Potato late blight epidemics and population structure of *Phytophthora infestans*

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M.J. Zwankhuizen

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Proefschrift

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op gezag van de rector magnificus
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Stellingen

1. Oösporen spelen een rol bij de jaarlijkse ontwikkeling van de aardappelziekte in Nederland.

   Dit proefschrift.

2. Door de aardappelziekte aangetaste biologische aardappelpercelen vormen een gevaarlijke infectiebron.

   Dit proefschrift.

3. Het vergelijken van genotypen van isolaten van *Phytophthora infestans* op basis van RG57 DNA merkers en enkele andere merkers in één grote database, zoals voorgesteld door Forbes *et al.* (1998), is niet erg zinvol wanneer wereldwijd steeds vaker sexuele recombinatie optreedt. Beter is het om een database op te stellen met genetische diversiteiten en afstanden.

   Dit proefschrift.

   Forbes GA, Goodwin SB, Drenth A, Oyarzun P, Ordonez ME and Fry WE (1998) A global marker database for *Phytophthora infestans*. Plant Disease 82: 811-818.

4. Het weergeven van alleen percentages of aantallen A1 en A2 isolaten van *P. infestans* zonder deze verder te karakteriseren (zoals bijvoorbeeld in Dowley *et al.*, 1995) is weinig informatief en dient te worden vermeden.

   Dit proefschrift.

   Dowley LJ, Bannon E, Cooke LR, Keane T and O'Sullivan E. (eds) (1995) *Phytophthora infestans* 150. Dublin, Boole Press.

5. Analyse van de epidemiologische context van een plantenziekte is noodzakelijk voor een goed begrip van de populatie-structuur van plantenpathogenen.

   Dit proefschrift.

6. Bij een studie van haarden en gradiënten van plantenziekten zou altijd de genetische samenstelling van de pathogeenpopulatie van de haard en van de gradiënt bekend moeten zijn.

Dit proefschrift.

7. Meerjarenplannen gewasbescherming vereisen meerjarenstudies van ziekten en plagen.

8. Afvalhopen met aardappelplanten vormen een sociaal probleem.

9. 'Een goede buur is beter dan een verre vriend' is zeker van toepassing op de afvalhopenproblematiek en de spuitvrije aardappelteelt.

10. In de notitie 'Biologische Landbouw' (Anonymus, 1992) wordt met geen woord gerept over de risico's van ziekten, plagen en onkruiden in de biologische teelt. Eigen waarnemingen in biologische (aardappel)gewassen geven aan dat schimmel-, bacterie- en virusziekten, insecten en onkruiden hoge niveaus respectievelijk dichtheden kunnen bereiken, hetgeen gevolgen heeft voor de ziektedruk in de omgeving en de instandhouding van gezond zaaizaad en pootgoed. Een risico-analyse betreffende de invoering van biologische landbouw is daarom wenselijk.

Anonymus (1992) Biologische landbouw. Notitie 22817, nr.1, Ministerie van Landbouw, Natuurbeheer en Visserij. Den Haag, SDU Uitgeverij.

11. Politici maken zich terecht zorgen over de groeiende kloof tussen burger en politiek. Burgers maken zich terecht zorgen over hoog op de verkiezingslijst geplaatste politici die reeds tijdens de kabinetsformatie een andere functie aanvaarden of een stapje terug doen.

12. Voetbal is heilige oorlog.

Stellingen behorende bij het proefschrift van Maarten J. Zwankhuizen: 'Potato late blight epidemics and population structure of *Phytophthora infestans*'.

Wageningen, 8 december 1998.

Author's abstract

Potato late blight is caused by the fungus *Phytophthora infestans*. To study the relative importance of oospores in the epidemiology, and to estimate the relative impact of various infection sources, late blight epidemics in Southern Flevoland (The Netherlands) were studied using epidemiological and DNA fingerprint analyses. Infested refuse piles were the most important infection source for late blight epidemics in 1994 and 1995. Infected seed tubers were of minor importance. The results suggest that oospores play a role in the development of late blight in commercial potato crops, although the importance is less pronounced than refuse piles. Infested organic potato crops were important mid-season infection sources in 1994, but not in 1995 and 1996 due to unfavourable weather. In allotment gardens, oospores appeared to be the major inoculum for disease on potatoes and tomatoes in 1995 and 1996. Influx of inoculum from the commercial potato fields was evident in 1994, a year with a major epidemic in the first half of the growing season. A long-term study of late blight epidemics in The Netherlands from 1950 through 1996 indicated that the disease level in the previous year and the number of days with precipitation during the growing season were the most important factors determining the current year's disease level. A multi-year pattern was observed. The results described in the thesis suggest that the disease pressure may increase in the future.

Aan mijn ouders en Arja

Voorwoord

Een voorwoord schrijven is altijd een van de leukere dingen van het schrijfwerk, zeker bij het afronden van een proefschrift. Ik heb het onderzoek dat hier beschreven wordt met enthousiasme uitgevoerd. Vooral het opzetten, organiseren, en uitvoeren van het omvangrijke veldonderzoek lag mij goed. Ik ging graag 'het land in en de boer op' om contacten te leggen, waarnemingen te verrichten en monsters te verzamelen. Zeker met mooi weer was het geen straf om het lab te verlaten. Overigens was het niet altijd rozegeur en maneschijn in de polder. De grilligheid van de aardappelziekte bezorgde me regelmatig verrassingen en de ziekte trad vaak daar op waar ik nu net niet van plan was te bemonsteren. Talloze keren ben ik naar de polder gegaan, peinzend hoe ik weer een nieuwe, onverwachte situatie het hoofd moest bieden. Vrijheid was er minder gedurende de winter. De resultaten analyseren was leuk, al dat labwerk kon me iets minder bekoren. Maar daar werd een oplossing voor gevonden (...).

Hoe graag ik ook wegzwierf van de vakgroep, de mensen die het meest hebben bijgedragen aan de totstandkoming van dit proefschrift waren juist daar te vinden. Ik bedank alle collega's van de vakgroep Fytopathologie en daarbuiten voor de samenwerking. Zonder mensen tekort te willen doen, wil ik een aantal personen met name noemen.

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Francine Govers bedank ik voor de begeleiding en de prettige samenwerking. Je hebt het aangedurfd om mij het 'DNA-werk' aan te leren en je hebt ook mijn manuscripten 'mogen' beoordelen. Van de kritiek heb ik veel geleerd, ik hoop dat jij ook wat van mij opgestoken hebt, bijvoorbeeld hoe Phytophthora zich in het wild gedraagt. Ook de overige mensen van het 'Pi-lab' bedank ik voor hun hulp bij het moleculaire werk en voor het feit dat ik veel mocht 'lenen'. Theo, je hebt aan dit werk bijgedragen door een deel van de isolaten te karakteriseren door middel van AFLP fingerprinting. Verder bedank ik Herman Frinking als begeleider; je was vooral in de eerste fase van mijn project bij het onderzoek betrokken. Roel, helaas hebben we de rit niet samen kunnen afmaken. Bedankt voor de samenwerking. André Drenth, je hebt in de beginfase van mijn project me 'een en ander' bijgebracht over RG57. Daarna heb ik veel voordeel gehad van onze discussies over de epidemiologie en populatiegenetica van deze enige echt belangrijke ziekteverwekker, zowel tijdens persoonlijke gesprekken als via e-mail.

Bill Fry, thank you for making my visit to Cornell University successful and for the valuable discussions about the epidemiology and population biology of the late blight fungus. Steve Goodwin, you answered many of my questions during your visit to

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Cees van Loon, Maarten Steeghs en Lo Turkensteen bedank ik voor hun sturende werk in de begeleidingscommissie. Het gaf me een goed gevoel dat jullie meedachten bij deze omvangrijke klus.

Zeer veel isolaten zijn gekarakteriseerd door Bert Uenk en Henry van Raaij (beiden als analist) en André van Esburg (als student). Bedankt voor het vele werk dat jullie gedaan hebben. Het werd vaak een vrolijke boel op 'ons' kleine labje. Maar eigenlijk was het helemaal niet 'ons' labje. Gitte, je hebt veel bijgedragen aan mijn onderzoek. Ten eerste door labruimte af te staan aan een stel grote jongens, ten tweede door me te helpen als 'systeembeheerder', en ten derde, het belangrijkste, je was een fijne collega/kamergenoot. Je hebt me ook geholpen bij het maken van de omslag. Je mag me nu nóg een keer helpen. Corné en Tonnie, ik herhaal de woorden uit jullie eigen proefschrift: bedankt voor de gezelligheid, jullie waren onmisbaar.

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Ik sluit af met een woord van dank aan mijn ouders en aan Arja. Veel dank ben ik verschuldigd aan mijn ouders. Mijn waardering voor hetgeen ik van jullie meegekregen heb en het medeleven is niet in enkele woorden uit te drukken. Datzelfde geldt voor mijn dank aan jou, Arja. Wat een geduld en een begrip in de afgelopen jaren. Bovenal past dankbaarheid jegens God Die kracht en inzicht gaf om dit werk te doen.

Maarten Zwankhuizen

Contents

Chapter 1

General introduction

Potato late blight and its causal agent *Phytophthora infestans*

Late blight disease of potatoes

In the nineteenth century, the potato *Solanum tuberosum* had become the staple food for north-western Europe. The first appearance of potato late blight in Europe in 1845 resulted in severely infested potato crops all over western Europe (Bourke, 1964). The effect of the disease was most dramatic in Ireland in 1845 and 1846, and resulted in the well-known Irish potato famine. More than one million people died and a similar number emigrated (Bourke, 1993; Large, 1940; Woodham-Smith, 1962). Potato late blight is caused by a fungus, which was named *Phytophthora infestans* by de Bary in 1876 (de Bary, 1876).

In The Netherlands, the late blight attacks in 1845 resulted in a significant reduction of potato yields. In that year, potato yields were less than 30 % of the normal yield. In one area, the Bommelerwaard, only 2 % of the normal yield could be harvested (Van der Zaag, 1956). The potato crops were again severely infested by the disease in 1846. As a result, social instability arose because food prices increased and many people of the working class became unemployed. After these two years, the disease occurred at relatively low levels for several years (Van der Zaag, 1956).

No outbreak of a plant disease, before or since, has received the public attention given to the epidemic of potato late blight in Europe in 1845 (Bourke, 1964). The impact of the *Phytophthora infestans* epidemics initiated research about the nature of plant disease and brought the scientific discipline of plant pathology into prominence and acceptance (Robertson, 1991).

The pathogen Phytophthora infestans

The oomycete *Phytophthora infestans* (Mont.) de Bary can reproduce asexually and sexually. In the absence of the sexual cycle, the fungus can only overwinter as mycelium in infected potato tubers (Van der Zaag, 1956; Hirst and Stedman, 1960). Diseased plants may arise from infected tubers and necrotic lesions are formed on leaves and stems. Numerous asexual sporangia are produced on diseased tissue. These sporangia are dispersed by the wind and can infect other plants, either by germinating directly (at temperatures above 12 - 15 °C), or indirectly via formation of motile zoospores (below 12 °C). If the weather conditions are favourable, many generations of asexual spores can be formed and dispersed, leading to late blight epidemics (Harrison, 1992).

Sexual reproduction in this heterothallic fungus only occurs when thalli of opposite mating type (A1 and A2) mate. Mating results in the production of oospores in host plant tissue. Oospores are persistent, thick-walled structures which can overwinter in the soil in

the absence of a host. Oospores of *P. infestans* can survive in soil for at least one year (Drenth *et al.*, 1995), but most likely for longer periods (L.J. Turkensteen, *unpublished*). Under favourable conditions, oospores can germinate and act as inoculum, infecting host plants in the next seasons (Drenth *et al.*, 1995).

Re-emergence of potato late blight

Prior to the 1980s, the A1 mating type was distributed throughout the world, whereas the A2 mating type was detected only in central Mexico, the centre of origin of *P. infestans* (Niederhauser, 1956; Hohl and Iselin, 1984). Consequently, sexual reproduction only occurred in the highlands of central Mexico (Gallegly and Galindo, 1958; Tooley *et al.*, 1985). Populations of *P. infestans* outside of Mexico were limited to asexual reproduction and vegetative overwintering in potato tubers.

The detection of the A2 mating type in Europe in the early eighties, in East Germany (Dagget *et al.*, 1993), Switzerland (Hohl and Iselin, 1984), The Netherlands (Frinking *et al.*, 1987) and the UK (Tantius *et al.*, 1986), indicated that populations of *P. infestans* were changing. Analysis of *P. infestans* populations using allozyme markers (Spielman, 1991) and DNA fingerprint markers (Goodwin *et al.*, 1992a) confirmed that populations of *P. infestans* in Europe had changed due to recent migrations. Prior to the 1980s, world-wide populations of *P. infestans* were dominated by a single clonal lineage, as a result of the first migration of the fungus in the 1840s from its centre of origin Mexico (Goodwin *et al.*, 1994b). The old populations were displaced by 'new', genotypically diverse populations (Fry *et al.*, 1992; 1993; Goodwin, 1997). The displacement of old populations by new populations was probably initiated by the import of large quantities of potato tubers in the late 1970s from Mexico into Europe (Niederhauser, 1991). Subsequent migrations in the 1980s and 1990s resulted in increasingly diverse *P. infestans* populations all over the world (Goodwin, 1997).

Also in The Netherlands, the *P. infestans* population in the 1980s and early 1990s was much more diverse than the population prevailing before 1980 (Drenth *et al.*, 1993; 1994). Moreover, oospores were found in naturally infected potato and tomato plant tissue collected in commercial potato fields and in allotment gardens (L.J. Turkensteen, *unpublished*). Drenth *et al.* (1995) showed that oospores could survive in natural soil under Dutch winter conditions and could infect potato plants in the next season. So it was concluded that sexual reproduction can occur in the field and that oospores might play a role in the development of late blight after the introduction of the new A1 and A2 mating type populations in The Netherlands.

Control of potato late blight in The Netherlands

Chemical control. Since the late 1880s, fungicides have become available for the control of late blight (Egan *et al.*, 1995). Chemical control is still the most important measure for the control of potato late blight. However, the use of fungicides is questioned due to growing environmental awareness. The Multi-Year Crop Protection Plan, introduced in The Netherlands in 1991 (Anonymous, 1991), aims at (*i*) a reduction of the dependence on pesticides, (*ii*) a reduction of the volume of pesticides used, and (*iii*) a reduction of the emission of pesticides. On the short term, a reduction of the volume of fungicides used can be achieved by increased efficacy of compounds (Egan *et al.*, 1995), and, to a lesser extent, by substitution of calendar spraying with supervised control via decision support systems (Schepers, 1995). On the long term, the dependence on fungicides can be reduced by the use of resistant cultivars.

Host resistance. Resistance in potato to *P. infestans* can be divided in vertical (monogenic) resistance and horizontal (polygenic) resistance. Vertical resistance (R-gene resistance) is not durable, because of the well-known generation of races of *P. infestans* which are no longer recognised by the R-gene containing cultivar. Therefore, cultivars with a high level of horizontal resistance, effective against all races of the pathogen, are desirable for sustainable potato growing. To breed cultivars with durable resistance, further research is needed on the molecular and genetic aspects of the potato - *P. infestans* interactions.

Cultural practices. By the mid-twentieth century, important information on the epidemiology of *P. infestans* became available which enabled more effective control of the disease. Bonde and Schultz (1943) were the first to report that infested refuse piles can be important inoculum sources. The comprehensive research of Van der Zaag (1956) provided essential information about the overwintering and spread of the pathogen in The Netherlands, and he also investigated some new possibilities of control. The fungus overwinters as a mycelium in infected seed tubers (Van der Zaag, 1956; Hirst and Stedman, 1960). These infected tubers may result in diseased plants in the next season, either on a refuse pile where the tuber was discarded, or in a potato field where the tuber was used as planting material. Van der Zaag (1956) found for his research area, De Streek, that foci, originating from infected seed tubers, were more important infection sources for late blight epidemics than infested refuse piles. In the 1960s, Lohuis *et al.* (1967) and Davidse *et al.* (1968) gathered conclusive evidence that refuse piles were more important sources in Noord-Friesland and in the Noordoostpolder, respectively. The studies of Mooi (1968; 1971) provided insight into the variability of the pathogen population on potatoes by characterising field isolates for virulence.

Recent developments affecting control of late blight

Sexual reproduction and oospores. As a consequence of the genetic changes in the pathogen population, the epidemiology of late blight might change, and control and forecasting of the disease might become more difficult. Oospores, formed by sexual reproduction, can survive in the soil and thus may serve as overwintering inoculum, in addition to tuber-borne inoculum. 'New' genotypes can have increased pathogenic fitness compared to genotypes of the old population (Day and Shattock, 1997; Kato *et al.*, 1997). Consequently, development of late blight epidemics under the new situation has to be investigated.

Organic potato growing. A complicating factor for the control of late blight concerns organic agriculture. Since no fungicides are used in organic potato crops, high disease levels may be found in those crops, notwithstanding the use of moderately resistant cultivars. Thus, infested organic potato crops may affect the overwintering and spread of the disease. Organic agriculture has been increasingly stimulated by the Dutch government (Anonymous, 1992b). The total hectareage with organic agriculture and horticulture in The Netherlands increased from 2724 ha in 1986 to 12789 ha in 1995 (Anonymous, 1996). Approximately 43 % of this area is grown with arable crops and grasses. Most organic farms are located in the province of Flevoland. Of the total area of arable crops in this province, 4.2 % was in use by organic growers in 1995 (Anonymous, 1996).

In the late 1980s and early 1990s, conventional potato growers in Southern Flevoland, the youngest polder of the province of Flevoland, began to complain about the occurrence of high levels of disease in the crops of their colleagues who were growing potatoes organically. The conventional growers claimed that the late summer infestation of their potato crops was caused by inoculum originating from severely infested organic potato fields. The organic growers in turn argued that the infections arose from infested refuse piles distributed throughout the area. This resulted in a social conflict between conventional and organic growers and questions were posed in the Dutch parliament (Anonymous, 1992a).

About this thesis

The need for reinvestigation of potato late blight in The Netherlands

The possibly changed epidemiology due to the introduction of a 'new' *P. infestans* population and the possibly increased disease pressure due to the presence of infested organic potato crops required renewed research into the overwintering and spread of the late blight pathogen.

The disease can occur in various sites, *i.e.* refuse piles, fields with volunteer potato plants, conventional and organic potato fields, and potato and tomato plots in allotment gardens. Differences among sites of host plants and among years may exist with respect to the relative importance of oospores as the initiating inoculum. The importance of the different infection sources, *e.g.* infested refuse piles, infested conventional and organic potato fields, and infested allotment gardens, may vary over the course of the growing season and among years. The fluctuations in the severity of late blight epidemics over the years may follow a multi-year pattern, which largely determines the occurrence of the disease and disease level in a certain year.

Research questions

Three major research questions are addressed in this thesis:

i) What is the importance of oospores as overwintering inoculum relative to tuber-borne inoculum?

ii) What is the relative impact of the various infection sources on epidemic development of *P. infestans*?

iii) What are the key factors determining the polyetic (= multi-year; Zadoks, 1978) pattern of potato late blight epidemics in The Netherlands?

Methodology

Combined epidemiological and genotypic research. In order to address the first two research questions, the epidemiology and the population structure of *P. infestans* were investigated by studying natural late blight epidemics. Epidemiology is the study of the development and spread of disease and of the factors affecting these processes (Zadoks and Schein, 1979). Knowledge of the epidemiology of a plant pathogen is necessary to effectively manage plant diseases (Van der Plank, 1963). Population structure refers to (*i*) the amount of genetic variation among individuals in a population, (*ii*) the ways in which this variation is partitioned in time and space, and (*iii*) the genetic relationships within and between (sub)populations (Leung *et al.*, 1993). The analysis of the structure of the *P. infestans* populations and the way the population structure changes in time and space provides insight into the processes which shape population structure, and contributes to the understanding of epidemiology and the effect of disease control measures (Leung *et al.*, 1993; Milgroom and Fry, 1997).

