltd, Dublin.
- Hansen, J. G., Andersson, B. 1996. *Development and practical implementation of a system for potato late blight forecasting in potatoes*. In: International Symposium on Applied Agrometeorology and Agroclimatology. European Commission. Cost 77, 79, 711. (Eds. Dalezios, N.R) 251-258
- Hansen, J.G., Nielsen, B.J., Bødker, L., Andersson, B., Yuen, J., Wiik, L., Hermansen, A., Nærstad, R., Le, V.H., Brurberg, M.B., Hannukkalla, A & Lehtinen, A. 2006. *Blight management in the Nordic countries*. In: Proceedings of the Ninth Workshop of a European network for development of an Integrated Control Strategy of potato late blight, 2006. Tallinn, Estonia. (Eds. Westerdijk, C.E. and Schepers H.T.A.M). PPO Special report no. 11. 39-56.
- Hanson, K. & Shattock, R.C, 1998. Formation of oospores of *Phytophthora infestans* in cultivars with different levels of race-nonspecific resistance. *Plant Pathology* 47, 123-129.
- Harrison, J. G. 1992. Effects of the aerial environment on late blight of potato – a review. *Plant Pathology* 41. 384-416.
- Hermansen, A. Hannukkala, A. Hafskjold Nærstad, R & M. B. Brurberg, M. B. 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* 49, 11-22
- Hjelm, H. 2003. Oosporer av *P. infestans* och som inokulumkälla. *Examensarbete 2003:59 Inst för ekologi och växtproduktionslära*.
- Hirst, J.M. 1955. The early stages of a potato blight epidemic. *Plant Pathology* 4, 44-50.
- Hirst, J.M. 1960. The epidemiology of *Phytophthora infestans*. II. The source of inoculum. *Annals of Applied Biology* 48, 489-517.
- Hohl, H.R. & Iselin, K. 1984. Strains of *Phytophthora infestans* with A2 mating type behaviour. *Transactions of the British Mycological Society* 83, 529-530.
- Jones, L.R. 1909. Resting spores of the potato fungus. *Science* 30, 813-814.
- Jones L.R & Giddings, N.J. 1909. Studies on the potato fungus, *Phytophthora infestans*. *Science* 29, 271.
- Judelson, H.S. & Blanco A.B. 2005. The spores of *Phytophthora*: Weapons of the plant destroyer. *Microbiology* ., 47-58
- Kadir, S. & Umærus, W. 1987. *Phytophthora infestans* A2 compatibility type recorded i Sweden. In: *Book of abstracts, 10th triennial conference EAPR, Aalborg, Denmark*, 223
- Ko, W.H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. *Annual review of Plant Pathology*. 26, 57-73
- Ko, W.H. 1994. An alternative possible origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology*, 84, 1227-1227.
- Krause, R. A., Massie, L. B. & Hyre, R. A. 1975. BLITECAST: a computerized forecast of potato late blight. *Plant Disease Report*, 59, 95-98.

