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Monitoring Primary Sources of Inoculum of *Phytophthora infestans* in The Netherlands 1999 - 2005

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Summary
In the period 1999 till 2005, a project on monitoring early outbreaks of late blight in Dutch potato fields was performed in the context of the so-called Master Plan Phytophthora (MP) and Umbrella Plan Phytophthora.
In the course of eight years, 184 fields with primary foci were surveyed and the farmers concerned were interviewed according to a question list. 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.
Regulations issued to cover potato pile culls reduced the impact of cull piles on early infections considerably but cull piles continue to be one of the important initial inoculum sources. It was learned that early during the growing season, and already within the first week of emergence, potato crops may attract infections of *P. infestans*. More than 80% of the fields visited were not protected when the first infections occurred, despite warnings for relevant critical periods for late blight infection.
The occurrence of very small numbers per acreage of isolated, heavily affected single plants resulting into large foci around mid June could be assigned to latently infected seed potatoes. Depending on the region, either latently infested seed potatoes and/or oospores proved to be the most important inoculum sources.

Introduction
A range of initial sources of inoculum is reputedly held responsible for primary and early outbreaks of potato late blight. Infected tubers (cull piles and volunteer potatoes), latently infected seed tubers and oospores are distinguished as primary sources of inoculum. In the frame of minimizing the use of fungicides to control late blight, but at the same time effectively controlling the disease with the help of disease forecasting systems, it was concluded that more regional information was needed on sources of initial inoculum and date of primary infections occurring in farmers' fields.
Fostered by the so-called Master Plan Phytophthora and Umbrella Plan Phytophthora, a cooperative

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project of farmer organizations (LTO, HPA), scientific institutions (PPO, PRI) private organizations (Extension services, Pesticide firms, DSS builders) and The Dutch Ministry of Agriculture, Nature and Food Quality was performed in the period from 1999 till 2005. This article gives an overview of the results obtained during these surveys.

Materials and Methods

Four Dutch potato-growing regions were selected:

1. A seed and ware potato-growing region in the South West, growing potatoes on clay soil.
2. A similar region in the North West.
3. A mainly ware potato-growing region in the South East on sandy soils.
4. A mainly starch potato-growing region in the North East on sandy and reclaimed peat soils.

Fields with early development of late blight were reported by extension services of private companies (Cebeco Agrochemie, Profyto, Nestlé, Syngenta, HLB and De Landbouw Voorlichting), providers of Decision Support Systems (Dacom Plant Service, Prolion) and farmers. Experts visited the reported fields with the consent of the farmer. Evaluation of the primary focus was based on several criteria:

1. Number of foci encountered
2. Focus size (area plus number of lesions)
3. Infection place, specifically affected leaf layer or layers
4. Infected plant parts (leaf, stem, seed tuber)
5. Classification of lesions based on their development and size (relative age), and determination of the number of classes
6. Number of leaf layers of diseased and healthy stems
7. Length of diseased and healthy stems
8. Crop history (date of planting, weed control, emergence, etc)
9. Spray schedule and fungicides applied
10. Weather data and specifically periods critical for infection using Plant Plus
11. Assessment of the age of the classes of lesions based on information under points 3 to 10
12. Crop rotation
13. Presence of volunteer potato plants in the foregoing three years and in the field examined
14. Presence of *Phytophthora* tuber rot during storage or in the seed lot

The farmer concerned was interviewed to obtain information over the abovementioned points. Infected plant material was sampled and *P. infestans* isolated. Isolates were stored in liquid nitrogen and genetically characterized by determining:

- Mating type (A1, A2)
- Haplotype
- AFLP- fingerprint patterns

The genetic information of the isolates was used to back-up assessment of primary inoculum sources. Primary inoculum sources distinguished are:

- (Latently) infected seed potatoes
- Oospores
- Sources nearby and far away
- Unknown

In case of a nearby source, the actual primary source was found (cull pile; neighbouring field, volunteers, etc) and/or the disease dissemination pattern in the filed indicated a nearby source. In case of a far

away source no potential primary source was found and the disease dissemination pattern in the field suggested a far away source. Else if no original source could be identified the origin was classified as unknown. This event was commonly associated with a single focus in a large region.