Disease development in the different host plant sites was monitored and quantified over the course of three growing seasons. Disease foci were studied to identify the initiating inoculum. Disease gradient analysis (Gregory, 1968) was conducted to follow the

spread of disease from infection sources within and among potato fields. Populations were analysed by collecting isolates which were characterised for mating type and DNA fingerprint pattern using the moderately repetitive RFLP probe RG57 (Goodwin *et al.*, 1992a). This probe recognises many unlinked loci dispersed over the genome. The use of the supposedly selection-neutral DNA markers allows to identify asexual and sexual populations unambiguously and to follow genotypes during epidemic development in consecutive years (Goodwin *et al.*, 1992b; Drenth *et al.*, 1994).

Research area. Late blight epidemics were studied in a part of the ware and seed potato area of Southern Flevoland in 1994, 1995, and 1996. The research area, including about 3000 ha of potatoes, measured approximately 10 by 15 km. The most important reasons for choosing this specific area were: (*i*) the occurrence of severe late blight epidemics in the late 1980s and the early 1990s; (*ii*) a social conflict between conventional and organic potato growers about the role of infested organic potato fields with respect to late blight development in the region; (*iii*) the occurrence of relatively high densities of volunteer plants because tubers, left in the soil after harvest, survive easily in the young, well-aerated reclaimed soil (M.J. Zwankhuizen, *unpublished*), which might affect the development of late blight epidemics; and (*iv*) its distance (over 30 km) from the nearest commercial potato-growing area located upwind, which reduces the probability of influx of inoculum.

Long-term study. To investigate the multi-year pattern of disease outbreaks, the severity of potato late blight epidemics from 1950 through 1996 was analysed using agricultural and meteorological data. Disease data and agricultural data were retrieved from the literature and several databases. Meteorological variables were obtained from the Royal Netherlands Meteorological Institute (KNMI) at De Bilt.

Levels of scale. The research described in this thesis considers late blight at several levels of scale. The lower levels are the subject of Chapters 2, 3, and 4, in which the first two research questions are addressed. In the epidemiological part, development of disease is considered over a range of scales, from individual diseased plants, originating from (overwintering) inoculum, via disease at the field level, to disease spread at the regional level. The distance over which the disease was studied varied from less than one meter in the study of disease foci, via hectometers at the field level, to a few kilometers at the level of regional spread. The genotypic analysis considered the 'epidemiological' levels as well as 'genotypic' levels. An isolate, obtained from a single lesion on a plant at a given location and time, belongs to a certain genotype, and this genotype may belong to a certain (sub)population. The time scale ranged from the daily and weekly level to a few years, the research period.

The longest time scale is related to the polyetic pattern of late blight epidemics (Chapter 5). *P. infestans* epidemics were analysed at a national level, covering a range of years (47), which is long relative to the period of research in Southern Flevoland (from autumn 1993 through autumn 1996). The third research question places the first two

research questions in a long-term and national perspective, to better understand the short-term results obtained at the regional level.

Outline of the thesis

The small-scale epidemiology of potato late blight in Southern Flevoland is described in Chapter 2. The results of the characterisation of a large number of isolates of *P. infestans*, collected in Southern Flevoland and adjacent areas, are presented in Chapter 3. The changes in genotypic composition of *P. infestans* populations were analysed to further elucidate the importance of the oospores relative to tuber-borne inoculum and to identify the relative impact of the various types of infection sources, *i.e.* refuse piles, infested conventional and infested organic potato fields, and potato and tomato plots in allotment gardens. Chapter 4 focuses on the relation between the epidemiology and population structure. The three-year study in Southern Flevoland was placed in perspective by the long-term study on the polyetic pattern of potato late blight epidemics in The Netherlands (Chapter 5). In Chapter 6, the results of the research described in this thesis are integrated and discussed, and some consequences are elaborated.

Chapter 2

Development of potato late blight epidemics: disease foci, disease gradients, and infection sources

M.J. Zwankhuizen, F. Govers, and J.C. Zadoks

Phytopathology 88: 754-763

Abstract

Natural potato late blight epidemics were studied to assess the relative impact of various inoculum sources of *Phytophthora infestans* in Southern Flevoland (The Netherlands) from 1994 through 1996. Disease surveys were combined with characterization of isolates for mating type and DNA fingerprint pattern using probe RG57. Seventy-four percent of the commercial potato fields with early foci were clearly associated with nearby infested refuse piles. Characterization of isolates from refuse piles and fields confirmed the association. Infected seed tubers, volunteer plants, and infested allotment gardens appeared to be of minor importance for late blight development in potato fields. Several foci in refuse piles, potato fields, and allotment gardens contained more than one genotype. Due to favorable weather in August 1994, infested organic potato fields became major inoculum sources, resulting in the spread of *P. infestans* to adjacent conventional potato fields. Analyses of disease gradients, both at field and regional levels, confirmed the role of the organic fields as mid-season infection sources. The mean slope of field gradients downwind of refuse piles (point sources) was significantly steeper (100-fold difference) than the mean slope of field gradients downwind of organic fields (area sources). The genotypic composition of the *P. infestans* populations along the gradient and of the source populations in the organic potato crops did not differ significantly. Analysis of the region gradient revealed genotype-specific disease gradients. Control measures are recommended.

Introduction

Effective plant disease management requires knowledge of the epidemiology of the pathogen in order to assess the impact of control measures (Van der Plank, 1963). Control measures may reduce disease at the beginning of the season, or they may decrease the rate of disease development during the growing season (Zadoks and Schein, 1979). Information about how a plant pathogen overwinters and about the relative importance of infection sources gives crop protectionists a handle to recommend effective disease control.

Phytophthora infestans (Mont.) de Bary, the causal agent of potato and tomato late blight, is one of the most damaging fungi of potatoes (Hooker, 1981). *P. infestans* can reproduce asexually and sexually. Sexual reproduction in this heterothallic fungus only occurs when thalli of opposite mating type (A1 and A2) mate. Pairings result in the production of oospores, which can survive in the absence of a host (Drenth *et al.*, 1995). Prior to the 1980s, the A1 mating type was distributed throughout the world, whereas the A2 mating type was detected only in central Mexico (Hohl and Iselin, 1984). *P. infestans* populations outside of Mexico were limited to asexual reproduction. In the absence of the sexual cycle, *P. infestans* survives between seasons in infected potato tubers, either used as seed potatoes or discarded, often in refuse piles. Van der Zaag (1956) and Davidse *et al.* (1989) showed that infected seed tubers were the most important infection sources for late

blight epidemics in The Netherlands in the 1950s and 1980s, respectively. Others, including Bonde and Schultz (1943) in the United States, Boyd (1974) in Scotland, J. C. Zadoks and H. Lohuis (*unpublished*), and J. C. Zadoks and L. C. Davidse (*unpublished*) in The Netherlands, provided evidence that refuse piles were the more important infection sources. Overwintering in tubers, remaining in the soil after harvest, and subsequent development of late blight on volunteer plants seemed of minor importance (Croxall and Smith, 1976; Easton, 1982; Hirst and Stedman, 1960).

In the early 1980s, there were indications that the *P. infestans* populations in Europe were changing. Isolates with the A2 mating type were detected in several European countries (Frinking *et al.*, 1987; Hohl and Iselin, 1984). Migration of A1 and A2 genotypes from central Mexico to Europe in the 1970s was demonstrated (Fry *et al.*, 1993; Goodwin, 1997). Populations of *P. infestans* have become increasingly diverse, and sexual reproduction now also occurs outside of Mexico (Drenth *et al.*, 1993; Sujkowski *et al.*, 1994). Drenth *et al.* (1993; 1994) found the *P. infestans* population in The Netherlands to be highly diverse, and concluded that sexual reproduction was the driving force behind the generation of new genotypes. In Dutch potato fields and allotment gardens with natural infections, oospores were encountered in leaves and tomato fruits (L. J. Turkensteen, *pers. com.*). Drenth *et al.* (1995) showed that oospores could survive in natural soil under Dutch winter conditions and could infect potato plants in the next season.

As a consequence of the changes, current late blight epidemics in The Netherlands might be different from those prior to the 1980s, both from a quantitative and a qualitative point of view. Oospores surviving in the soil could serve as an infection source in addition to tuber-borne inoculum. Genotypes of sexual origin might have increased pathogenic fitness, compared with old genotypes (Day and Shattock, 1997; Kato *et al.*, 1997), changing the rate at which epidemics develop.

A complicating factor concerns organic agriculture. Organic agriculture has been increasingly endorsed by the Dutch government (Anonymous, 1992b). Since no fungicides are used on organic potato crops, high disease intensities may be found in these crops. Thus, infested organic potato crops might have a profound effect on spread and overwintering of the disease.

Prior to the 1980s, potato late blight epidemiology in The Netherlands was fairly well understood. The establishment of a new, sexually reproducing population requires reassessment of the relative impact of the various inoculum sources on epidemic development. Therefore, development of natural epidemics of *P. infestans* was studied from 1994 through 1996 in Southern Flevoland, one of the IJsselmeerpolders in the central part of The Netherlands. The aim of this chapter is to analyze the development of potato late blight within and between various forms and locations of potato sites (*i.e.* refuse piles, volunteer plants, allotment gardens, and commercial fields), from the initial establishment of foci to the spread at a regional scale. We combined field observations with analysis of population structure, using DNA fingerprinting with probe RG57 (Goodwin *et al.*, 1992a).

Drenth *et al.* (1994) found this probe to be a useful tool to identify genotypes during epidemic development in consecutive years.

Materials and methods

Selection of potato-growing area

Late blight epidemics were studied in a part of Southern Flevoland, the youngest polder in The Netherlands (Fig. 1), in 1994, 1995, and 1996. Land in this polder was reclaimed in the 1960s and the first farms were established in the 1970s (soil type: marine clay). The research area (Fig. 2) was approximately 10 by 15 km, including approximately 3,000 ha of ware and seed potatoes (mainly cultivar Bintje). This area included approximately 170 farms, of which 6 were organic and had approximately 140 ha of potatoes.

Reasons for choosing this specific area were: (*i*) the occurrence of severe late blight epidemics in the early 1990s; (*ii*) an apparent conflict between conventional and organic potato growers about the role of infested organic potato fields with respect to late blight development in the region; (*iii*) the occurrence of relatively high densities of volunteer plants because tubers, left in the soil after harvest, survive easily in the young, well-aerated reclaimed soil (M.J. Zwankhuizen, *unpublished*), which might have a profound effect on the development of late blight epidemics; and (*iv*) its distance (over 30 km) from the nearest commercial potato-growing area located upwind, which reduces the probability of influx of inoculum.

Scouting for potato late blight

Field surveys were carried out by M.J. Zwankhuizen, in cooperation with officers of the Plant Protection Service and students of an agricultural college. In addition, growers and salesmen of the local pesticide suppliers were contacted frequently during the growing season. Our own findings, together with the information of local people, actual weather conditions, and weather forecasts determined the selection of fields to be inspected and the inspection frequency. The interval between inspection rounds varied from 1 week in periods favorable for late blight development, to 2 or 3 weeks in dry periods.

The observational units were sites where potatoes grow. We distinguished four types of potato sites: refuse piles (R); fields with volunteer plants (V); commercial potato fields, either conventional (Fc) or organic (Fo) fields; and potato plots in allotment gardens (A) .Potatoes in allotment gardens are grown by private citizens who hire an allotment situated within a large compound. Usually, no fungicides are applied in these gardens. Refuse piles and plots in allotment gardens were inspected completely. Fields with volunteer plants and commercial potato fields were inspected along two transects, one in

each half of the field. A transect consisted of 10 potato rows, 5 to the left and 5 to the right of the observer, covering a width of 7.5 m over the full length of the field. On average, about 7.5% of the field area was inspected. In each compound of allotment gardens, 40 randomly selected plots (average size varied between 25 and 30 m^2) were inspected per visit, which is approximately 22% of the area of potatoes per compound of allotment gardens (approximately 0.5 ha).

Inspections were most frequent in the Nz-section (the polder area has been subdivided into several sections to which codes are assigned; Fig. 2). The Nz-section was the area where the first organic farms of the region were established. In this area, severely infested organic potato fields led to discussions between growers. The annual survey began by drawing a random sample of farms to be visited. In addition to these random samples, a selection of sites was visited more frequently, consisting of organic farms and infested

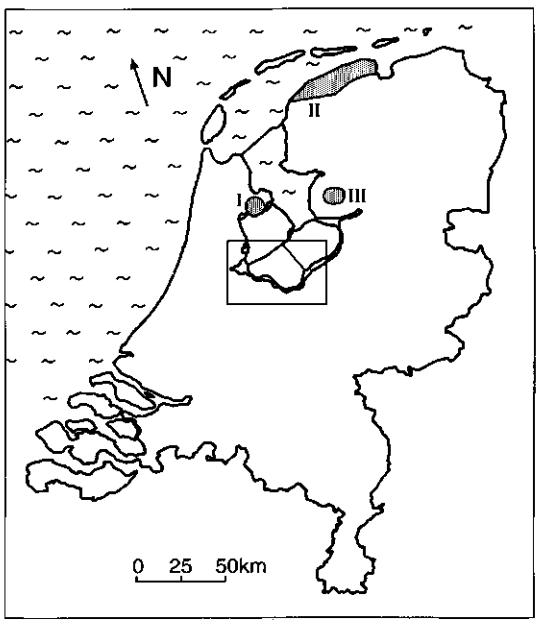

Figure 1. The Netherlands. Potato late blight epidemics were studied in Southern Flevoland (indicated by a rectangle) in 1994, 1995, and 1996. Earlier studies on late blight epidemics were carried out in areas I (De Streek) from 1952 to 1955 (Van der Zaag, 1956), II (Friesland) in 1965 (J.C. Zadoks and H. Lohuis, *unpublished*), and III (Noordoostpolder) in 1968 (J.C. Zadoks and L.C. Davidse, *unpublished*). Results of these studies are used in this chapter.

Table 1. Numbers of inspected potato sites and numbers of infested potato sites found (in parentheses) [1].

Year	Potato site									
	Refuse piles		Fields with volunteer plants		Potato fields (conventional and organic)		Allotment gardens		Total	
1994	63	(9)	29	(1)	145	(33)	760	(70)[2]	997	(113)
1995	129	(2)	36	(0)	59	(20)	720	(45)	944	(67)
1996	91	(0)	8	(0)	132	(9)	1880	(35)	2111	(44)
Total	283	(11)	73	(1)	336	(62)	3360	(150)	4052	(224)

1 Total number of infested potato sites found in the research area (including those found by others) was only slightly more, except for the potato fields in 1994. In that year, more than 90 % of all commercial potato fields in the research area was infested in June, so the total number of infested fields in 1994 was much higher than the number of fields recorded by us. Many potato sites were inspected more than once.

2 The minimum number of infested potato plots encountered in compounds of allotment gardens.

potato sites and their immediate surroundings. We inspected at least 75% of the farms for refuse piles in the beginning of each season. At least 30 fields with volunteer plants and at least 30 commercial fields (10% of the total) were inspected each year to find early infections of late blight. An overview of the survey efforts made is given in Table 1.

Disease foci

A focus is a patch of crop with disease, limited in time and space (Anonymous, 1953). Disease intensity of foci in fields and allotment garden plots was determined by counting diseased stems, expressed as the number of diseased stems per m^2.

To determine whether a focus originated from an infected seed tuber (as described by Van der Zaag, 1956), diseased plants within foci were thoroughly inspected and seed tubers of plants in the focus center were excavated to inspect them visually for *P. infestans*

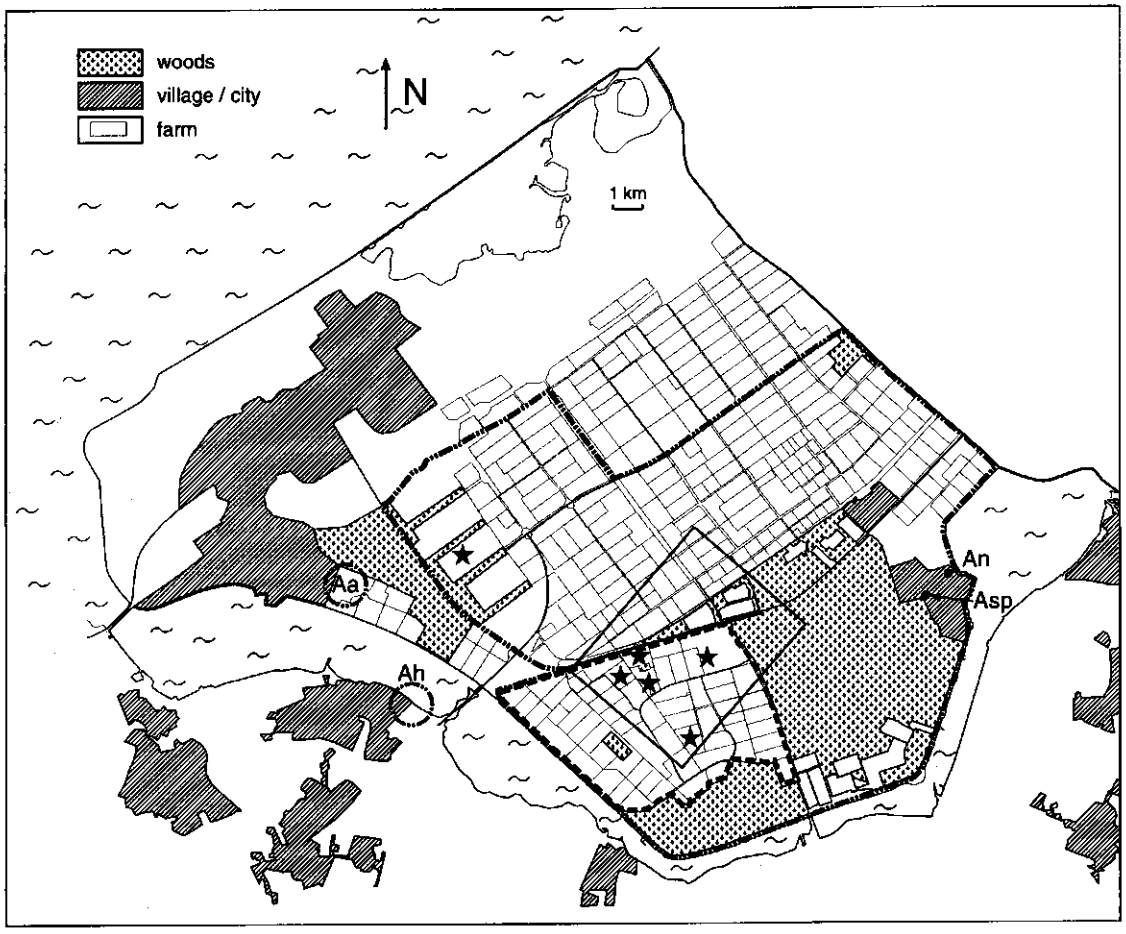

Figure 2. Southern Flevoland and adjacent areas. The research area (·····), the Nz-section (---), the location of compounds of allotment gardens (Aa, Ah, An, and Asp), and organic farms (*) are indicated. The rectangle indicates the area in which spread of late blight from organic fields to conventional fields was studied (shown in detail in Fig. 3).

symptoms. Since the focus could have been initiated by soilborne oospores, 1 kg of soil was collected from its center and infectivity was determined using the bioassay described by Drenth *et al.* (1995). Six foci from refuse piles, seven foci from potato fields, and three foci from allotment gardens were assayed.

Disease gradients

The change in disease intensity along a straight line from one point to another is termed a disease gradient (Zadoks and Schein, 1979). To study disease gradients, the distribution of late blight within commercial potato fields was determined using grid sampling. The rectangular sampling grid consisted of 16 plots of 15 or 30 m^2 (four transects per field, with four plots along each transect). Early in the season, disease intensity was determined as the fraction of plants diseased per sampling plot of 15 m^2. Later in the season, it was difficult to distinguish separate plants, so late blight intensities were determined by counting leaf and stem lesions per plot of 30 m^2.

Downwind disease gradients were constructed by using the distance from the probable source to every plot of the sampling grid within the field and the disease intensity in the sampled plot. Due to frequent fungicide applications, disease intensities near infested refuse piles were generally low. Therefore, data of only one field from our study could be used in the disease gradient analysis and data from fields near refuse piles studied previously and elsewhere in The Netherlands were included (Fig. 1).