- Lacey, J. 1965. The infectivity of soils containing *Phytophthora infestans*. *Annals of Applied Biology* 56, 363-380.
- Large, E.C. 1946. The advance of the fungi. *Henderson & Spalding. London W1*
- Lebreton, L., Lucas, J.M. & Andrivon, D. 1999. Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. *Phytopathology* 89, 679-86
- Lehtinen, A. & Hannukkala, A. 2004. Oospores of *Phytophthora infestans* in soil provide an important new source of primary inoculum in Finland. *Agricultural and Food Science* 13, 399-410.
- May, K.J. & Ristaino, J.B. 2004. Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish Potato Famine. *Mycological Research* 108, 471-479.
- Malcolmson, J. 1985. *Phytophthora infestans* A2 compatibility type recorded in Great Britain. *Transaction of the British Mycological Society*, 85, 531.
- McDonald, B.A. & Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* 40, 349-379.
- Mosa, A.A., Kato, M., Sato, N., Kobayashi, K. & Ogoshi, A. 1989. Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Japan. *Annals of the Phytopathological Society of Japan*. 55, 615-620.
- Niederhauser, J.S. 1991. *Phytophthora infestans: The Mexican connection*. In: *Phytophthora. Symposium of the British Mycological Society for Plant Pathology and the society of Irish Plant Pathologists held at Trinity College, Dublin, September 1989*. (Eds. Lucas, J.A., Shattock, R.S., Shaw, D.S & Cooke, L.R.) Cambridge University Press, Cambridge
- Sandström, M. & Andersson, B. 1998a. Bladmögel - en ny sjukdom på potatis? *Potatisodlaren, nr 1*.
- Sandström, M. & Andersson, B. 1998b. Parningstypsinventering av *Phytophthora infestans*. *Potatisodlaren, nr 4*.
- Salaman, R. 1949. The History and Social Influence of the Potato. *Cambridge University press. Cambridge, USA*.
- 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 from the U. K. *Plant Pathology* 34, 552-554.
- Smart, C.D, Willman, M.R, Mayton, H, Mitzbuti, E.S.G, Sandrock, R.W, Muldoon, A.E. & Fry, W.E. 1998. Self-fertility in two clonal lineages of *Phytophthora infestans*. *Fungal Genetics and Biology* 25, 134-142.
- Smith, L. P. 1956. Potato late blight forecasting by 90 % humidity criteria. *Plant Pathology* 5, 83-87.
- Smoot, J.J., Gough, F.J., Lamey, H.A., Eichenmuller, J.J. & Gallegly, M.E. 1958. Production and germination of oospores of *Phytophthora infestans*. *Phytopathology* 48, 165-171.
- Spielman, L.J., Drenth, A. Davidse, L.C., Sujkowski, L.J., Gu, W., Tooley, P.W. & Fry, W.E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? *Plant Pathology* 40, 422-430.
- Tantius, P.M., Fyfe, A.M., Shaw, D.S. & Shattock, R.C. 1986. Occurrence of the A2 mating type and self fertile isolates of *Phytophthora infestans* in England and Wales. *Plant Pathology* 35, 578-581.
- Turkensteen, L.J., Flier, W.G., Wanningen, R & Mulder, A. 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* 49, 688-96.
- Turner, R.S. 2005. *After the famine: Plant pathology, Phytophthora infestans, and the late blight of potatoes, 1845-1960*. In: *Historical studies in the physical and biological sciences*. 35, 341-370
- Ullrich, J. & Schrödter, H. 1966. Das Problem der Vorhersage des Auftretens der Kartoffelkrautfäule (*Phytophthora infestans*) und die Möglichkeit seiner Lösung durch eine "Negativprognose". *Nachrichtenblatt Deutsch. Pflanzenschutzdienst (Braunschweig)* 18, 33-40.

- Wahlberg, P.F. 1847. Bidrag till kännedomen om potäternas sjukdom I Sverige åren 1845 och 1846. *Meddelande i Kongliga Lantbruks-Akademien*
- Westrin, T. (Ed.) 1915. Nordisk familjebok. Andra utgåvan. Nordisk familjeboks förlags aktiebolag. Nordisk familjeboks tryckeri. Stockholm.
- Whetzel, H.H. 1918. An outline of the history of phytopathology. W.B. Saunders Company, Philadelphia, PA, 130 pages.
- Widmark, A.- K., Andersson, B., Cassel-Lundhagen, A. Sandström M., & Yuen, J. 2007. *Phytophthora infestans* in a single field in southwest Sweden early in spring: symptoms, spatial distribution and genotypic variation. *Plant Pathology*. 56, 573-576
- van der Zaag, D.E. 1956. Overwintering en epidemiologie van *Phytophthora infestans*, tevens enige nieuwe bestrijdingsmogelijkheden. *Tijdschrift over Plantenziekten* 62, 89-156

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