Results and discussion

In the period of 1999 till 2005, 184 primary and early foci of *P. infestans* were visited and their most likely origin investigated (Mulder & Turkensteen, 1999; Baarlen & Raatjes, 2001; Turkensteen, 2003; Turkensteen *et al.*, 2004, Turkensteen *et al.,* 2005; Evenhuis *et al.*, 2006). Table 1 shows the relative importance of the various primary inoculum sources of late blight for the four selected regions during the surveying period. During this period 2075 isolates of *P. infestans* were sampled and stored in liquid nitrogen.

Information obtained on primary inoculum sources was used to optimize the late blight control strategy. Different strategies have to be used to control each particular primary inoculum source and their effects. Flyers explaining the consequences for the late blight control strategy were disseminated to the farmers through Master Plan Phytophthora (LTO-Nederland, 2005 & 2006).

Table 1. Relative importance of primary inoculum sources of late blight in four regions of The Netherlands during the survey period 1999 – 2005.

Region	Source of primary infection					Number of fields examined
	Seed	Oospores	Far away	Nearby	Unknown	
North East	37	32	13	12	6	90
South East	24	9	46	12	9	33
North West	26	0	40	30	4	27
South West	53	0	32	15	0	34
Weighed average[a]	36	17	27	15	5	184

[a] *Percentage of fields infected by late blight originating from the primary inoculum source indicated in the heading (n=184).*

First spray Prevention of primary late blight development is an essential part of an effective control programme. Hence, eradication of primary inoculum sources is the first step. Furthermore the crop has to be protected from primary infections by spraying in good time before critical periods occur. From 42 fields in our survey sufficient data on spray applications were available. A large percentage (83%) of the visited fields (n=42) with early foci was not sprayed at all at the time of the first relevant critical period for late blight for the crop concerned. With respect to these fields, 39% should already have been sprayed as early as during the first week at the start of crop emergence. In 47 % of these cases, late blight development was initiated by oospores, the remaining part was mostly due to infection originating from sources outside the potato field.

Cull piles Cull piles are long known as a primary inoculum source of *P. infestans* (Hänni, 1949). Cull piles were found to serve as a primary source of inoculum in 74% (n=19) of the infested potato fields during a survey from 1994 till 1996 in South Flevoland (Zwankhuizen *et al.,* 1998). Since regulations (HPA) to cover cull piles have been enforced, the numbers of cull piles carrying late blight infected plants decreased initially. However, the last three years (2005-2007) the number of uncovered cull piles stabilized at a non-acceptable level (LTO-Nederland, 2005 & 2006; NAK-Agro personal communication). The first reports of occurrences of late blight in the season were usually from cull piles, during the years of our surveys. It indicates that cull piles continued to act as an important early source of primary inoculum, although to a lower level. It implies that regulations to cover cull piles

have to be enforced even more stringently. Nevertheless, with the reduction of uncovered cull piles, which in most years are the earliest inoculum sources, the other inoculum sources, (latently) infected seed and oospores, became more notable.

Cultivation under cover Cultivation of early potatoes under plastic cover is vulnerable to late blight infection. Soil and canopy under cover remain longer moist than in the open field. The moist conditions and the higher temperatures early in the season favour late blight infection and development. Usually covered crops are not sprayed to control late blight, before the cover is removed. Considering the earliness of this type of crop, these fields form primary inoculum sources for late blight to surrounding potato fields. An early and heavy epidemic in such crops occurred both in 1999 (Mulder & Turkensteen, 1999; Hadders, 2003) and 2005 (Evenhuis *et al.*, 2006) going along with large areas with widely isolated, single infections that took place around the time the cover was removed. To control late blight, these crops should be sprayed even when fields are still covered. Preliminary experiments showed effectiveness of over cover sprays (Spits & Bus, 2003). It is assumed that the initial infection sources are diseased or latently infected tubers. Oospore initiated epidemics were not found with these type of cultivation, but may be feasible as well.

Latently infected seed Every year, single heavily infected plants were found in the season around mid June, which for an initial source is relatively late in the growing season. Symptoms usually occurred on most if not all stems of these plants. After the stage of infected stems, the pathogen spread to the leaves. Under conditions favourable to late blight, heavy sporulation occurred leading to large foci of 100 to 1000 m^2. These foci were characterized by the presence of only one or two age-class-lesions in full-grown crops. Each of the original single diseased plants started off from a diseased mother tuber, which again originated from a latently infected seed tuber. These findings concerning the acting and timing of *P. infestans* through latent infected seed tubers were supported by results from Germany, which showed that the pathogen could be latently present in tubers in storage, and in sprouts and stems formed on these tubers in storage and in the field (Adler, 2001). If these plants were raised under moist conditions they showed similar late blight symptoms (Adler, 2001) as found in the field during our survey.