To describe the spread of late blight from the organic fields to the conventional fields, the probable source was represented by a virtual source (Fig. 3), calculated by averaging the coordinates of a group of closely located organic fields. Within-field gradients and one among-fields gradient (further referred to as the region gradient) were constructed. Representative conventional fields along the region gradient were selected based on their relative positions to the organic fields and their disease levels. The region gradient was constructed using the data of downwind fields Fc5, Fc6, Fc7, Fc8, Fc9, Fc10, and Fc11. A crosswind disease gradient at 1,306 m downwind from the virtual source was constructed using disease intensities measured in conventional fields Fc5, Fc6, Fc8, and Fc11.

The genotypic composition of the *P. infestans* population along the region gradient was determined.

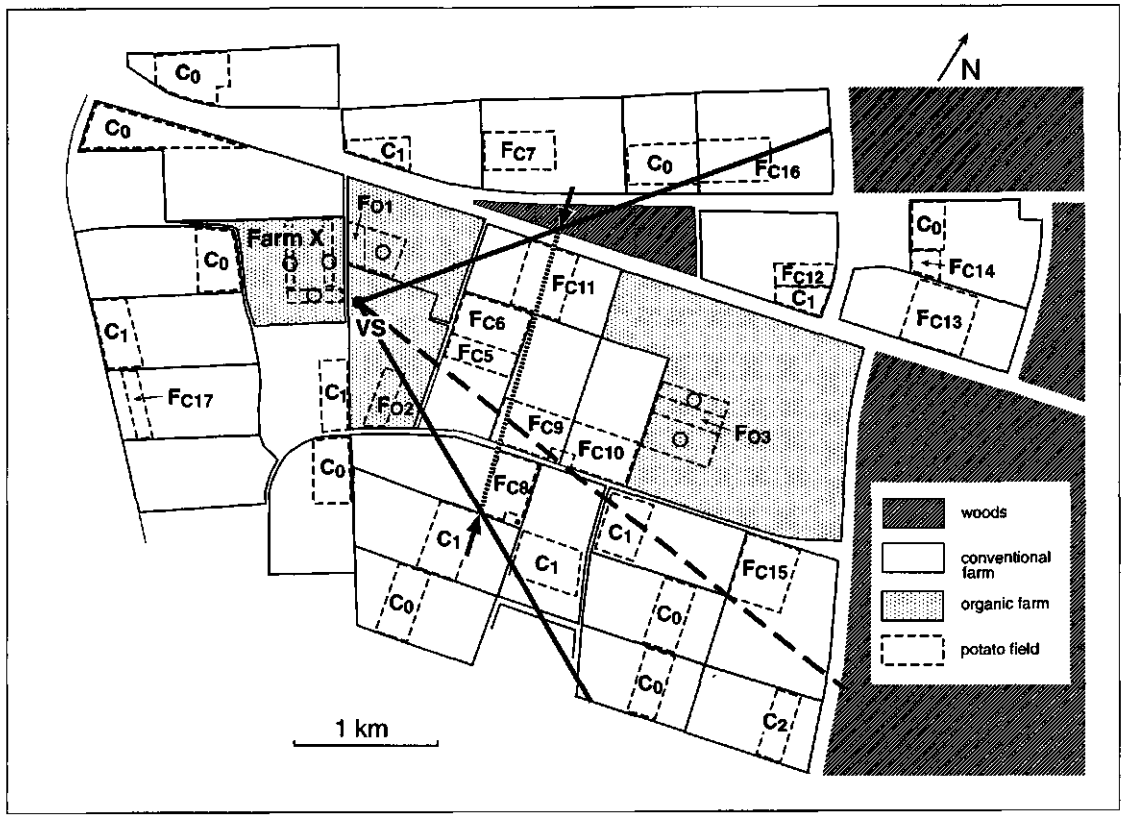

Figure 3. Conventional and organic potato fields in Southern Flevoland in the summer 1994. The cone of dispersal from the virtual source (VS; descibed in text), its calculated bisector, and the approximate position of the crosswind gradient (arrows) are indicated. Grid sampling for gradient analysis was applied in fields Fc5, 6, 7, 8, 9, 10, 11, and 12. Isolates for characterization were collected in all potato fields indicated with Fc and Fo. No late blight was found during inspections between 31 August (day 243) and 21 September (day 264) in conventional fields indicated with C_0. Conventional fields indicated with C_1 were not sampled; disease intensities were very low (one to four lesions per field) between 31 August (day 243) and 21 September (day 264). Conventional field C_2 was severely infested in the last week of August, but canopy was destroyed before sampling. Other organic fields (O) were infested in the last decade of August, but no isolates could be collected because of rapid haulm killing.

Collection of isolates

Isolates were collected in several ways. In small foci (< 30 diseased plants) 10 single lesion samples, and in large foci (≥ 30 diseased plants) 30 single lesion samples were obtained from separate plants scattered along the downwind and crosswind axes of the focus.

From the infested organic fields, 30 isolates were collected randomly along two transects. From conventional fields, located downwind of organic fields, in which late blight intensity was determined using grid sampling, 1 isolate per grid point was collected, when possible. From other conventional fields near the organic fields, 5 to 10 isolates were collected randomly along the disease gradient. If no disease gradient was visible, isolates were collected randomly along two transects. Formal sampling requirements could not always be satisfied (*e.g.* due to the fact that growers had already started to remove diseased plants, or because of contaminations and loss of isolates during culturing.

Individual isolates were obtained from single lesions on stems or leaves according to the method of Davidse *et al.* (1989). Pure cultures were obtained by plating the mycelium on Rye A medium (Caten and Jinks, 1968), amended with antibiotics. Pure cultures were kept on Rye A medium at 18°C in the dark, or stored in liquid nitrogen.

Characterization of isolates

All isolates were characterized for mating type and RG57 fingerprint pattern (Goodwin *et al.*, 1992a). To assess mating type, isolates were grown on clarified Rye A agar medium in proximity to a strain of known mating type (A1 or A2). Each isolate was tested against a known A1 strain and a known A2 strain. Oospore formation usually was recorded after 1 week.

For DNA fingerprinting, isolates were grown in liquid Rye A medium in the dark at 18°C for approximately 3 weeks. Mycelium was harvested and stored at –80°C for further use. DNA isolation, *EcoRI* digestion, Southern blotting, and hybridization with probe RG57 were performed as described by Drenth *et al.* (1993).

A multilocus genotype was constructed for each isolate by combining data for mating type and DNA fingerprint loci (Goodwin *et al.*, 1995; Sujkowski *et al.*, 1994). A genotype was designated unique if one or more isolates of this genotype were detected only in one sampling site in the research area (from 1993 through 1996).

Source-target relations

A source (or an infection source) is defined as a plant or a group of plants within a potato site with sporulating lesions from which spores are dispersed. A target is defined as a plant or group of plants within a potato site on which spores, originating from the source, are deposited, resulting in diseased tissue.

To determine whether an infested potato site had acted as an infection source for another potato site (the target), two items were considered: the disease gradient and the genotypic composition of source and target. If disease intensity in the target potato site is high enough, a disease gradient can be determined (Gregory, 1968). A primary disease gradient consists of all first-generation infections coming from the source under consideration (Zadoks and Schein, 1979), suggesting a source-target relation with decreasing disease intensities at increasing distance from the source. If late blight in a given target originates from a given source, the genotypic compositions of source and target populations must match (Zadoks, 1988).

Statistical analyses

Disease gradients were analyzed by nonlinear regression, using the exponential model of Kiyosawa and Shiyomi (1972), $y = ae^{-bx}$, in which y is the disease intensity at distance x, a is the y intercept, and b is the slope of the gradient. Slope (b), y intercept (a), coefficient of determination (R^2), and significance of the regression (P) were estimated. Data of the crosswind disease gradient were fitted to a Gaussian model ($y = a_0 + a_1\ exp(-0.5[(x-a_2)/a_3]^2)$ using nonlinear regression.

Slopes of field gradients downwind of refuse piles and slopes of field gradients downwind of organic fields (from significant regressions) were compared using the nonparametric Mann-Whitney U-test (Sokal and Rohlf, 1981).

Chi-square tests were conducted to identify differences in genotype frequencies encountered in target fields and their respective sources.

Results

Late blight epidemics in 1994, 1995, and 1996

Late blight epidemics differed dramatically from each other with regard to the date of first appearance of late blight, the overall disease level, and the development pattern during the growing season (Table 2). The pattern of disease development during the growing seasons was quite similar to the pattern of alternating periods with favorable and unfavorable

Table 2. Development [1] of late blight in Southern Flevoland during the growing seasons of 1994 through 1996.

Year	May	June	July	August	September
1994	+	++++	-	++[2]	+[2]
1995	0	++	+	+/-[2]	-[2]
1996	0	0	+	+/-[2]	+/-[2]

1 0 = no late blight observed; - = decrease of disease intensity due to hot, dry weather; +/- = only very slight increase of disease intensity and number of infested potato sites; + = few potato sites infested, slight increase of disease intensity; ++ = 1-10 % of potato sites infested, moderate increase of disease intensity and number of infested potato sites; ++++ = > 90 % of potato sites infested and rapid increase of disease intensity.

2 Decrease in number of infested potato sites due to vine killing on organic and conventional fields.

weather conditions. The distribution of the total precipitation over the course of the growing season of 1994, 1995, and 1996 is an example (Fig. 4). The number of days from May through September with precipitation were 67, 66, and 67, respectively, whereas the total amounts of precipitation during those periods were 448, 347, and 239 mm, respectively, for the different years.

1994. During the surveys from April through June, 93 farms were visited. Of 62 refuse piles found, 52 (84%) were not covered or were incompletely covered by plastic foil. On 9 of these refuse piles (17%), diseased plants were found. Five of the infested refuse piles were encountered in the Nz-section (Fig 2). Conditions were favorable for late blight development until the third week of June. Around mid-June, *P. infestans* was present in most potato fields. Due to hot and dry weather in July, disease intensities decreased and almost no newly infested potato sites were found. After this dry period, weather conditions again were favorable for late blight, and actively sporulating foliar lesions were found in organic field Fo1 on 16 August (Julian day 228) (Fig. 3). The foliage in this field was killed by flaming one day after our visit on 19 August (day 231). On 25 and 26 August (days 237 and 238), the organic growers began to destroy the foliage of their severely diseased crops (disease intensities 100-250 lesions per 30 m^2). From 26 August (day 238), disease began to appear in the conventional fields located downwind (east) of the organic fields (Fig. 3). Far less disease was found in conventional fields in the upwind direction. *P. infestans* was recorded in allotment gardens from the beginning of July until the beginning of August. Disease developed slowly due to hot and dry weather in this period.

1995. Weather conditions were more or less favorable for late blight development until the first half of July (Fig. 4). During the rest of the growing season (until the third week of September), weather was almost continuously unfavorable. Of 124 refuse piles identified on 139 farms, 65 (52%) were not covered or were incompletely covered by plastic foil. Only 2 diseased refuse piles were encountered, 3% of the incorrectly covered piles. Early disease foci in commercial fields were found 17 days later than in 1994 (day 171). No disease spread from the organic fields to the conventional fields was observed, despite high disease intensities (comparable to 1994) in most of the organic fields at the end of July. After mid-July, disease development was limited due to hot and dry weather. In allotment gardens, disease was found from the last week of June till the end of the growing season, but disease progressed slowly and never reached high levels.

1996. Seventy-nine refuse piles, located at 135 farms, were inspected. Even though 58% of the refuse piles were not covered or were incompletely covered, no diseased plants were found. Weather was unfavorable for late blight until the second half of July (Fig. 4). Thereafter, weather conditions were rather unstable, sometimes being favorable, sometimes not. Disease in the area was first found in an organic field on 24 July (day 206), 88 and 42 days later than in 1994 and 1995, respectively. The organic fields were the only potato fields where late blight was found until mid-September. Only very late in the growing

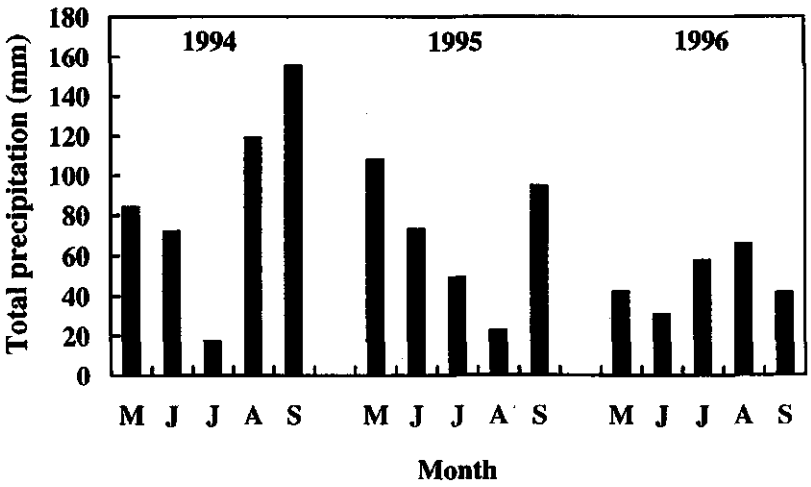

Figure 4. Total precipitation in May (M), June (J), July (J), August (A), and September (S) of 1994, 1995, and 1996. Data from the Royal Netherlands Meteorological Institute (KNMI) at De Bilt, located at 20 km south of the research area.

season, when many growers already had started haulm killing, were some lesions found in a few conventional fields. Initially, disease developed slowly in the allotment gardens after the first detection on 31 July (day 213), but at the end of August, when most potatoes were harvested, nearly all tomato plots were found to be infested.

In 1994 and 1996, densities of volunteer plants were low (≤ 1,000 plants/ha). In 1995, average density was approximately 5,500 volunteer plants/ha. No significant disease development was recorded in fields with volunteer plants throughout the three growing seasons.

Disease foci

Typical foci of *P. infestans* (Van der Zaag, 1956) in refuse piles were difficult to recognize because of the use of herbicides and mechanical disturbance. Six distinct foci were identified in three refuse piles (Table 3). In all infested refuse piles, including those without typical foci, many rotten tubers were found, some with symptoms of *P. infestans*. Symptoms were visible on both below-ground and above-ground stem parts, and foci appeared to have developed from infected tubers. Only 2 foci were genotypically uniform (Table 3), indicating that several independent infections occurred, even within small foci consisting of only 3 to 7 diseased stems. Most isolates (91%) collected on refuse piles in 1994 and 1995, including those without typical foci, belonged to predominant genotypes NL-41, NL-69, NL-75, and NL-76.

During the 3 years of the study, 34 early foci were found in 20 potato fields and were inspected thoroughly. In 1994 and 1995, these foci were encountered from the first week of June through the first week of July. In 1996, only 1 focus was found in an organic field on 24 July (day 206), the first disease record of that year in the research area. In general, a focus (disease intensity ranging from less than 5 to 100 diseased plants) consisted of 1 severely diseased plant with reduced growth, all stems blighted, and nearly all leaflets infected. Surrounding plants had one to three diseased stems, with 10% of the leaflets diseased. Plants outside the focal center generally had one diseased stem, with 1 leaf or stem lesion. Symptoms on below-ground stem parts were never found in foci in potato fields. In only a few cases, the planted seed tubers were slightly infected with *P. infestans*. A selection was made for the characterization of foci from potato fields (Table 3). Six foci from three fields in 1994 represented the foci encountered during the early outbreak of late blight: the four foci in fields Fc1 and Fc2 were the first detected foci in the research area in1994; the two foci in field Fc4 were selected because the field was located downwind of field Fc3 and refuse pile Rc2, and the question was whether disease in this field could have originated from refuse pile Rc2. The two foci characterized in 1995 were the first foci found in the area early in the season in that year. No other foci were found to be appropriate for characterization, either because foci were found too late or because they

Table 3. Characteristics of foci of *Phytophthora infestans* found in Southern Flevoland in 1994, 1995, and 1996.

Year	Sampling day	Potato site[1,2]	Size in m^2	Disease intensity[3]	No. isolates characterized	Genotypes detected (frequency)	Unique genotypes	Comments
1994	118	Rc1[4] 1	0.3	24	3	NL-76 (3)		Distance between foci varied from 0.5 to 1 m. Small scale dispersal between foci cannot be excluded.
		Rc1 2	0.3	16	3	NL-41 (2), NL-100 (1)	NL-100	
		Rc1 3	0.3	28	5	NL-76 (4), NL-78 (1)	NL-78	
		Rc1 4	0.3	12	3	NL-41 (1), NL-76 (2)		
1994	147	Rc2	4	13	17	NL-41 (13), NL-42(1), NL-75 (3)	NL-42	Observed focus might have originated from several initial foci
1994	147	Rc4	1	8	5	NL-69 (5)		
1994	154	Fc1 1	1	8	5	NL-41 (5)		An infested refuse pile (Rc3)[5] was found at 750 m southeast from this field. Only one isolate from this refuse pile could be characterized, which had genotype NL-76. It cannot be excluded that this refuse pile contained also genotype NL-41, but also another (unknown) refuse pile might have been involved.
		Fc1 2	1	6	4	NL-41 (4)		
		Fc1 3	1	6	2	NL-41 (2)		
1994	165	Fc2	375	0.09	13	NL-75 (13)		An infested refuse pile (Rc6)[5] was found at 325 m northeast from this focus on day 154, with genotypes NL-75 and NL-76.
1994	181	Fc4 1	20	2.5	5	NL-41 (5)		These foci were located at 1030 m northeast from refuse pile Rc2.
		Fc4 2	13	3.1	5	NL-41 (4), NL-75 (1)		

1995	171	Fc1	120	2	30	NL-41 (30)		An infested refuse pile (Rc2)[5] was found at 500 m west from this focus, with genotypes NL-41 and NL-76.
1995	186	Fo1	4	4	10	NL-41 (10)		
1995	178	Aa39	4	11	10	NL-17 (10)	NL-17	Aa39 and Aa89 were 2 out of 3 early infested plots in that compound of allotment gardens, all containing different genotypes.
1995	178	Aa89	5	5.4	10	NL-3 (10)	NL-3	
1995	186	Asp81	7	14.3	10	NL-54 (1), NL-57 (1), NL-70 (6), NL-71 (2)	NL-54, NL-57, NL-70, NL-71	Second infested plot in this compound of allotment gardens.
1996	206	Fo1	8	1.4	9	NL-126 (8), NL-127 (1)	NL-126, NL-127	This focus was the first diseased potato site in S. Flevoland in 1996.

1 Refuse pile on conventional farm (Rc), conventional potato field (Fc), organic potato field (Fo), allotment garden (A). Aa39 = plot 39 in the compound of allotment gardens Aa, Asp81 = plot 81 in the compound of allotment gardens Asp.

2 Cultivar: Bintje, except for potato sites Fc1 (cv. Spunta), Fo1 (cv. Vital), Aa89 (cv. Eersteling), Asp81 (cv. Doré). Susceptibility values (foliage/tuber) of the cvs. Bintje, Spunta, Eersteling, Doré, and Agria are 3/3, 5/5, 2/3, 2.5/7, and 5.5/7, respectively, according to the Dutch Descriptive List of Varieties of Field Crops (1996). A high value means low susceptibility. Values for Vital not known.

3 Number of diseased stems per m^2.

4 In some cases, more than one focus was sampled per potato site (e.g., 4 foci were sampled on Rc1).

5 This refuse pile was not considered in this paper, because no focus could be identified.

had been (partially) removed by the grower. The only focus found in 1996 was selected for DNA fingerprinting. Seven foci, found in 1994 and 1995, were each genotypically uniform and contained the predominant genotypes NL-41 or NL-75. One focus in field Fc4 in 1994 had two genotypes, probably resulting from the simultaneous establishment of at least two infections from source Rc2, with isolates belonging to genotypes NL-41 and NL-75. The focus on field Fo1, found in 1996, contained two unique genotypes. In this field, six genotypes were detected in total, five of which were unique or rare (Zwankhuizen *et al.*, *subm.(a)*).

In allotment gardens, disease levels were generally low. Disease usually began with a few infected leaves, scattered over the plot; the typical focal pattern of disease described for the commercial fields was rarely evident. Out of 41 potato sites sampled in allotment gardens from 1994 through 1996, only three typical foci were found and characterized (Table 3). The structure of these foci was quite similar to those described for the commercial potato fields. Two foci appeared to be genotypically uniform, but one focus had isolates belonging to four (unique) genotypes. In the latter case, almost all plants in the plot were infected.

Soil samples collected in foci from refuse piles, potato fields, and allotment gardens were not infectious, according to the bioassay.

Disease gradients

Disease intensity in commercial potato fields near infested refuse piles was generally low due to frequent fungicide applications. Data from only one field of our study (Fc3 near refuse pile Rc2) were included in the disease gradient analysis.

In September 1994, disease was assessed and isolates were collected in most of the infested conventional fields located downwind (east) of the organic fields (Fig. 3). To analyze the spread of disease over the region, organic potato fields Fo1, Fo2, and the potato fields on farm X were used to construct the virtual source (VS). Although no inspections were done on farm X (this grower did not cooperate), we assume that disease intensity on this farm was as high as on Fo1 and Fo2, in accordance with reports of neighbors who had visited this farm. The region gradient was visible as a sector with its origin in the virtual source. Seventeen conventional fields located upwind of the organic fields were inspected between 31 August (day 243) and 21 September (day 264; seven of these fields were visited twice). Disease was detected in only three upwind fields and only the disease intensity of field Fc17 was relatively high (10 lesions per 30 m^2) (Fig. 3).

Slopes of the field gradients downwind of the refuse piles varied considerably, whereas slopes of the field gradients downwind of the organic fields were all in the same order of magnitude, despite differences in disease intensity (Table 4). For field Fc3, the exponential model gave a poor fit. The mean slope of the field gradients downwind of the

refuse piles and the mean slope of the field gradients downwind of organic fields were – 0.35 and –0.0045 m^{-1}, respectively (Table 4). This 100-fold difference is statistically significant ($P < 0.01$).

The slope of the region gradient (Fig. 5A) and the mean slope of its individual field gradients were in the same order of magnitude (–0.0029 and –0.0045 m^{-1}, respectively; Table 4). The crosswind gradient (Fig. 5B) was described by the simplified Gaussian equation: $y = 0.78 + 8.24\ exp(-0.5[x/211.14]^2)$ ($R^2 = 0.70$, $P < 0.001$), with the bisector of the cone pointing to the east (Fig. 3).

Results of the characterization of isolates revealed remarkable differences between disease gradients of genotypes NL-41 and NL-76 in the *y* intercept and the slope of the gradient (Table 4, Fig. 5C and D).

Source-target relations

An infested refuse pile was found at a median distance of 600 m, with 50% of the distances between 160 and 900 m, for 14 out of 19 potato fields (74%) in which early foci were studied in 1994 and 1995. An infested refuse pile appeared the most likely infection source for 7 out of 9 foci in potato fields from which isolates were characterized (Table 3). Most isolates from refuse pile Rc2 and from downwind fields Fc3 and Fc4 belonged to the predominant genotypes NL-41 and NL-75 (94 and 97%, respectively). No significant disease gradient was present in field Fc3, but genotypic compositions of the target populations in fields Fc3 and Fc4 and the putative source population of refuse pile Rc2 did not differ significantly ($P = 0.88$, Table 5). The result of this test may be unreliable because 50% of the expected values were lower than 5. When only the predominant genotypes NL-41 and NL-76 were considered, there was no difference in genotypic composition according to Fisher's exact test ($P = 0.62$) (Sokal and Rohlf, 1981).

Field gradients and the region gradient suggested organic fields Fo1, Fo2, and the fields on organic farm X to have been the infection sources for nearby conventional fields in 1994 (Table 4). Three organic fields (Fo1, Fo2, and Fo3, with moderately resistant cvs. Provento, Santé, and Escort, respectively; Fig.3) could be sampled just before growers destroyed the foliage by flaming. Most isolates of fields Fo1 and Fo2 (80%) belonged to genotypes NL-41 and NL-76. The majority of isolates (89%), collected in conventional fields located downwind and upwind also belonged to genotypes NL-41 and NL-76. Genotypic compositions of source and target populations were not significantly different ($P = 0.11$, Table 6). Field Fo3 appeared not to have acted as a source. All 14 isolates from this field had genotype NL-48, and this genotype was not found in any other field.

The relative importance of organic fields Fo1 and Fo2 might be different for different conventional fields, according to the results of disease gradient analysis and the isolate characterizations. If real sources are used instead of the virtual source, regressions

Table 4. Disease gradients of *Phytophthora infestans* in potato fields downwind of refuse piles (in Southern Flevoland in 1994, in Friesland in 1965, and in Noordoostpolder in 1968) and in fields downwind of organic fields in Southern Flevoland in 1994.

Field - probable source[1]	Year	Disease intensity[2]	Day of first late blight observation	Day of sampling	Distance to source (m)[3]	n[4]	y-intercept (a)[5]	slope (b) (m^{-1})[5,6]	R^2 [7]	Significance (P)
Field gradients										
Fcr1 - Rc007	1965	3.3	183	183	34	30	6.6	-0.033	0.53	< 0.001
Fcr2 - Rc007	1965	0.8	183	183	59	30	1.0	-0.016	0.15	< 0.05
Fca - Rc058	1965	1.9	189	189	70	13	7.3	-0.051	0.86	< 0.001
Fch - Rc109	1965	1.9	174	174	30	4	6.8	-0.30	0.97	< 0.05
Fcb - Rc1	1965	1.5	161	172	15	25	60.3	-1.35	0.71	< 0.001
Fco - Rc107	1968	0.2	164	164	155	8	0.4	-0.0078	0.76	0.46
Fc3 - Rc2	1994	0.2	167	168	365	16	0.33	-0.0025	0.15	0.14
Fc7 - VS	1994	15.3	243	244	1570	16	29.3	-0.0036	0.25	< 0.05
Fc11 - VS	1994	1.7	241	244	1335	16	3.2	-0.0035	0.14	0.16
Fc6 - VS	1994	2.1	238	244	973	16	5.6	-0.0074	0.67	< 0.001
Fc5 - VS	1994	10.2	238	244	950	16	19.4	-0.0044	0.83	< 0.001
Fc8 - VS	1994	1.7	243	250	1660	14	4.9	-0.0076	0.20	0.11
Fc9 - VS	1994	5.1	239	244	1508	15	8.1	-0.0027	0.35	< 0.05
Fc12 - VS	1994	32.1	242	250	3140	15	53.8	-0.0046	0.39	< 0.01

Region gradients

Region[8] - VS	1994	3.5	..[9]	..	..	92	11.5	-0.0029	0.44	< 0.001
Region-41+76[10] - VS	1994	3.7	..	..	..	52	11.5	-0.0017	0.41	< 0.001
Region-41[10] - VS	1994	4.5	..	..	..	13	20.0	-0.0050	0.98	< 0.001
Region-76[10] - VS	1994	3.7	..	..	..	39	6.3	-0.00059	0.18	< 0.01

1 Conventional refuse pile (Rc), conventional field (Fc), and virtual source (VS, see text). Cultivar: Bintje, except for potato sites Fcr (Doré), Fcr2 (Eigenheimer), Fco (Sirtema), and Fc6 (Marijke). Susceptibility values (foliage/tuber) of the cvs. Bintje, Doré, Eigenheimer, Sirtema, and Marijke are 3/3, 2.5/7, 4.5/3, 2/6, and 5/4, respectively, according to the Dutch Descriptive List of Varieties of Field Crops (1996). A high value means low susceptibility.

2 Disease intensity for fields Fcr1, Fcr2, Fca, Fch, Fcb, and Fco: = percentage of leaf area diseased; for field Fc3 = proportion of diseased plants; for other fields: = number of lesions per 30 m^2. For Fc10 (Fig. 3), no disease was found at any grid point; only one isolate could be found near the edge of the field. For the fields Fc13, 14, 15, 16, and 17 (see Fig. 3), no grid sampling was applied, but disease intensities, estimated during collection of isolates, were 1.8, 0.0, 0.0, 1.0, and 10.0, respectively.

3 Distance from center of source to center of infested field. Distances of fields Fc10, 13, 14, 15, 16, and 17, were 1,995, 4,065, 3,975, 3,395, 2,835, and 1,640 m, respectively.

4 Number of observation points (grid or line sampling).

5 Determined by non-linear regression of the data to the model $y = ae^{-bx}$.

6 Mean slope for the disease gradients downwind of refuse piles = -0.35 ± 0.26 m^{-1} (± standard error); for disease gradients downwind of organic fields = -0.0045 ± 0.00079 m^{-1} (± standard error); slopes of the significant regressions used only.

7 Coefficient of determination.

8 Gradient over fields Fc5, 6, 8, 9, 10, and 11. For selection of fields, see text.

9 See field data.

10 Region gradients based on characterization of isolates. Region 41 + 76: gradient on fields Fc5 to 16 (except Fc7 and Fc12), based on isolates with genotypes NL-41 or NL-76. Region 41: gradient on fields Fc5, 8, 10, 11, 14, 15, and 16, based on isolates with genotype NL-41 only. Region 76: gradient on fields Fc5, 6, 8, 9, 11, 13, 14, and 16, based on isolates with genotype NL-76 only.

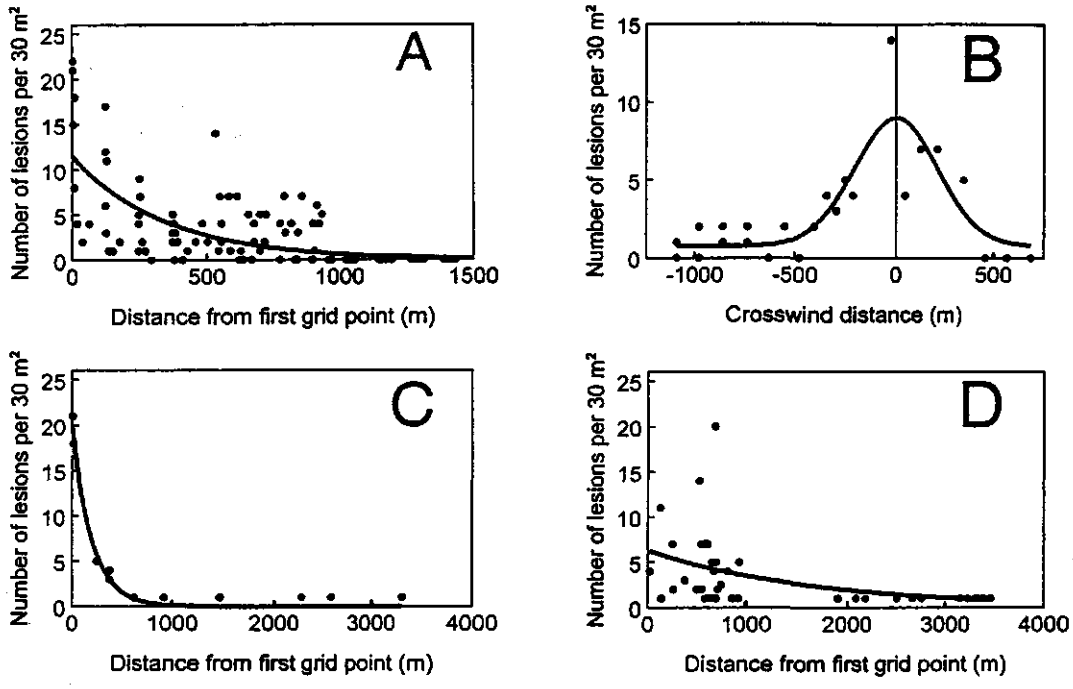

Figure 5. Downwind and crosswind region gradients of *Phytophthora infestans* in Southern Flevoland in September 1994. **A,** Region gradient (all genotypes) over conventional potato fields Fc5, 6, 8, 9, 10, and 11. The distance from the virtual source (VS) to the first grid point (in Fc5) was 765 m. **B,** Crosswind gradient (all genotypes) over conventional fields Fc5, 6, 8, 9, and 11, located approximately 1,300 m downwind of the VS. Crosswind distance measured relative to the bisector of the infected cone (Fig. 3). See text for details. The gradient was described by the Gaussian equation $y = 0.78 + 8.24\ exp(-0.5[x/211.14]^2)$; $R^2 = 0.70$, $P < 0.001$, $n = 24$. **C,** Region gradient, including only isolates belonging to genotype NL-41, over conventional fields Fc5, 8, 10, 11, 14, 15, and 16. The distance from VS to the first grid point (on Fc5) was 765 m. **D,** Region gradient, including only isolates belonging to genotype NL-76, over conventional fields Fc5, 8, 10, 11, 14, 15, and 16. The distance from VS to the first grid point (on Fc5) was 780 m. Downwind disease gradients (A, C, and D) were fitted by the negative exponential model ($y = ae^{-bx}$); the parameters are given in Table 4.

gave a better fit for some fields (for target Fc7 and source Fo2, $R^2 = 0.48$, $P < 0.01$; for targets Fc8, Fc9, and Fc11 and source Fo1, R^2 was 0.28, 0.44, 0.31, $P < 0.01$, 0.01, 0.05, respectively). The rare genotype NL-77 was found for the first time in Fo1 and later in downwind field Fc12, indicating that Fo2 was the most important source for Fc12.

Discussion

Muddy epidemics

Waggoner's (1962) statement "we shall find the real epidemic muddy and uncomfortable" is certainly applicable to our subject. Late blight epidemics differed dramatically between years due to weather conditions. Several other confounding factors, such as differences in spray regime, crop growth, and removal of diseased plants by growers, resulted in variation of disease development among fields. The statistical design was far from unbiased. Fields were not always selected randomly for inspection and sampling, and isolates could not always be sampled at random within potato sites. The system under study was also influenced by the researcher. Frequent inspections alerted growers, reducing the representativeness of the results. The inconvenience of a strong bias in the sample surveys had to be accepted in view of the inevitable mix of research questions. Notwithstanding these objections, field-oriented research is essential to obtain a true understanding of the survival and spread of the late blight fungus *P. infestans*. We tried to overcome the methodological problems by selecting representative potato sites for the analysis.

Disease foci

Foci on refuse piles had a similar structure as the foci described by Van der Zaag (1956) and appeared to have originated from infected tubers. Foci encountered in potato fields did not include plants that could have originated from infected seed tubers, according to the description of Van der Zaag (1956). In a few cases, diseased seed tubers were found but they were only slightly infected. It is likely that these tubers were infected by spores washed down from infected leaves and stems. Experiments are needed to test this hypothesis. In allotment gardens, the focal pattern of disease was usually not evident, probably because most infections resulted from oospores (Zwankhuizen *et al.*, *subm.(a)*).

Our hypothesis was that isolates of *P. infestans*, collected from a single focus, all belong to one genotype, as the result of the successful colonization by a single genotype. For foci in potato fields, this was generally the case. Exceptions were a focus in a field with high disease intensity and a focus in which oospores were thought to be involved. In

Table 5. Numbers of isolates belonging to genotypes NL-41, NL-75 and others found in refuse pile Rc2 and fields Fc3 and Fc4 in Southern Flevoland, 1994 [1].

Potato site	Genotypes			
	NL-41	NL-75	Others	Total
Refuse pile Rc2	14	3	1	18
Conventional fields Fc3[2] and Fc4	29	6	1	36
Total	43	9	2	54

1 Genotypic composition of the population in refuse pile and fields not significantly different (P = 0.88) according to the Chi-square test. P-value may be inaccurate because 3 expected values were < 5.

2 In this field, 30 isolates were collected along 5 transects.

allotment gardens, where oospores are thought to be the most important infection source (Zwankhuizen *et al.*, *subm.(a)*), a focus was found with four unique genotypes. Isolates collected from foci on refuse piles generally belonged to more than one genotype, most isolates having the predominant genotype NL-41, NL-69, NL-75, or NL-76. The occurrence of more than one genotype within a disease focus may affect the rate at which a focus expands. To our knowledge, genotypic composition of foci of *P. infestans* has not been analyzed so far.

Disease gradients

Slopes of the gradients downwind of refuse piles varied considerably. Differences might have been caused by secondary spread along the gradient or by infections arising from other sources (Gregory, 1968). Slopes of gradients of fields downwind of refuse piles were significantly steeper than slopes of gradients downwind of organic fields (–0.35 versus –0.0045 m^{-1}). Although the distance from refuse piles to target fields was roughly one-tenth of the average distance from the virtual source to the targets, the areas of the two sources differed more than a 1,000-fold. Therefore, the 100-fold difference between the two types of slope may be largely attributed to the size of the respective sources relative to that of the targets. Infested refuse piles can be regarded as point sources, whereas infested organic

Table 6. Numbers of isolates belonging to genotypes NL-41, NL-76, and others found in organic fields Fo1 and Fo2 and downwind conventional fields (Fc5-16) in Southern Flevoland, 1994 [1].

Potato site	Genotypes			
	NL-41	NL-76	others	Total
Organic fields Fo1 and Fo2	3	21	6	30
Conventional fields Fc5-16	22	48	9	79
Total	25	69	15	109

[1] Genotypic composition of the population in organic and conventional fields not significantly different (P = 0.11), according to the Chi-square test.

fields are area sources (Zadoks and Schein, 1979). Other, unknown sources could have contributed to the flattening of the gradients downwind of the organic fields. However, we estimate their effects to be limited, because the organic fields, included in the virtual source, were the only infection sources in the area where spread of disease was observed during that time. The region gradient was cone-shaped with a slope of $-0.0029\ m^{-1}$, with only minor disease intensities upwind of the virtual source. The observable length of the gradient was approximately 4 km. Fields Fc7 and Fc12 were excluded because disease intensity in those fields was much higher than in the other fields, taking into account their distance to the virtual source. The probable reason is mismanagement, since the two growers did not check their fields regularly. Crosswind gradient analysis showed the width of the cone to be at least 0.41 radians, with the calculated bisector pointing to the east. Spread to the east is in accordance with the prevailing wind directions at this time of the year. Clear differences in disease intensities existed in the cone. If other factors are constant, these differences should only be determined by the relative position of the field in the cone (*i.e.* by the distance from the source, along the downwind gradient) and the distance from the bisector of the cone (along the crosswind gradient). Differences in control practices (*e.g.* inspection frequency by the grower, amount and type of fungicide used) and crop development confounded the theoretical pattern.

A general shortcoming in the use of disease gradients for the localization of infection sources is the disregard of the genotypic composition of target and source population (Zadoks, 1988). Unknown sources can have a profound effect on disease

development in target fields (Gregory, 1968). Therefore, we combined gradient analysis with characterization of isolates. Gradient analysis and isolate characterizations were in good agreement in this study. Consideration of the genotypes found along the region gradient revealed that the observed gradient consisted of at least two genotype-specific gradients. Genotype dependency of disease gradients might be an additional explanation of the relatively poor statistical fit (R^2 = 0.44) of the gradient. Differences between the genotype-specific gradients may have occurred simply by chance, but differences in pathogenic fitness (*e.g.* the number of spores produced per unit of lesion area) between genotypes cannot be excluded and needs further research.

Infection sources

Both field observations and characterization of isolates suggest that infested refuse piles were major infection sources for the development of late blight epidemics in Southern Flevoland in the spring of 1994 and 1995. The decrease in number of infested refuse piles over the years was probably caused by reduced tuber infections in the autumn of 1994 and1995, compared to 1993. The majority (74%) of fields with early foci was associated with nearby infested refuse piles and, in most cases, putative sources and targets had the same genotypes. Ninety-one percent of the isolates from the refuse piles collected in 1994 and 1995 belonged to the predominant genotypes NL-41, NL-69, NL-75, and NL-76 (Zwankhuizen *et al., subm.(a)*). The majority (70%) of isolates collected in the commercial fields also belonged to these genotypes.