Infection from latently infected seeds occurred in each region. If circumstances were favourable for tuber infection in the previous year, a relative high percentage of latently infected seed tubers might be present. However expression of late blight in spring depends on weather circumstances during May and June. Infection risks from seed tubers increase when the soil is moist. A period of 5 to 7 days of moist soil between emergence and closing of the canopy favours expression of late blight originating form (latent) tuber infection (Adler, 2001; Bässler *et al.,* 2002). Usually stem lesions developed. Fungicides with a curative or systemic mode of action showed to be effective to prevent the stem blight, when sprayed at due time (Kalkdijk *et al.*, 2005).

Oospores During a survey in 2000, oospores were encountered in 78%, 50%, 30% and 15% of two lesion single leaflets samples collected from regular crops in North East, South East, Central and South West of the Netherlands, respectively (Flier *et al.*, 2004). On unprotected potato plants oospores can be formed abundantly, especially on volunteer potato plants (Kessel *et al.*, 2002; Förch *et al.*, 2004). To prevent oospore formation, volunteer potato plants should be destroyed. Oospores can survive up to four years in a sandy soil, whereas survival is limited to three years in a clay soil (Turkensteen *et al.*, 2000).
The North East of the Netherlands is characterized by growing potatoes for starch production. To save

costs occasionally sprays at the end of the season are left out. Further crop rotation schemes are narrow and soils are sandy. All these circumstances favour oospore formation and consequently infection through oospores.
Infections originating from oospores were mainly found on sandy soils, and were most common in the North East of the Netherlands. In case of oospore infections, large numbers of plants synchronously attract single or a few primary infections on infested fields. At the same time neighbouring potato fields with a same protection history may be totally free of late blight. From three of such fields with a recently emerged crop, AFLP-fingerprints were made from four isolates per field in 2005. The fact that for each of the fields, the four isolates from the same field had different AFLP patterns is in agreement with what to expect from an epidemic initiated through oospores, since every oospore has a unique AFLP-pattern. A mix of genotypes of both mating types also indicated that oospores might be the most logical source of infection, as was found in Sweden (Widmark *et al.*, 2007).
Oospores based infections frequently occur after heavy rainfall. Sometimes even in the first week of crop emergence, infection of the canopy occurred. Contrary to popular believe, a spray to control late blight might be necessary when weather circumstances are critical during or shortly after crop emergence, and especially so in case oospore are present in the field. Furthermore, spray application schemes should be followed through until the haulm is killed.

Main conclusions

- Foci of late blight yielded useful information on moments of primary and secondary infections, inoculum sources and importance of (failures in) timing of fungicide spraying.
- As many as 83% (n=42) of the fields with an early outbreak of late blight were not treated with fungicides prior to the initial infections, indicating the importance of proper timing of the first application. Decision support systems can assist the grower to do so.
- Reports on first infections often concerned diseased plants on potato cull piles in spite of regulations to cover cull piles. These regulations have to be implemented even more strictly.
- The importance of latently infected potato tubers in the onset of epidemics was revealed. It is a relatively late acting source of initial inoculum. Nevertheless it formed an important source of primary inoculum in all regions and especially so for the ware and seed growing regions on clay soils. Strict potato late blight control in seed potato crops to prevent infection of the next season seed is therefore imperative.
- Expression of infection from (seed) tuber to plant is strongly dependent on weather conditions. When plant infection from infected seed tubers is to be expected spray application with a (local) systemic fungicide in the early season is recommended.
- Oospore based infections are found on sandy soils mainly and most frequently in the starch potato growing area in the North East of The Netherlands. Oospore based infections may already occur within the first week of above ground crop development at emerging.
- Potato late blight should be strictly controlled during the whole growing season to avoid both tuber infection and oospore formation.
- Volunteer potato plants should be destroyed to prevent a boost of the late blight epidemic and to avoid mass production of oospores.

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