In 1994, five infested refuse piles were found in the Nz-section of Southern Flevoland (Fig. 2), which equals 0.2 infection sources per km^2 (25 ha of potatoes). This relatively low density of initial infection sources resulted in a general occurrence of late blight in June. Van der Zaag (1956), J. C. Zadoks and H. Lohuis (*unpublished*), and J. C. Zadoks and L. C. Davidse (*unpublished*) identified 1, 1.5, and 0.7 foci per km^2, respectively. Although other sources cannot be excluded in our study, (*e.g.* infected seed tubers or undetected infested refuse piles), the occurrence of a general epidemic can be explained by the presence of the few initial foci found in infested refuse piles. Most infested refuse piles were found before the time growers began to apply fungicides (*i.e.* the first week of June). The putative increase in pathogenic fitness of the new *P. infestans* population (Day and Shattock, 1997; Kato and Fry, 1997) may have contributed to a rapid spread of disease.

Van der Zaag (1956) found 1 focus per km^2 originating from an infected seed tuber in potato fields. He also identified 0.2 infested refuse piles per km^2. For only one potato field was the distribution of the disease in the field found to be associated with an infested refuse pile. He concluded that refuse piles were of minor importance. Boyd (1974), J. C.

Zadoks and H. Lohuis (*unpublished*), and J. C. Zadoks and L.C. Davidse (*unpublished*) found infested refuse piles to be the major infection sources, with little or no indications that infected seed tubers had contributed significantly to disease development. However, other studies indicate that the contribution of infected seed tubers cannot be excluded. The results of Hirst and Stedman (1960) suggest that foci can develop from diseased tubers by infection of above-ground plant parts via spores originating from diseased tubers and passing through the soil without invading stems. In addition, several population studies showed that seed tubers can be important as an infection source (Davidse *et al.*, 1989; Fry *et al.*, 1993; Goodwin *et al.*, 1995). Moreover, our own studies (Zwankhuizen *et al.*, *subm.(a)*) indicate that infected seed tubers might play a role in the development of disease in organic crops.

To determine whether early foci could have originated from oospores, infectivity of the soil was tested using a bioassay. None of the soil samples appeared to contain oospores. Nevertheless, characterization of isolates from disease foci suggested infection from oospores in some cases. The sensitivity of the bioassay might be too low to allow detection of infectious oospores in soil, especially when these propagules are not distributed evenly in the soil.

Volunteer plants seemed to be of minor importance, probably due to relatively low volunteer plant densities in 1994, and minor epidemics in 1995 and 1996. No foci originating from infected tubers were found, and no volunteer plants were involved in secondary spread. Although Hirst and Stedman (1960) showed that the fungus can overwinter in tubers in the soil, conditions in the open field are less favorable for survival of the pathogen and development of foci than in refuse piles.

Disease gradient analysis and characterization of isolates demonstrated convincingly that at least two organic fields were major secondary inoculum sources in the Nz-section of Southern Flevoland in August and September 1994. Gradient analyses suggest that not all infested organic fields contributed to disease spread (*e.g.* field Fo3 was exempted) (Fig. 3). In 1995 and 1996, due to unfavorable weather conditions, hardly any field-to-field disease spread was observed. In 1994 and 1995, the majority of infections in organic fields probably originated from refuse piles, surrounding conventional fields, and organic seed tubers.

Some infections in the commercial potato fields could have originated from allotment gardens, but the evidence is scanty (Zwankhuizen *et al.*, *subm.(a)*).

This study is the first to provide combined epidemiological and genotypic evidence that infested refuse piles were important sources for the establishment of early foci within fields, which may lead to a general epidemic of late blight in an agricultural area. In 1994, infested organic crops acted as late or secondary infection sources, resulting in dispersal of at least two genotypes of the late blight fungus over an area of 25 km^2 in a period of 2 weeks. Effective elimination or covering of all the refuse piles is needed to prevent early

introduction of late blight. The longer the epidemic is retarded, the shorter will be the period during which sexual reproduction with successful formation of oospores can occur. The foliage of organic crops has to be destroyed by flaming immediately after the first appearance of the disease, in order to prevent dispersal to neighboring fields, to reduce the risk of oospore formation, and to avert infection of seed potatoes.

Chapter 3

Population studies on *Phytophthora infestans* in Southern Flevoland and adjacent areas (The Netherlands). 1. Genotypes and inoculum sources

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Submitted

Abstract

Genotypic changes in populations of *Phytophthora infestans* in Southern Flevoland (150 km^2) were analysed by characterising isolates from potato refuse piles, conventional and organic potato fields, and potatoes and tomatoes in allotment gardens for mating type (1712 isolates) and DNA fingerprint pattern using probe RG57 (1048 isolates). The overall percentages of isolates that were A2 varied from 4 % in 1994 with weather favourable for late blight to 56 % in 1996 with unfavourable weather. The percentages of genotypes that were A2 ranged from 32 % in 1994 to 45 % in 1996. Among 981 isolates collected on potato, 146 genotypes were identified, of which 116 (79 %) were unique. Most unique genotypes were encountered in organic potato fields and in allotment gardens. Four genotypes predominated during 1994 and 1995. Highest percentages of isolates with these genotypes were encountered in refuse piles and conventional potato fields. Refuse piles were identified as the most important infection source in 1994 and 1995. The predominant genotypes from these years were nearly extinct in 1996, suggesting that the population had passed through a bottleneck at the transition from 1995 to 1996. The high numbers of unique genotypes detected every year indicate a role of oospores in commercial potato fields. In 1996, disease in organic fields was probably initiated by a few genotypes, originating from seed tubers. Subsequently, in-the-green crop sexual recombination occurred, followed by multiplication and spread of recombinants. In allotment gardens, oospores appeared the most important infection source. Evidence for potato-tomato specialisation was not convincing. Probe RG57 was used to identify (sub)populations and to directly track individual genotypes. AFLP fingerprinting was conducted to further distinguish between isolates with identical RG57 fingerprints from unrelated sites within and outside the research area, found during or prior to the present study. Evidently, sexual reproduction plays a role in the development of *P. infestans* populations in The Netherlands.

Abbreviations: Rc = refuse pile, Fc = conventional potato field, Fo = organic potato field, and A_p = potato plot in allotment garden, A_t = tomato plot in allotment garden.

Introduction

Since the 1840s, when *Phytophthora infestans* (Mont.) de Bary migrated from Mexico to other parts of the world (Goodwin *et al.*, 1994b), late blight has been a serious threat to potato growing world-wide (Hooker, 1981). Despite frequent fungicide applications, late blight epidemics sometimes appear to be uncontrollable, especially in recent years (Fry *et al.*, 1993; Goodwin *et al.*, 1995). Several authors (*e.g.* Spielman *et al.*, 1991; Fry *et al.*, 1992; 1993) showed that 'new' genotypes of *P. infestans*, originating from central Mexico, displaced the old populations, consisting mainly of one clonal lineage. Prior to the 1970s

migration, sexual reproduction was confined to central Mexico (Tooley *et al.*, 1985; Goodwin *et al.*, 1992b). Sexual reproduction has now been found to occur in Europe as a result of the introduction of 'new' genotypes of both A1 and A2 mating type (Drenth *et al.*, 1993, 1994; Sujkowski *et al.*, 1994).

The appearance of a new, genetically diverse population may alter the epidemiology of *P. infestans* in two ways. First, if sexual reproduction occurs, oospores are formed which can survive in the soil in the absence of a host (Drenth *et al.*, 1995). These oospores may serve as an important additional inoculum source. Second, 'new' immigrant genotypes, or recombinant progeny from oospores, may have higher pathogenic fitness (Day & Shattock, 1997; Kato *et al.*, 1997), resulting in increased epidemic growth rates.

Knowledge of the population biology of *P. infestans* may help to better understand its possible altered epidemiology (Milgroom & Fry, 1997). The availability of molecular markers, *e.g.* probe RG57 (Goodwin *et al.*, 1992a) has stimulated research on population structure and population biology of *P. infestans*. Most of the studies in which probe RG57 has been used concern large geographic areas, *i.e.* counties, states, countries, or continents (Drenth *et al.*, 1993; Forbes *et al.*, 1997; Goodwin *et al.*, 1994a, 1995; Koh *et al.*, 1994; Sujkowski *et al.*, 1994). These studies focused on migrations, population changes, and the possible occurrence of sexual reproduction.

However, to assess the relative impact of the different types of overwintering inoculum (oospores and mycelium in tubers) and of the various infection sources (foci originating from infected seed tubers, refuse piles, volunteer plants, allotment gardens) on the development of late blight epidemics, population development has to be analysed also at a smaller scale, *i.e.* from the initial establishment of disease foci at the within-field level to the development of disease within a limited geographic area.

Previously, we analysed the small scale epidemiology of late blight in the seed and ware potato area of Southern Flevoland (Chapter 2; Zwankhuizen *et al.*, 1998). It was shown that refuse piles with asexual inoculum were the most important sources for the development of epidemics. In late summer 1994, infested organic fields appeared to be major infection sources. The results of the characterisation of isolates from disease foci indicated that oospores might have played a role as initial inoculum in a few cases.

To make further inferences about the overwintering and spread of the late blight pathogen, especially concerning the role of oospores, changes in the genotypic composition of the pathogen populations within and between growing seasons were analysed. In this chapter, two major questions are addressed. The first question concerns the occurrence of oospore initiated disease: is there evidence for oospores as initiating inoculum sources additional to tuber-borne inoculum? If so, the second question applies, concerning the impact of oospores: what is the relative importance of this sexual inoculum source, among the different sites throughout the research area? The present chapter focuses on the results of the characterisation with DNA fingerprinting of a large number of isolates collected within a relatively small area, whereas chapter 4 (Zwankhuizen *et al.*, *subm.(b)*) further analyses the structure of the *P. infestans* population in relation to its epidemiology.

Materials and methods

Research area and surveys

During the growing seasons of 1994, 1995 and 1996, late blight development was monitored in Southern Flevoland, a polder in the central part of The Netherlands, in an area of 150 km^2. The research area and the surveys are described in detail in Zwankhuizen *et al.* (1998). We distinguished four types of host plant sampling sites: refuse piles with potato plants (Rc), fields with volunteer potato plants (V), conventional and organic potato fields (Fc and Fo, respectively, and potato and tomato plots in allotment gardens (A_p and A_t, respectively).

At the start of each growing season, a random selection of farms was visited for inspection of refuse piles. Subsequently, (random) selections of fields with volunteer plants, potato fields, and allotment gardens were inspected. In each compound of allotment gardens, 40 randomly chosen potato plots were inspected per inspection round. From the time tomatoes were planted in the allotment gardens, tomato plots were inspected in each individual garden in which potatoes were inspected. Sites were visited regularly throughout the growing season. Depending on the actual late blight recordings, some potato sites were visited more frequently, in addition to the inspection of randomly selected potato sites. Occasionally, potato production areas adjacent to the research area were visited, particularly when late blight appeared earlier in those areas than in the research area.

Collection of isolates

The sampling units were either disease foci, or entire sites. In the early phase of the epidemics, disease occurred in foci (Zwankhuizen *et al.*, 1998). Ten or 30 isolates per focus were collected as described by Zwankhuizen *et al.* (1998). Entire potato sites (refuse piles, conventional and organic fields) were sampled when disease levels were so high that disease was found all over the potato site (*e.g.* potato fields sampled later in the season), or when no typical disease focus could be found (*e.g.* in refuse piles because of mechanical disturbance). In the infested organic fields, 30 isolates were collected randomly along two transects, unless stated otherwise. From conventional fields, 10 to 30 isolates were obtained, generally along two transects. For more details we refer to Zwankhuizen *et al.* (1998). In potato plots of allotment gardens, 10 isolates were collected from 10 randomly chosen plants. From tomato plots in allotment gardens, one isolate per diseased plant was selected at random.

After isolation in pure culture, cultures were kept on Rye A medium (Caten & Jinks, 1968) at 18 °C in the dark, or stored in liquid nitrogen.

Characterisation of isolates

All isolates were analysed for mating type (Fry *et al.*, 1991) and the majority of isolates was analysed for nuclear DNA fingerprint using probe RG57 (Goodwin *et al.*, 1992a). Culturing of isolates, DNA extraction, digestion with restriction enzyme *Eco*RI, and Southern blot analysis were carried out as described by Drenth *et al.* (1993).

A multilocus genotype (often referred to as genotype) was constructed for each isolate by combining the data for mating type and DNA fingerprint loci (Goodwin *et al.*, 1995). Genotypes were arbitrarily assigned to numbers, with a prefix NL. The NL genotype numbers used in this chapter do not correspond to the NL genotype numbers used by Drenth *et al.* (1993), Goodwin and Drenth (1997), and Forbes *et al.* (1998). A genotype was designated unique when one or more isolates of this genotype were detected in only one sampling site in the research area (from 1993 through 1996). All genotypes were compared with those available in the WAU data collection. This collection contains genotypes of isolates collected in The Netherlands between 1966 and 1996 and were identified by Drenth *et al.* (1993, 1994).The genotype of an isolate of unknown origin collected in 1951 was also included in the comparison.

A subset of isolates was also characterised with AFLP DNA fingerprinting (Vos *et al.*, 1995). The purpose was to distinguish between isolates with identical genotypes, as determined by mating type and RG57 RFLP fingerprinting. Template preparation, selective preamplification of primary template, selective restriction fragment amplification, and separation of labelled fragments and autoradiography were carried out as described by van der Lee *et al.*, (1997), who optimised the AFLP-DNA fingerprinting technique for *P. infestans*. Total DNA was digested with the restriction enzymes *Eco*RI and *Mse*I. The primers used in the first PCR were 5'-GACTGCGTACCAATTCA for the *Eco*RI restriction site and 5'-ATGTCCTGAGTAAC for the *Mse*I restriction site. In the second PCR, the *Eco*RI restriction site primers were 5'-GACTGCGTACCAATTC-AA and -CG, and the primers fitting the *Mse*I restriction site were 5'-ATGAGTCCTGAGTAA-CA and -CT. Presence (indicated by 1) and absence (0) of bands was scored visually. The analysis was based on 51 polymorphic loci.

Bioassay

Many growers in allotment gardens discard diseased potato and tomato foliage on waste piles. In the next season, they use the 'composted' material to enrich the soil in their garden with organic matter. Six samples from tomato waste piles and 6 samples from potato waste piles were taken and 'composted' from September, 1996, to May, 1997. To check whether oospores could survive in the waste during the winter period, infectivity was tested by subjecting the ground material to a bioassay (Drenth *et al.*, 1995).

Selection of sites for characterisation

An overview of the sampling efforts is given in Table 1. Isolates of *P. infestans* were collected in autumn 1993 and during the course of the growing seasons of 1994, 1995, and 1996.

Nine infested refuse piles were encountered in 1994, 7 of which were sampled. In 1995, only 2 infested piles were found. No infested refuse piles were found throughout the research area in 1996. However, in Wezep, located 28 km north-east of the research area, there was a large infested refuse pile (ca. 500 m^2). Isolates were collected from that pile.

In 1994, isolates were collected from 30 conventional fields. Isolates from 17 of these fields were selected for analysis with DNA fingerprinting. Four of these conventional fields, sampled during the early outbreak in June, were amongst the first infested fields in the area, and were regarded representative for all other fields with early infections (Zwankhuizen *et al.*, 1998). The other 13 conventional fields were sampled during the second outbreak of the disease in August and September (Zwankhuizen *et al.*, 1998). In 1995 and 1996 when disease pressure was low, isolates of nearly all infested conventional and organic potato fields were characterised.

In each compound of allotment gardens, 5 to 8 of the first diseased potato plots were sampled each year. In 1994 and 1996, only isolates from the compounds An and Ah were included in the fingerprint analyses. In 1996, 5 randomly selected tomato plots from the compounds An and Ah were sampled for isolate characterisation.

Data analysis

The frequencies and relative frequencies of genotypes per complete sampling site are the response variables in this study. Contingency chi-square analyses were conducted to detect differences between the genotypic composition of *P. infestans* populations in time and space. In case of 2 x 2 tables, Fisher's exact test (Sokal & Rohlf, 1981) was applied. All contingency tables analysed in this chapter can be derived from Tables 1, 3, 4, and 6.

The probability of isolates sharing the same multilocus genotype by chance was estimated as follows. The mating type locus and the 23 fingerprint fragments were considered as single genetic loci. Using the presence/absence of bands within the individual genotypes, the relative frequency of each band within the total population or in subpopulations was determined. The probability of obtaining a certain genotype by random recombination was calculated as the product of the relative frequencies for each locus. These simple calculations will give overestimated probabilities, because homozygosity and heterozygosity of bands was not taken into account. Neither did we include linkage between the loci, because it does not change the results qualitatively.

Table 2. Genotypes of 1048 isolates of *Phytophthora infestans* collected from September 1993 to September 1996 in Southern Flevoland and adjacent areas.

Genotype[1]	Mating type	RG57 fingerprint[2]	*N*[3]	Year[4]
NL-1	A1	0 1 0 0 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	94
NL-2	A2	0 1 0 1 1 0 0 1 0 1 1 1 1 1 0 1 0 1 1 1 0 0 1 1	3	95
NL-3	A2	0 1 0 1 1 0 0 1 0 1 1 1 1 1 1 0 1 1 0 1 0 0 1 1	10	95
NL-4	A2	0 1 0 1 1 0 0 1 1 1 1 1 1 1 0 1 0 1 1 1 0 0 1 1	1	95
NL-5	A1	1 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 1 0 1 0 1	3	95
NL-6	A2	1 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 0 0 1 1 0 1 0 1	3	93
NL-7	A2	1 0 0 1 0 0 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 1 0 1 0 1	1	95
NL-8	A1	1 0 0 1 0 0 0 0 0 0 1 1 1 1 0 0 0 0 0 1 1 0 1 0 1	13	95,96
NL-9	A2	1 0 0 1 0 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 1 0 1 0 1	17	95,96
NL-10	A1	1 0 0 1 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 1 1 1 0 1 0 1	2	95,96
NL-11	A2	1 0 0 1 0 0 0 0 0 0 1 1 1 1 1 0 1 0 1 1 1 0 1 0 1	9	95
NL-12a	A1	1 0 0 1 0 0 1 0 0 0 0 1 1 1 0 0 0 0 0 1 1 0 1 0 1	5	95
NL-12b	A2	1 0 0 1 0 0 1 0 0 0 0 1 1 1 0 0 0 0 0 1 1 0 1 0 1	5	95
NL-13	A2	1 0 0 1 0 0 1 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	95
NL-14	A2	1 0 0 1 0 0 1 0 0 1 1 1 1 1 1 0 0 1 1 1 0 0 0 1	2	95
NL-15	A1	1 0 0 1 0 0 1 0 0 1 1 1 1 1 1 0 1 0 1 1 0 1 0 1	2	95
NL-16	A1	1 0 0 1 0 0 1 1 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-17	A1	1 0 0 1 1 0 0 1 0 1 1 1 1 0 1 0 1 0 0 1 1 1 0 1	10	95
NL-18	A1	1 0 0 1 1 0 1 1 0 1 1 1 1 1 0 0 0 1 1 1 0 1 0 1	1	95
NL-19	A2	1 0 1 1 0 0 0 0 0 1 1 1 1 1 0 0 0 0 1 1 0 1 0 1	5	94
NL-20	A2	1 0 1 1 0 1 0 0 0 0 1 1 1 0 0 1 0 1 1 0 1 0 1	1	95
NL-21	A1	1 0 1 1 0 1 0 0 0 0 1 1 1 0 1 0 1 1 1 1 1 0 1	3	95
NL-22	A1	1 0 1 1 0 1 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-23	A1	1 0 1 1 0 1 0 0 0 1 1 1 1 1 0 1 0 0 1 1 0 1 0 1	1	95
NL-24	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	11	95,96
NL-25	A2	1 0 1 1 0 1 0 1 0 1 1 0 1 1 0 0 1 1 1 0 1 0 1	2	95
NL-26	A1	1 0 1 1 0 1 0 1 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	8	95
NL-27	A2	1 0 1 1 0 1 1 0 0 0 1 1 1 0 0 0 0 0 1 1 0 1 0 1	1	94
NL-28	A1	1 0 1 1 0 1 1 0 0 0 1 1 1 1 0 0 0 0 0 1 0 1 0 1	1	95
NL-29	A1	1 0 1 1 0 1 1 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	14	93,95
NL-30	A1	1 0 1 1 0 1 1 0 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	9	93
NL-31	A2	1 0 1 1 0 1 1 0 0 1 1 1 1 1 1 0 0 1 1 1 1 1 0 1	1	95
NL-32	A2	1 0 1 1 1 1 0 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	9	95
NL-33	A1	1 0 1 1 1 1 0 0 0 1 1 1 1 1 0 0 0 0 1 0 1 0 1	1	95
NL-34	A1	1 0 1 1 1 1 0 0 0 1 1 1 1 1 1 0 0 1 1 1 1 1 0 1	2	95
NL-35	A1	1 0 1 1 1 1 0 1 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-36	A1	1 0 1 1 1 1 0 1 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	3	94,95,96
NL-37	A1	1 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 0 0 0 0 1 0 1 0 1	8	95
NL-38	A1	1 0 1 1 1 1 1 1 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	93
NL-39	A1	1 0 1 1 1 1 1 1 0 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	94
NL-40	A1	1 0 1 1 1 1 1 1 1 0 1 1 1 1 1 0 0 0 1 1 1 0 1 0 1	1	95

NL-41	A1	1 0 1 1 1 1 1 1 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	186	93,94,95,96
NL-42	A1	1 0 1 1 1 1 1 1 0 1 1 1 1 1 0 1 1 1 1 0 1 0 1	1	94
NL-43	A2	1 1 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1	1	95
NL-44	A2	1 1 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	9	95
NL-45	A2	1 1 0 0 0 0 0 0 0 0 1 1 1 1 0 0 1 1 1 1 0 1 0 1	11	95
NL-46	A1	1 1 0 0 0 0 1 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-47	A1	1 1 0 0 1 0 0 0 0 1 1 1 1 0 0 1 1 1 1 0 1 0 1	1	95
NL-48	A1	1 1 0 0 1 0 0 0 0 1 1 1 1 0 1 0 1 1 1 0 1 0 1	29	94,95
NL-50	A2	1 1 0 1 0 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	95
NL-51	A1	1 1 0 1 0 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	3	95
NL-52	A1	1 1 0 1 0 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 1 1	1	95
NL-53	A1	1 1 0 1 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	95
NL-54	A2	1 1 0 1 0 0 0 1 0 0 1 1 1 0 0 0 0 1 1 0 1 1 1	1	95
NL-55	A2	1 1 0 1 0 0 0 1 0 1 1 0 1 1 0 0 1 1 1 0 0 1 1	1	94
NL-56	A1	1 1 0 1 0 0 1 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	11	95
NL-57	A1	1 1 0 1 0 0 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 1 1	1	95
NL-58	A2	1 1 0 1 0 0 1 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	2	94
NL-59	A2	1 1 0 1 1 0 0 0 0 1 1 1 1 0 1 0 0 1 1 0 1 0 1	1	95
NL-60	A2	1 1 1 1 0 1 0 0 0 0 1 1 1 1 0 0 1 1 1 0 1 0 1	1	95
NL-61	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 1 1 1 0 1	1	95
NL-62	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	10	95,96
NL-63	A2	1 1 1 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-64	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	4	95,96
NL-65	A2	1 1 1 1 0 1 0 1 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	95
NL-66	A2	1 1 1 1 0 1 0 1 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	4	95
NL-68	A2	1 1 1 1 0 1 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	95,96
NL-69	A1	1 1 1 1 0 1 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	39	94,95
NL-70	A2	1 1 1 1 0 1 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 1 1	6	95
NL-71	A1	1 1 1 1 0 1 1 0 0 1 1 1 1 1 0 0 1 1 1 0 0 1 1	2	95
NL-72	A1	1 1 1 1 1 1 0 0 0 1 1 1 1 1 0 0 0 0 1 0 1 1 1	10	95
NL-73	A1	1 1 1 1 1 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-74	A1	1 1 1 1 1 1 0 1 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	4	94
NL-75	A1	1 1 1 1 1 1 0 1 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	62	94,95,96
NL-76	A1	1 1 1 1 1 1 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	163	93,94,95
NL-77	A2	1 1 1 1 1 1 1 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	3	94
NL-78	A1	1 1 1 1 1 1 1 0 1 1 1 1 1 1 0 1 0 1 1 0 1 0 1	1	94
NL-79	A1	1 1 1 1 1 1 1 1 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	95
NL-80	A1	1 1 1 1 1 1 1 1 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	2	95,96
NL-81	A2	1 0 0 1 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 0 0 1 1	1	96
NL-82	A2	1 0 0 1 0 0 0 0 0 0 0 1 1 1 0 0 1 1 1 0 0 1 1	19	96
NL-83	A1	1 0 0 1 0 0 0 0 0 0 0 1 1 1 0 0 1 1 1 0 0 1 1	2	96
NL-84	A1	1 0 0 1 0 0 0 1 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-85	A1	1 0 0 1 0 0 1 0 0 0 1 1 1 0 0 1 1 1 1 0 1 0 1	1	96
NL-86	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	4	96
NL-87	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 0 0 0 1 1 1 0 0 0 1	1	96
NL-88	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 1 0 1 0 1	1	96
NL-89	A1	1 0 1 1 0 1 0 1 0 1 1 1 1 0 1 0 1 1 1 0 0 0 1	2	96
NL-90	A1	1 0 1 1 0 1 0 1 0 1 1 1 1 1 0 0 1 1 1 0 0 0 1	3	96

Table 2 continued

NL-91	A1	1 0 1 1 0 1 0 1 0 1 1 1 1 1 0 1 0 1 1 0 1 0 1	2	96
NL-92	A1	1 0 1 1 0 1 1 0 0 1 1 1 1 0 0 0 1 1 1 0 0 0 1	1	96
NL-93	A2	1 0 1 1 1 1 0 0 0 1 1 1 1 0 0 0 0 0 1 0 1 0 1	6	96
NL-94	A1	1 0 1 1 1 1 0 1 0 1 1 1 1 0 1 0 1 1 1 0 0 1 1	1	96
NL-95	A2	1 0 1 1 1 1 1 0 0 0 1 1 1 0 1 0 0 1 1 0 1 0 1	2	96
NL-96	A2	1 0 1 1 1 1 1 0 0 1 1 1 1 0 1 0 0 1 1 0 1 0 1	2	96
NL-97	A2	1 0 1 1 1 1 1 0 1 1 1 1 0 0 1 0 0 1 1 0 0 1 1	1	96
NL-98	A2	1 0 1 1 1 1 1 0 1 1 1 1 0 0 1 0 0 1 1 0 1 0 1	6	96
NL-99	A2	1 0 1 1 1 1 1 0 1 1 1 1 1 0 1 0 0 1 1 0 1 0 1	1	96
NL-100	A2	1 1 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 1 1 1 0 0 0 1	1	94
NL-101	A1	1 1 0 0 0 0 0 0 0 0 1 1 1 1 0 1 0 0 1 1 0 0 1 1	1	96
NL-102	A1	1 1 0 0 0 0 0 0 0 0 1 1 1 1 0 1 0 0 1 1 0 1 0 1	1	96
NL-103	A1	1 1 0 0 0 0 0 0 0 0 1 1 1 1 0 1 0 0 1 1 0 1 1 1	1	96
NL-104	A1	1 1 0 1 0 0 0 0 0 0 0 1 1 1 0 1 0 1 1 1 0 0 1 1	1	96
NL-105	A2	1 1 0 1 0 0 0 1 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	94
NL-106	A2	1 1 0 1 0 0 1 0 0 0 0 1 1 1 1 0 0 1 1 1 0 0 1 1	1	96
NL-107	A1	1 1 0 1 0 1 1 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	94
NL-108	A1	1 1 0 1 1 0 0 0 0 0 0 1 1 1 0 1 0 1 1 1 0 1 0 1	1	96
NL-109	A2	1 1 1 1 0 1 0 0 0 1 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-110	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 1 1	1	96
NL-111	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 1 0 0 1 1 0 0 1 1	1	96
NL-112	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 1 0 0 0 1	22	96
NL-113	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 1 0 0 1 1	30	96
NL-114	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 1 0 1 0 1	1	96
NL-115	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 1 0 0 1 1 1 0 0 0 1	1	96
NL-116	A1	1 1 1 1 0 1 0 0 1 1 1 1 1 0 1 0 1 1 1 0 0 1 1	1	96
NL-117	A2	1 1 1 1 0 1 1 0 0 1 1 1 1 0 1 0 0 1 1 0 1 0 1	2	96
NL-118	A2	1 1 1 1 0 1 1 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-119	A2	1 1 1 1 1 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-120	A2	1 1 1 1 1 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 1 1	1	96
NL-121	A1	1 1 1 1 1 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 1 1	1	96
NL-122	A1	1 1 1 1 1 1 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-123	A1	1 1 1 1 1 1 0 0 0 1 1 1 1 1 0 0 1 1 1 1 1 0 1	24	96
NL-124	A1	1 1 1 1 1 1 0 0 1 1 1 1 1 0 1 0 1 1 1 0 0 1 1	1	96
NL-125	A2	1 1 1 1 1 1 0 1 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96
NL-126	A1	1 0 1 1 0 1 0 0 0 0 1 1 1 0 1 0 1 1 1 0 0 0 1	8	96
NL-127	A1	1 0 1 1 0 1 0 0 1 1 1 1 1 0 1 0 1 1 1 0 0 1 1	1	96
NL-128	A2	1 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	1	96
NL-129	A1	1 0 0 1 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 0 1 1	2	96
NL-130	A2	1 0 0 1 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-131	A1	1 0 0 1 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 1 1	4	96
NL-132	A2	1 0 0 1 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	3	96
NL-133	A1	1 0 0 1 0 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-134	A2	1 0 0 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96
NL-135	A1	1 0 0 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	96
NL-136	A2	1 0 0 1 1 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	7	96

NL-137	A2	1 0 0 1 1 0 0 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	38	96
NL-138	A1	1 0 0 1 1 0 0 0 0 1 1 1 1 0 0 0 1 1 1 0 1 0 1	6	94,96
NL-139	A2	1 0 0 1 1 0 0 0 0 1 1 1 1 0 0 1 1 1 1 0 1 0 1	3	96
NL-140	A2	1 0 0 1 1 0 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-141	A2	1 0 0 1 1 1 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-142	A2	1 0 1 1 0 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	96
NL-143	A1	1 0 1 1 0 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	3	96
NL-144	A1	1 0 1 1 0 1 0 0 0 1 1 1 1 0 0 1 0 1 1 0 1 1 1	1	96
NL-145	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1	1	96
NL-146	A1	1 0 1 1 0 1 0 1 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-147	A1	1 0 1 1 0 1 1 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-148	A2	1 0 1 1 0 1 1 0 0 1 1 1 1 0 0 0 0 1 1 1 1 0 1	4	96
NL-149	A1	1 0 1 1 0 1 1 0 0 1 1 1 1 0 0 0 1 1 1 0 1 1 1	1	96
NL-150	A1	1 0 1 1 0 1 1 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96
NL-151	A1	1 0 1 1 1 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-152	A1	1 0 1 1 1 1 0 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-153	A1	1 0 1 1 1 1 1 1 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	94
NL-154	A1	1 1 0 1 0 0 0 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1	2	96
NL-155	A1	1 1 0 1 0 0 0 0 0 0 0 1 1 1 0 1 0 1 1 1 0 1 0 1	1	96
NL-156	A1	1 1 0 1 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 0 0 0 1	1	96
NL-157	A2	1 1 0 1 0 0 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	2	96
NL-158	A2	1 1 0 1 0 0 1 0 0 1 1 1 1 0 0 0 1 1 1 0 0 0 1	1	96
NL-159	A1	1 1 0 1 0 0 1 0 0 1 1 1 1 1 1 0 1 1 1 1 0 1 0 1	1	96
NL-160	A2	1 1 0 1 1 0 0 0 0 0 1 1 1 1 0 0 0 1 1 0 1 0 1	6	96
NL-162	A1	1 1 1 1 0 1 0 0 0 1 1 1 1 1 1 0 0 1 0 1 0 1 0 1	3	96
NL-163	A2	1 1 1 1 0 1 0 0 0 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-164	A1	1 1 1 1 0 1 0 1 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96
NL-165	A1	1 1 1 1 1 0 0 0 0 1 1 1 1 1 1 0 0 0 1 1 0 1 0 1	7	94
NL-166	A2	1 1 1 1 1 1 0 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	2	96
NL-167	A2	1 1 1 1 1 1 0 0 0 1 1 1 1 0 1 0 1 1 1 1 0 1 0 1	1	96
NL-168	A2	1 1 1 1 1 1 0 0 0 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-169	A1	1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 1 0 1 0 1	1	96
NL-170	A2	1 0 1 1 0 1 1 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 1	1	96
NL-171	A1	1 0 1 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96
NL-172	A2	1 0 1 1 0 1 0 0 0 1 1 1 1 1 0 0 0 1 1 0 1 0 1	1	96

[1] Genotypes were arbitrarily numbered; numbers do not correspond to those used by Drenth *et al.* (1993), Goodwin and Drenth (1997), and Forbes *et al.* (1998). Genotype numbers do not range from 1 to 170 due to the fact that some genotypes (initially identified as separate genotypes) were assigned to existing numbers.

[2] DNA fingerprint bands revealed by the moderately repetitive probe RG57 (Goodwin *et al.*, 1992a). Presence of a band is indicated by 1, absence by 0. Bands listed from left to right are: 1, 2, 3, 5, 6, 7, 8, 9, 9a, 10, 13, 14, 14a, 16, 17, 18, 19, 20, 21, 23, 24, 24a, 25. Band numbers correspond to those used by Goodwin *et al.* (1992a; 1992b, 1994b) and Drenth *et al.* (1993; 1994). Bands 4 and 22 were excluded from the analyses because these bands

Table 2 continued

could not be scored unambiguously. Other bands not included in the analyses were 11 and 15 because they have never been detected in isolates collected in The Netherlands.

[3] Number of isolates.

[4] Year of occurrence: 1993, 1994, 1995, and 1996, respectively.

Genotypic composition of P. infestans populations on potato

Over the years, 149 genotypes were identified among 981 isolates collected from potatoes in refuse piles, commercial fields and allotment gardens.

Predominant genotypes. Four genotypes were found in many sampling sites and in at least 2 years. These predominant genotypes (mating type A1) were NL-41, NL-69, NL-75, and NL-76. The fraction of isolates with these genotypes was highest in refuse piles and conventional fields in 1994 (Table 3). The frequencies of isolates belonging to these genotypes differed significantly over the years ($P < 0.001$), and were also different between commercial potato sites (Rc + Fc + Fo) and allotment gardens within years ($P < 0.001$). At least 90 % of the isolates collected from refuse piles in Southern Flevoland in 1994 and 1995 had one of these predominant genotypes. On average 70 % of the isolates from the commercial fields (conventional and organic) of 1994 and 1995 belonged to these genotypes. Only a very low fraction (0.031) of isolates collected from the commercial fields in 1996 had a predominant genotype. All isolates collected from the infested refuse pile in Wezep in 1996 had one of the predominant genotype NL-41 or NL-75. The predominant genotypes apparently overwinter well in tubers.

Unique genotypes. The majority of the genotypes (79 %), encountered on potato from 1994 through 1996, was unique (116 out of 146 genotypes, identified among 941 isolates; Table 4). The total frequencies of isolates with unique genotypes were significantly different over years ($P < 0.001$), and over potato sites within years ($P < 0.05$). When genotypes are considered instead of isolates, these differences become smaller. Frequencies of unique genotypes were almost significantly different across the years ($P = 0.057$). The frequencies of unique genotypes in the commercial potato sites were not significantly different from the frequencies in the allotment gardens (at $P = 0.05$), except in 1995 ($P = 0.023$). Highest numbers of unique genotypes were found in the organic fields (1996), and in the allotment gardens (1995 and 1996) (Tables 1, 4, 5).

Genotypes in organic fields in 1996. Characterisation of 162 isolates, collected from 6 organic fields in 1996, revealed 44 different genotypes (Table 1), 37 of which were unique and 6 rare (see below). Two of these fields (Fo2 and Fo5) had an extremely diverse population, with 14 and 17 different genotypes, respectively. The first disease focus found in the research area in 1996 was in organic field Fo1. Two unique genotypes were found among 9 isolates initially collected from that focus. One week later, another 30 isolates

Table 3. Fractions [1] of isolates belonging to the predominant genotypes NL-41, NL-69, NL-75, and NL-76, found in potato sites in Southern Flevoland and adjacent areas, 1994 through 1996.

Year	Potato site					
	Refuse piles	Conventional fields	Organic fields	Allotment gardens	Total	No. of isolates
1994	0.92	0.94	0.63	0.52[3]	0.82	277
1995	0.90	0.52	0.52	0.23	0.42	383
1996	1[2]	0	0.03	0	0.12	281
Total	0.93	0.78	0.30	0.20[3]	0.20	941

1 See Table 1 for numbers of isolates tested for RG57 fingerprint pattern per potato site per year. The fraction of isolates belonging to genotypes NL-41, NL-69, NL-75, and NL-76 in 1993 was 0.65 (n= 40 isolates).

2 Isolates collected from a refuse pile in Wezep, located 28 km north-east from Southern Flevoland.

3 In 1994 and 1996, only isolates from the compounds A_pn and A_ph were characterised with DNA fingerprinting.

were collected. Characterisation of these isolates revealed another 4 genotypes, of which 1 genotype was unique, 2 were rare, and one was predominant (NL-75).

Genotypes in allotment gardens. The majority (52 %) of isolates collected in the allotment gardens in 1994 belonged to the predominant genotypes (occurring in 4 out of 9 sampled plots) (Table 5). In 1995 and 1996, most plots contained unique genotypes. In 1995, all 4 plots in compound An contained predominant genotypes and in one plot, three of them, NL-41, NL-69, and NL-76, were identified. In the other three compounds in 1996, only 1 out of 17 plots contained a predominant genotype. In 1996, none of the 11 potato plots of 2 compounds contained one of the predominant genotypes, most of the genotypes being unique or rare.

Genotypes in field Fc2 (1995). The number of different genotypes encountered in conventional potato fields was usually 2 or 3. However, in one case the situation was different. In June 1995, a conventional grower found diseased leaves scattered over an area of about 2 ha of field Fc2. No sources of late blight were identified in the area surrounding this field. Analysis of the 30 collected isolates revealed a A1 : A2 mating type ratio of 12 : 18, not significantly different from 1 : 1. The isolates belonged to 10 genotypes, 6 of which

Table 4. Fractions [1] of isolates belonging to unique genotypes (FI), and fraction of unique genotypes (FG) relative to the total number of genotypes, found in potato sites in Southern Flevoland and adjacent areas, 1994 through 1996.

Year	Potato site								Total		No. of isolates
	Refuse piles		Conventional fields		Organic fields		Allotment gardens				
	FI	FG	FI	FG	FI	FG	FI	FG	FI	FG	
1994	0.085	0.50	0.014	0.25	0.070	0.33	0.18	0.25[3]	0.058	0.50	277
1995	0.13	0.50	0.29	0.55	0.19	0.47	0.61	0.79	0.37	0.73	383
1996	0[2]	0[2]	0.44	0.80	0.50	0.84	0.27	0.62[3]	0.28	0.76	281
Total	0.069	0.60	0.11	0.52	0.30	0.70	0.47	0.76	0.25	0.79	941

1 See Table 1 for numbers of isolates tested for mating type and RG57 fingerprint pattern, and number of genotypes detected per potato site per year. The fraction of isolates belonging to unique genotypes, and the fraction of unique genotypes in 1993 was 0.25 and 0.33, respectively (n=40 isolates).

2 Isolates collected from a refuse pile in Wezep, located 28 km north-east from Southern Flevoland.

3 In 1994 and 1996, only isolates from the compounds A_pn and A_ph were characterised with DNA fingerprinting.

were A1. Six genotypes were unique and 4 belonged to the category of rare genotypes, which will be described later.

Genotypic composition of P. infestans populations on tomato

In 1996, 28 different genotypes were detected on tomatoes in allotment gardens (Table 1). Most genotypes were unique (Table 6) whereas the majority of the other genotypes belonged to the category of rare genotypes. No significant differences existed (at P = 0.05) between the frequencies of isolates belonging to unique genotypes, and the frequencies of unique genotypes, between potato and tomato plots in each compound.

Table 5. Numbers of potato plots sampled in allotment gardens in Southern Flevoland and adjacent areas in 1994, 1995 and 1996, numbers of isolates characterised and numbers of genotypes detected.

Year	Site	Plots sampled	Isolates characterised	Genotypes identified	Unique genotypes (fraction)	Isolates belonging to predominant genotypes[1] (fraction)	Plots with predominant genotypes[1] (fraction)
1994	A_{pn}	4	13	4	1 (0.25)	5 (0.39)	1 (0.25)
	A_{ph}	5	20	4	1 (0.25)	12 (0.60)	3 (0.60)
Total 1994		9	33	8	2 (0.25)	17 (0.52)	4 (0.44)
1995	A_{pn}	4	36	7	3 (0.43)	32 (0.89)	4 (1)
	A_{ph}	6	50	15	13 (0.87)	0 (0)	0 (0)
	A_{ps} p	5	48	10	7 (0.70)	9 (0.19)	1 (0.20)
	A_{pa}	6	46	8	8 (1)	0 (0)	0 (0)
Total 1995		21	180	39	31 (0.80)	41 (0.23)	5 (0.24)
1996	A_{pn}	5	20	14	9 (0.64)	0 (0)	0 (0)
	A_{ph}	6	61	12	7 (0.58)	0 (0)	0 (0)
Total 1996		11	81	26	16 (0.62)	0 (0)	0 (0)

[1] Genotypes NL-41, NL-69, NL-75, NL-76.

In compound An, only 2 genotypes (NL-62, NL-131) out of 28 (7.1 %) were detected on both tomato and potato. Genotype NL-62 was found in one potato plot and in 3 tomato plots, genotype NL-132 was found in one potato plot and one tomato plot. In compound Ah, 3 genotypes (NL-136, NL-137, NL-143) out of 21 genotypes (14.3 %) were detected on both tomato and potato plants. Genotype NL-136 was found in 2 potato plots and in 1 tomato plot, genotype NL-137 was found in 4 potato plots and 3 tomato plots, and genotype NL-143 was found in one potato plot and one tomato plot. Some exchange of genotypes between hosts appeared evident.

Rare genotypes

The majority of genotypes appeared to be unique (138 of the 170 genotypes, Table 1). The other 32 genotypes were detected at least twice. Four of these genotypes, the predominant ones (NL-41, NL-69, NL-75, NL-76), were discussed earlier. The remaining 28 genotypes are called rare genotypes because they were encountered in only 2 to 7 sampling sites. The rare genotypes could be subdivided into two categories: one for which there is an epidemiological explanation for the occurrence in more than one site (16 genotypes), and the other for which such an explanation could not be given (12 genotypes).

The first category consists of genotypes which were often found in contiguous potato fields or adjacent plots in allotment gardens, or in fields near an infection source containing the same genotype. For example, genotype NL-48 was detected in organic potato fields Fo3 and Fo4 (distance 8 km) in 1994, and was found in 1995 in field Fo4 (at ca. 2 km from the location of field Fo3 in 1994). Genotype NL-113 was identified in fields Fo1 and Fo2 in 1996, which were two contiguous organic potato fields. Genotypes NL-136 and NL-137 were found in several potato and tomato plots in allotment garden Ah in 1996. The largest distance between those plots was 80 m.

The second category consists of genotypes often found in allotment gardens and in organic fields. No reasonable epidemiological explanation other than sharing the same genotype by random recombination is applicable to these genotypes. For example, genotype NL-56 was found on day 178 in plot 3 of allotment garden Asp in 1995, which was the first infested plot in that garden. One week later in 1995, this genotype was also recovered from conventional field Fc2. Fc2 was one of the first infested potato fields in the area, had a very diverse population (see before), and was located at approximately 14 km west from allotment garden Asp. The probability of obtaining this RG57 genotype by random recombination is 5.0×10^{-4}. To distinguish between those identical RG57 genotypes, 2 identical isolates from each site with genotype NL-56 were characterised for AFLP fingerprint pattern. Isolates with genotype NL-56 in field Fc2 differed at 8 out of 51 loci from isolates of genotype NL-56 in Asp (data not shown).

The average probability of obtaining a unique genotype by random recombination was 4.6×10^{-4}, and 8.2×10^{-4} for the non-unique (predominant and rare) genotypes. This difference is statistically significant ($P < 0.01$; Mann-Whitney *U*-test; Sokal & Rohlf, 1981). The average probabilities for the two categories of rare genotypes (one with epidemiological explanations for occurrence at more than one site and the other without) were 4.9×10^{-4} and 1.4×10^{-3}, respectively. Again this difference is significant ($P < 0.01$). It seems plausible that isolates found at unrelated sites could share the same RG57 genotype by chance because of random recombination.

Table 6. Numbers of isolates characterised, and numbers of genotypes detected on potato and tomato in the compounds of allotment gardens An and Ah in 1996.

Site	Isolates characterised for mating type and RG57 fingerprint pattern	Genotypes detected	Fraction isolates with unique genotypes	Fraction unique genotypes
A_pn	20	14[1]	0.75	0.64
A_tn	33	16[1]	0.55	0.75
A_ph	61	12[2]	0.13	0.58
A_th	34	12[2]	0.44	0.67

[1] Total no. of genotypes found in compound An was 28, 2 of which occurred on potato and tomato.

[2] Total no. of genotypes found in compound Ah was 21, 3 of which occurred on potato and tomato.

Comparison of genotypes with genotypes found previously in The Netherlands

Twenty-three genotypes (13.5 %) identified in the present study had been identified earlier as they are listed in the WAU data collection containing genotypes of isolates collected previously in The Netherlands. Eleven of these genotypes were unique genotypes in our study (6.5 % of our genotypes). Isolates from the previous collections with identical genotypes were obtained from fields throughout The Netherlands and were not restricted to one sampling area. Two genotypes, detected in our research area, had genotypes identical to those found in the 'old' population prior to the 1980s. Genotype NL-22, found in organic potato field Fo2 in 1995, had a genotype identical to an isolate collected in 1951. Genotype NL-26, identified in a potato plot in allotment garden Ah in 1995, had been encountered in many sampling sites in The Netherlands between 1966 and 1978 (Drenth *et al.*, 1994) and was similar to the US-1 genotype (Goodwin *et al.*, 1994a).

To discriminate between isolates from both data sets, having identical RG57 genotypes, 13 genotypes (34 isolates) found in our research area between 1993 and 1995 were compared with identical RG57 genotypes (29 isolates) from previous collections using AFLP fingerprinting. Ten of the RG57 genotypes in the AFLP characterisation were rare or unique to the population of the research area, and 3 were predominant (NL-41, NL-75, and NL-76). AFLP fingerprinting revealed differences between isolates from the research area and isolates with identical RG57 genotypes of previous collections (Table 7).

Table 7. Differences between isolates of *P. infestans*, revealed by AFLP fingerprinting [1]. Isolates belonged to identical genotypes, as determined by RFLP probe RG57 and mating type. Two groups of isolates were compared: isolates from previous collections in The Netherlands and isolates from Southern Flevoland and adjacent areas [2].

Genotype	No. of isolates characterised from previous collections (1951-1990)	Average no. of loci at which isolates were different (within previous collections)	No. of isolates characterised from the present study (1993-1995)	Average no. of loci at which isolates were different (within present collection)	Average no. of loci at which isolates were different (between previous and present collections)
Predominant					
NL-41	5	4.1	4	4.8	5.2
NL-75	5	2.3	5	5.3	4.4
NL-76	2	3.0	5	4.7	4.0
	12	3.1	14	4.9	4.5
Unique or rare					
NL-9	1		1		13
NL-22[3]	1		1		21
NL-26[4]	5	1.5	4	2.7	21.8
NL-30	1		5	2.4	7.2
NL-34	1		2	3	16.5
NL-62	1		1		13
NL-64	1		3	3.3	13.0
NL-68	1		1		16
NL-79	1		1		9
NL-80	4	7.8	1		11.5
	17	4.7	20	2.9	14.2

[1,2] See text for details.

[3] Genotype NL-22, detected in 1951 and in 1995, differs only one RG57 fragment from NL-26.

[4] Genotype NL-26 had the US-1 genotype (Goodwin *et al.*, 1994b) and was identified between 1966 and 1978 and in 1995 (Drenth *et al.*, 1994).

The average number of loci at which isolates of the respective genotypes differed was 12. The 10 rare or unique RG57 genotypes differed at 14.2 AFLP loci on average, whereas the 3 predominant RG57 genotypes differed at 4.5 AFLP loci. This difference is statistically significant (*t*-test, $P < 0.001$). The range of variation between isolates of the same RG57 genotypes was equal for both the genotypes from the research area and for the genotypes from previous collections (3.7 loci on average). Highest numbers of differences existed between isolates from the 'old' population and the 'new' population (having genotypes NL-22 and NL-26), indicating that these isolates had the same RG57 fingerprint pattern by chance.

Infectivity of potato and tomato waste in allotment gardens

In the bioassay, infections were recorded in 4 of the 6 trays with tomato waste samples. None of the trays with the potato waste samples showed infected leaflets. Five isolates, collected from the trays with tomato waste, were characterised for mating type and fingerprint pattern. The isolates had unique genotypes which had not been found in the population of the research area during the research years (fingerprint data not shown). A few samples of infected tomato leaves were comminuted in a commercial blender. Microscopic examination of the suspensions showed that the waste material contained oospores.

Discussion

General

Our sampling was far from unbiased. The statistically appropriate sampling scheme designed in advance was overruled by the facts. Consequently, some potato sites had to be visited and sampled more frequently than others, depending on the late blight development. In 1995 and 1996, years with limited late blight development, almost every infested site was sampled, whereas in 1994, a selection had to be made for the analysis. To compensate for the unbalanced sampling and to satisfy the objectives of our research, choices had to be made among the sites to be selected for characterisation with DNA fingerprinting.

The main result of the isolate characterisations is that sexual reproduction appears to occur within the research area and that the relative importance of oospores varied among the different sampling sites. In the next sections, the importance of oospores as the initiating inoculum, relative to tuber-borne inoculum within the different sampling sites is discussed.

Inoculum sources of P. infestans in commercial potato fields

Asexual inoculum and infection sources. Infection sources initiated by asexual inoculum (*i.e.* refuse piles and foci originating from infected seed tubers) were of major importance for late blight development in the commercial potato fields, compared to infection sources initiated by oospores.

Field observations indicated that refuse piles were the most important infection source for the commercial potato fields in 1994 and 1995 (Zwankhuizen *et al.*, 1998). This conclusion was supported by the results of the isolate characterisations. Ninety-one percent of the isolates collected from infested refuse piles belonged to the predominant genotypes NL-41, NL-69, NL-75, and NL-76, and 70 % of the isolates collected from the commercial potato fields belonged to these genotypes. Field observations did not indicate a significant role of infected seed tubers (Zwankhuizen *et al.*, 1998). However, several publications (*e.g.* Van der Zaag, 1956; Davidse *et al.*, 1989; Goodwin *et al.*, 1995) show that infected seed tubers can be important inoculum sources. Circumstantial evidence from this study indicates that this source cannot be excluded. Comparison of genotypes identified in our research area between 1993 and 1996 with those found before 1990 (Drenth *et al.*, 1993; 1994) revealed that some genotypes could have survived asexually for years. Furthermore, genotype NL-48 was only detected in organic fields of our research area in 1994 and 1995, but not in conventional fields area. So we conclude that infected seed tubers were of minor importance, except for the organic crops (see below).

Sexual inoculum and infection sources. The evidence for the role of oospores in the development of disease in 1994 and 1995 is sparse and indirect. The ratios of A1 and A2 genotypes, and the high fractions of unique genotypes observed every year indicate that sexual reproduction did occur. In the absence of sexual reproduction, one would expect the number of genotypes to decrease over time. The analysis of the genotypic composition of field Fc2 in 1995 provides evidence for the involvement of oospores as initiating inoculum. Ten genotypes (all unique or rare) were found in this field. No infection source was found outside the field. The grower told the first author that the 1992 potato crop in this field had been severely diseased. In 1994, he found severely diseased volunteer plants in this field. These observations strongly support the suggestion that infections in this field originated from oospores.

The year 1996. Disease development in 1996 showed a pattern quite different from the previous two years. No disease was found in refuse piles, except in the refuse pile at Wezep, located outside the research area. Our hypothesis was that the isolates from this refuse pile, containing potato waste from Flevoland and deposited by a potato processing company, belonged to the predominant genotypes. The results of the genotyping of isolates was consistent with this hypothesis. In Southern Flevoland, only one of the predominant genotypes (NL-75) was found at a low frequency (only 5 isolates) in two adjacent organic fields. In these fields, disease in these fields could thus have been initiated by infected seed tubers, but the high number of unique genotypes cannot be explained by massive survival

of asexual genotypes within seed tubers. In August 1995, conditions at harvest time were very unfavourable for the infection of seed tubers, and no infected seed tubers were found during inspections of seed tuber lots in the spring of 1996. The high diversity cannot be explained by immigration of genotypes from other sources in Southern Flevoland or elsewhere, because the organic fields were among the earliest infested fields in The Netherlands in 1996, and other sources were practically absent.

Recombination in the potato crop. Based on the 1996 observations, we venture the following hypothesis, which is compatible with the observed facts: (*i*) a few genotypes survived the 1995-1996 winter in infected seed potatoes and initiated disease early in the 1996 season, (*ii*) asexual descendants from these genotypes spread throughout the crop and recombination between isolates with opposite mating type took place during the season, (*iii*) a highly diverse progeny re-infested the crop. So most of the isolates which were collected could have been progeny of crosses established in the potato foliage earlier in the season. So far, we have not found any data in the *P. infestans* literature which support this new hypothesis of in-the-green-crop sexual reproduction cycle. A similar hypothesis was brought forward for *Mycosphaerella graminicola* on wheat (Kema *et al.*, 1996).

Inoculum sources of P. infestans in allotment gardens

Sexual inoculum. In three compounds analysed in 1995, and in the two analysed in 1996, every plot contained different genotypes, most of them being unique or rare, and A1 and A2 genotypes were mostly in a 1 : 1 ratio. These results are strongly in favour of the hypothesis that disease in allotment gardens generally originates from oospores, and are in agreement with the findings of Turkensteen *et al.* (1996). Since growers in allotment gardens often use the potato and tomato waste to enrich the soil with organic matter, it was hypothesised that this material contains oospores which can survive the winter period and infect host plants in the next season. Our finding that 'composted' waste material collected in allotment gardens was infectious is consistent with this hypothesis. The results suggest that tomato leaves are a better substrate for formation and survival of oospores than potato leaves.

Asexual inoculum. In the allotment gardens in 1994 and compound An in 1995, the majority of isolates belonged to predominant genotypes. In 1994, these genotypes were already prevalent throughout the commercial potato growing area and spores from the commercial fields might have initiated disease in allotment gardens. For compound An in 1995, influx of inoculum was the most likely source again. Disease in this compound was observed about two weeks later than in the other compounds. Some infections might have originated from oospores, but may have been overruled by infections with inoculum from commercial potato fields. Our fingerprint results do not suggest that seed tubers played an important role in the development of disease in allotment gardens.

Potato-tomato specialisation. Turkensteen (1973), Fry *et al.* (1991), Legard *et al.* (1995) and Lebreton and Andrivon (1998) showed that some specialisation occurs. Our data are inconclusive with respect to host specialisation. Here, less than 15 % of the genotypes per compound of allotment gardens occurred in both tomato and potato plots. The disparity could be an indication of specialisation, but it might also be caused simply by the fact that the majority of infections in every infested tomato and potato plot arose from oospores, so that almost every plot contained unique genotypes.

RFLP fingerprinting versus AFLP fingerprinting

Fingerprinting with probe RG57 allowed to identify populations and subpopulations, and to follow the spread of individual genotypes. We found isolates at different, unrelated sites with an identical genotype, probably due to frequent recombination. This is a drawback of using a single RFLP probe. Application of AFLP fingerprinting, with a much higher capacity to discriminate between isolates than RFLP fingerprinting, confirmed that there were differences in an apparently single genotype occurring at two epidemiologically unrelated sites.

The genotypes predominant from 1993 through 1996 were also found among the samples collected in The Netherlands between 1985 and 1990. These genotypes might have survived asexually for many years. Similarly, some genotypes, unique or rare in our study, were also found during previous collections. But because these isolates were often encountered in sites were oospores were important infection sources, it is likely that the same genotypes were formed by chance. If so, AFLP fingerprints would reveal fewer differences between isolates of the predominant genotypes from this study and from previous collections, than between isolates of the unique genotypes in the two studies. AFLP fingerprinting confirmed this. The simple probability calculations also indicate that sexual recombination may lead to the generation of identical RG57 fingerprints by chance.

The occurrence of sexual reproduction in world-wide populations of *P. infestans* may confine the value of using marker databases (Forbes *et al.*, 1998) for comparing genotypes from different locations, if genotyping is only based on mating type, allozymes, and RG57 fingerprints.

Heuristics

DNA fingerprinting was used to answer epidemiological questions. Analysis of the available data makes some answers more plausible than others and several hypotheses were brought forward. We demonstrated that both asexual and sexual inoculum sources initiated disease. The relative importance of inoculum sources differed within and among seasons, resulting in differences in genotypic composition among (sub)populations of *P. infestans*.

For the commercial potato fields, refuse piles were the most important asexual infection source. It is clear that refuse piles should be effectively covered or eliminated to prevent the early introduction of late blight in an area and to reduce the risk of sexual reproduction. The occurrence of highly diverse populations in organic potato fields makes these potential infection sources the more hazardous. The foliage of these crops has to be destroyed as soon as the disease appears. Flaming of the foliage will be more effective than mechanical destruction, because of the possible presence of oospores in the leaves.

The present study shows that sexual reproduction of *P. infestans* occurs in a commercial potato growing area. Although the effect on epidemics in a quantitative sense currently appears of minor importance, it is feasible that the highly diverse populations include strains with higher pathogenic fitness which might significantly affect the rate at which epidemics develop in the near future. When oospores have acquired a more predominant role, better fungicide application methods are needed, because it can be expected that disease will begin in the lower canopy layers.

Chapter 4

Population studies on *Phytophthora infestans* in Southern Flevoland and adjacent areas (The Netherlands). 2. The relation between epidemiology and population structure

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Submitted

Abstract

Genotypic diversities and genotypic distances among populations of *Phytophthora infestans* on potato and tomato were analysed in space and time. Over the period 1994 through 1996, *P. infestans* isolates were collected from 99 sites, including refuse piles, conventional potato fields, organic potato fields, and allotment gardens in Southern Flevoland and adjacent areas (The Netherlands). Genotypes were determined by mating type assessment and DNA fingerpinting. Genotypic diversity was high in allotment gardens and in organic potato fields. Significant differences in diversity levels existed between compounds of allotment gardens. The diversity changed both within the season and among seasons. Within one season (1994), diversity was highest in refuse piles and organic fields, and lowest in early infested conventional potato fields. Diversity increased over the years. For the total populations, the normalised Shannon index ranged from 0.34 in 1994, with weather relatively favourable to late blight development, to 0.61 in 1996, a year with relatively unfavourable weather conditions. Accordingly, genotypic distances increased over the years. In 1994, only 20 % of the sampling sites had an average genotypic distance $D \geq 0.07$. For 1995 and 1996, the percentages of sites with an average $D \geq 0.07$ was 29 % and 35 %, respectively, indicating a population shift. No relation was found between the Euclidean distance and the genotypic distance of isolates. The genotypic distance between isolates was negatively correlated with the level of genotypic diversity ($r = -0.91$). Analysis of the *P. infestans* populations in Southern Flevoland suggests an annual supply of new genotypes originating from sexual reproduction, resulting in diversification between sites and generation of unique genotypes. The structure of a *P. infestans* population developed during a severe epidemic may differ significantly from that of a population developed under unfavourable conditions.

Abbreviations: Rc = refuse pile, Fc = conventional potato field, Fo = organic potato field, and A_p = potato plot in allotment garden, A_t = tomato plot in allotment garden.

Introduction

Plant pathogen populations are genetically variable and able to adapt to changes in their environment (Wolfe & Caten, 1987). In agricultural crops, cultural and disease control measures, including application of fertilisers and pesticides, and the choice cultivars, cause changes in environmental conditions. The changes may have significant effects on the genetic composition and the structure of plant pathogen populations.

Population structure refers to (*i*) the amount of genetic variation among individuals in a population, (*ii*) the ways in which this variation is partitioned in time and space, and (*iii*) the genetic relationships within and between (sub)populations (Leung *et al.*, 1993). The processes which affect population structure are: mutation, selection, genetic drift, gene flow, and mating system.

Analysis of the structure of plant pathogen populations, and the way the population structure changes in time and space, provides insight into the processes which shape population structure, and thus contribute to the understanding of epidemiology and the effect of disease control measures (Leung *et al.*, 1993; McDonald, 1997; Milgroom & Fry, 1997). The availability of easily assayed genetic markers (*e.g.* isozymes, RFLPs) has stimulated research on population biology and population structure in plant pathology (Milgroom & Fry, 1997). Especially for fungi which reproduce both asexually and sexually, population studies have proved to be useful in making inferences about the importance of sexual reproduction (McDonald *et al.*, 1995; Milgroom, 1996).

In the last decade, the potato late blight fungus *Phytophthora infestans* (Mont.) de Bary has been the subject of extensive population studies. Due to the detection of isolates with the A2 mating type outside Mexico (Hohl and Iselin, 1984), populations throughout the world have been studied. Based on allozyme (Spielman, 1991) and DNA fingerprinting analyses (Goodwin *et al.*, 1992a), it was demonstrated that a world-wide migration in the late 1970s resulted in the distribution of diverse *P. infestans* populations across Europe and other continents (Fry *et al.*, 1992; 1993; Goodwin *et al.*, 1994b; Goodwin, 1997). Old, asexual populations consisting mainly of one clonal lineage have been replaced. Studies in The Netherlands (Drenth *et al.*, 1993; 1994; Zwankhuizen *et al.*, *subm.(a)*), Poland (Sujkowski *et al.*, 1994) and Northern America (Goodwin *et al.*, 1995) showed that sexual reproduction of *P. infestans* now also occurs outside Mexico, thus creating even more diverse populations.

In most studies on population structure of *P. infestans*, the epidemiological context has been ignored. The question addressed in this chapter concerns the effect of epidemic development of late blight on the population structure of *P. infestans*. Previously, we focused on the small-scale epidemiology of late blight in Southern Flevoland (The Netherlands) (Chapter 2; Zwankhuizen *et al.*, 1998). Subsequently, the genotypic composition of populations of *P. infestans* during the growing seasons of 1994, 1995, and 1996 in that area was analysed (Chapter 3; Zwankhuizen *et al.*, *subm.(a)*). We demonstrated that both asexual and sexual inoculum sources initiated disease. The relative importance of inoculum sources differed within and among seasons, resulting in differences in genotypic composition among (sub)populations of *P. infestans*.

In this chapter, the results of the small-scale epidemiology study (Chapter 2) and the genotypic analyses (Chapter 3) are integrated to further elucidate the development of late blight on potato and tomato within a limited agricultural area.

Materials and methods

Sampling sites and data sets

During the growing seasons of 1994, 1995, and 1996, development of late blight epidemics, caused by *P. infestans*, was studied in a limited area (150 km^2) of Southern Flevoland, a polder in the central part of The Netherlands. A detailed description of the research area and the disease surveys is given in Zwankhuizen *et al.* (1998). Sampling sites included refuse piles (Rc), conventional (Fc) and organic (Fo) potato fields, and allotment gardens (A_p and A_t, for potatoes and tomatoes, respectively).

The data on genotypic composition of sampling sites from 1994 through 1996, presented in chapter 3 (Zwankhuizen *et al.*, *subm.(a)*), were used to study the change of the population structure in space and time. We explored genotypic diversity and distance measures to identify differences among sampling sites. The spatial component refers to the different categories of sampling sites and the geographical (Euclidean) distance between sampling sites. The temporal component refers to both within season and among season comparisons. Specific subsets were selected from the data base. These subsets allowed to test a number of *a posteriori* hypotheses on the relation between epidemiology and population structure.

Characterisation of isolates

Isolates, collected from the sampling sites, were characterised for mating type and RG57 fingerprint pattern, as described in Zwankhuizen *et al.* (1998). Mating type A1 was scored as '0', A2 scored as '1'. Absence of a fingerprint fragment was scored as '0', presence of a fragment as '1'. Each isolate was represented by a 24 binary code, including mating type and 23 fingerprint loci which together determine the genotype (Zwankhuizen *et al.*, *subm.(a)*). Out of 1048 isolates characterised for mating type and RG57 fingerprint, 170 genotypes were identified (Zwankhuizen *et al.*, *subm.(a)*). A genotype was designated unique if one or more isolates of this genotype were detected in only one sampling site in the research area during the research period.

Genotypic diversity

According to the dual-concept meaning of diversity (Peet, 1974), genotypic diversity includes both genotype richness (*i.e.* the number of different genotypes in the sample) and genotype evenness (*i.e.* the relative frequencies of the genotypes in the sample). The spatial component of diversity was considered in the analysis of the diversity among potato sites belonging to different categories, and between sites of the same category. A special case

concerned the diversity of *P. infestans* at different downwind distances along a disease gradient, originating from organic potato fields (Zwankhuizen *et al.*, 1998). Diversity in time was studied within and between seasons. Only the year 1994 provided sufficient data for the analysis of diversity over the course of the growing season. In 1994, isolates could be collected from April through September, whereas in 1995 and 1996, sampling in a limited number of fields had almost been finished by mid August. An overview of the number of sampled host plant sites was given in Zwankhuizen *et al.* (*subm.(a)*). Diversity of the *P. infestans* populations on potatoes and tomatoes in allotment gardens in 1996 was also considered.

The index used for diversity was the Shannon index (Bowman *et al.*, 1971) defined as:

$$h_0 = - \sum_{i=1}^{k} p_i \cdot \ln p_i$$

where p_i is the frequency of isolates with the i^{th} genotype in the sample and k is the number of genotypes in the sample. As normalised Shannon statistic we used $h'_0 = h_0 / \ln N$ where h_0 is the original Shannon index and N is the total number of isolates (Goodwin *et al.*, 1992b). This statistic, presenting the Shannon index as a fraction of the maximum diversity in the sample, has been found to be relatively stable and has provided a good measure when sample sizes varied (Sheldon, 1969). Depending on the hypotheses to be tested, either single sites or groups of sites were considered. When single sites were used, sites from which only one isolate was characterised were excluded from the analyses.

Differences between diversities of pairs of samples were calculated using the *t*-test of Hutcheson (1970). Calculations were made using BIODIV, version 5.1 (Baev and Penev, 1995).

Genotypic distance

Relationships among isolates were calculated in several situations. First, the average genotypic distance per sampling site was determined. Chi-square analysis and Fisher's exact test (Sokal and Rohlf, 1981) were applied to identify differences in average genotypic distance between seasons and among different categories of sampling sites. Analyses were made using total samples per site and censored samples, *i.e.* only one isolate of each different genotype was included in the sample to correct for repeatedly collecting the same genotype in a site.

Genotypic distance D was calculated as 1 - F where F is the Dice coefficient (Rohlf, 1993):

$$F = 2N_{xy} / (N_x + N_y)$$

and N_x and N_y refer to the number of fragments in isolate X and Y, respectively, and N_{xy} is the number of DNA fragments shared by the two isolates.

Second, the relationship between the genotypic distance and the geographic (Euclidean) distance between the positions where the respective isolates had been collected was analysed. The relation between the genotypic distance of isolates and the geographic distance between certain clusters of potato fields was also tested. To test whether the genotypic distance was correlated with the Euclidean distance, genotypic distance and Euclidean distance matrices were compared using a matrix comparison technique, originally developed by Mantel (1967) and used by Milgroom and Lipari (1995). Significance testing was done by comparing the observed correlation between the distance matrices with its permutational distribution (1000 random permutations). The P-value was estimated from the proportion of the null distribution smaller than the observed correlation. Calculation of genotypic distances and matrix comparisons were made using NTSYS-pc, version 1.80 (Rohlf, 1993). To account for the effect of repeated collection of the same genotype within a relatively short distance, censored samples were used to analyse the relation between genotypic distance and Euclidean distance in the allotment gardens and in clusters of potato fields.

Relation between genotypic diversity and genotypic distance

The relation between genotypic diversity and genotypic distance was investigated by using the diversity and distance data of individual sampling sites. Spearman's rank correlations (Sokal and Rohlf, 1981) were calculated for categories of sampling sites, for each of the years 1994 through 1996 and for the whole period. Both complete samples and censored samples were used.

Results

Genotypic diversity in space and time

Diversity along a disease gradient, 1994. Diversity along the gradient assessed at distances of about 0, 1, and 4 km from the source was independent of distance and remained practically constant (Table 1).

Table 1. Genotypic diversities of *P. infestans* collections, as measured by the Shannon index (h_o), in potato and tomato sampling sites of Southern Flevoland and adjacent areas (The Netherlands) from 1994 through 1996.

Site(s)	No. of sites	No. of isolates	No. of genotypes	h_o [1]
Diversity along disease gradient, 1994[2]				
VS	2	30	7	1.11a
FcI	7	51	7	1.08a
FcII	5	23	4	1.00a
Diversity among categories of sites, 1994				
Rc	7	47	8	1.57ab
Fc	17	140	8	1.27a
Fo	5	57	9	1.30a
A_p	9	33	8	1.85b
Diversity among categories of sites, 1995[3]				
Fo	6	105	21	2.28a
A_p	21	180	39	3.26b
Diversity among categories of sites, 1996[3]				
Fo	6	162	44	2.96b
A_p	11	81	26	2.46a
Diversity among compounds of allotments, 1994[4]				
A_pn	4	13	4	1.22a
A_ph	5	20	4	1.16a
Diversity among compounds of allotments, 1995[4]				
A_pn	4	36	7	1.44a
A_ph	6	50	15	2.36c
$A_p$sp	5	48	10	1.90b
A_pa	6	46	8	1.80ab
Diversity among compounds of allotments, 1996[4]				
A_pn	5	20	14	2.48bc
A_tn	5	33	16	2.55c
A_ph	6	61	12	1.71a
A_th	5	34	12	2.03ab
Diversity over time, 1994[5]				
Rc	7	47	8	1.57c
FcE	4	61	4	0.71a
Fo	5	57	9	1.30bc
FcL	13	79	8	1.10b

Diversity between seasons[6]				
Total 1994	38	277	22	1.89a
Total 1995	31	383	66	3.24b
Total 1996	30	348	97	3.43b
Diversity between seasons measured for organic fields (Fo)[3]				
Fo 1994	5	57	9	1.30a
Fo 1995	6	105	21	2.28b
Fo 1996	6	162	44	2.96c
Diversity between seasons measured for allotment gardens (A_p)[3]				
A_p 1994	9	33	8	1.85a
A_p 1995	21	180	39	3.26c
A_p 1996	11	81	26	2.46b

1 Diversities (h_0) within the sections of the column followed by a common letter are not significantly different at $P \leq 0.05$ according to the *t*-test of Hutcheson for pairwise comparisons (see text).

2 Two infested organic potato fields were sampled which appeared to be the infection source (called VS) for several downwind conventional potato fields (Zwankhuizen *et al.*, 1998). FcI and FcII are groups of conventional fields located at average distances of 1 and 4 km, respectively, from the infection source VS.

3 Refuse piles and conventional fields not included here, because only few sites could be sampled (Zwankhuizen *et al.*, *subm.(a)*).

4 In 1994 and 1996, only isolates from the compounds of allotment gardens An and Ah were characterised with DNA fingerprinting. In 1996, isolates from tomato were also characterised (Zwankhuizen *et al.*, *subm.(a)*).

5 The development of the epidemic in 1994 is described in detail in Zwankhuizen *et al.* (1998). FcE are early infested fields, sampled during the first outbreak of disease between June 3 and June 30. FcL are conventional fields sampled during the second outbreak of disease in August and September.

6 The normalised Shannon index h'_0, expressing the Shannon index as a fraction of the maximum diversity was 0.34, 0.54, and 0.61 for 1994, 1995, and 1996, respectively.

Diversity among categories of sampling sites, 1994-1996. Each year, diversities of organic fields and allotment gardens differed significantly, but their ranking order was not consistent (Table 1). Only in 1996 was the diversity of the samples from the organic fields higher than in the allotment gardens. In 1994, high diversities were found for refuse piles and allotment gardens, and relatively low values for commercial fields. Only the difference between commercial fields (Fc and Fo) and potato plots in allotment gardens (A_p) was significant ($P < 0.05$).

Diversity among compounds of allotment gardens, 1994-1996. High diversity levels were found in allotment gardens (Table 1). Significant differences in diversity existed among compounds of allotment gardens in 1995 and 1996, but not in 1994. No pattern is discernible. In 1996, no significant difference was found between potato and tomato plots in both compounds.

Diversity over time, 1994. Early-infested conventional potato fields (FcE) had remarkably low diversity (Table 1). The ranking order of diversities does not correspond with the ranking of the respective sampling periods. Refuse piles were sampled between April and June, the conventional fields were sampled in June (FcE) and between August and September (FcL), and the organic fields (Fo) were sampled in August. When considering the conventional fields only (FcE and FcL), diversity increased significantly over the sampling interval of about 2 months.

Diversity between seasons, 1994-1996. Total diversity was significantly lower in 1994 than in 1995 and 1996 (Table 1). The percentage of maximum diversity increased from 34 % in 1994 to 61 % in 1996. Analysis of the diversities of organic fields and allotment gardens separately indicated a significant increase of genotypic diversity for the organic fields over the three-year period, whereas the diversity in the allotment gardens fluctuated, with the diversity in 1994 being much lower than in the two following years.

Genotypic diversification between sampling sites. When the genotypic composition of sampling sites was considered, the effect of the year was also evident (Table 2). The frequency of sampling sites in which unique genotypes were found was significantly lower in 1994 than in 1995 and 1996.

Genotypic distance in space and time

Genotypic distance analysis was conducted both for complete samples (including all isolates) and censored samples (corrected for genotypes with more than one isolate). For most analyses, censoring of the samples gave similar results. The analyses with the censored samples will only be shown when there were considerable differences between the two methods. The genotypic distance of the sampling sites ranged from 0 in many sites where only 1 genotype was detected to 0.27 in a conventional field (Fc2) in 1996 where 4 genotypes, NL-84, NL-92, NL-106, and NL-115, were found which had substantially different DNA fingerprints (Zwankhuizen *et al., subm.(a)*).

Genotypic distance over the categories of sampling sites, 1994-1996. The distribution of sampling sites over three genotypic distance classes is shown in Table 3. Chi-square analysis did not indicate a significant interaction between genotypic distance class and category of sampling site. Analyses of 2 x 2 tables were carried out for the difference between the commercial sites (Rc+Fc+Fo) and the allotment gardens (A) within each year.

Table 2. Annual distributions of the frequency of sampling sites in Southern Flevoland and adjacent areas (The Netherlands), 1994 - 1996, with and without unique genotypes [1].

Year	No. of sites containing unique genotypes	No. of sites without unique genotypes	Total
1994	8	30	38
1995	24	7	31
1996	24	6	30
Total	56	43	99

[1] The frequency of sites with unique genotypes differed significantly among the years according to the Chi-square test ($P < 0.001$). Fisher's exact tests on 2 x 2 tables revealed no significant differences between 1995 and 1996 ($P = 0.11$), but the frequencies of sites with unique genotypes differed significantly between 1994 and 1995, and 1994 and 1996 ($P < 0.001$).

In 1994 and 1995, frequencies of commercial sampling sites and allotment gardens were distributed significantly different over relatively high ($D \geq 0.04$) and relatively low ($D < 0.04$) genotypic distance classes (most sites in 1994 had relatively low genotypic distance), but not in 1996 ($P < 0.05$).

Genotypic distance and Euclidean distance. Analysis of the relation between genotypic distance and Euclidean distance for a selection of situations gave almost no significant correlations (Table 4). For individual potato fields, all correlations were low and non-significant, including the 'control'. This control was constructed using the genotype data of three closely-located conventional fields which were assumed to be infested by inoculum from the same infection source (*i.e.* the infested organic potato fields in August 1994). The only compound of allotment gardens where a significant (negative) correlation (-0.72) existed was A_ph in 1994 ($P = 0.01$). Regional analysis of genotypic distance and Euclidean distance revealed very low correlations, of which the correlation for 1995 was slightly significant.

Genotypic distance over the seasons, 1994-1996. Table 5 shows the distribution of sampling sites over genotypic distance classes and years. Chi-square tests showed that the high genotypic distance class ($D \geq 0.07$) was underrepresented in 1994, whereas the low genotypic distance class ($D < 0.01$) was underrepresented in 1996. Apparently, a 'genotypic distance distribution shift' took place between 1995 and 1996. Using censored samples, this shift appeared between 1994 and 1995.

Table 3. Distributions of the frequencies of sampling sites in Southern Flevoland and adjacent areas (The Netherlands), 1994 - 1996, with relatively low, intermediate and high genotypic distance (*D*) over the categories of sampling sites[1].

Category of sampling sites[2]	No. of sites			Total
	$D < 0.01$	$0.01 \leq D < 0.07$	$D \geq 0.07$	
Refuse piles	2	5	2	9
Conventional fields	7	8	5	20
Organic fields	3	5	9	17
Allotment gardens	20	14	16	50
Total	32	32	32	96

1 Chi-square analysis did not indicate a significant interaction (P = 0.31) between genotypic distance and categories of sampling sites.

2 The average genotypic distance calculated over the three years was 0.04, 0.05, 0.08, and 0.05 for the refuse piles, conventional fields, organic fields, and allotment gardens, respectively. Using censored samples, the average genotypic distances were 0.10, 0.08, 0.14, and 0.07, respectively.

Genotypic diversity and genotypic distance

Correlations between the Shannon diversities and the average genotypic distances of the sampling sites were calculated. For the total data set (1994 through 1996), Spearman's rank correlation coefficient was -0.91 (-0.79 censored) ($P < 0.001$). The correlation coefficient varied slightly among the years, being -0.91 (-0.86 censored), -0.90 (-0.76 censored), and -0.89 (-0.69 censored) for 1994, 1995, and 1996, respectively ($P < 0.001$). The correlations between genotypic diversity and genotypic distance in the different categories of sampling sites were, -0.65 (-0.79 censored), -0.94 (-0.79 censored), -0.75, and -0.81 (-0.79 censored), for refuse piles, conventional fields, organic fields, and allotment gardens, respectively. These correlations were significant ($P < 0.05$) except for the refuse piles (complete sample and censored sample) and organic fields (censored).

Table 4. The relation between genotypic distance and Euclidean distance of isolates of *P. infestans*, collected in Southern Flevoland (The Netherlands), 1994 - 1996.

Site(s)	No. of isolates	No. of genotypes	D^1	E^2	r^3	P
Individual potato fields[4]						
Fcc 1994[5]	32	5	0.07	785	-0.01	0.42
Fc2 1995	28	10	0.14	172	0.04	0.37
Fo2 1995	25	9	0.09	127	0.18	0.05
Fo2 1996	21	14	0.18	158	-0.14	0.09
Fo5 1996	27	17	0.20	205	-0.10	0.37
Allotment gardens[6]						
A_pn 1994	5	4	0.16	71	0.15	0.43
A_ph 1994	6	4	0.10	115	-0.72	0.01
A_pn 1995	7	7	0.08	40	-0.34	0.08
A_ph 1995	15	15	0.17	89	0.09	0.27
A_ps 1995	10	10	0.20	112	-0.27	0.06
A_pa 1995	8	8	0.28	139	-0.05	0.34
A_pn 1996	14	14	0.16	93	-0.06	0.31
A_ph 1996	18	12	0.11	60	0.07	0.31
A_tn 1996	19	16	0.17	99	-0.13	0.08
A_th 1996	14	12	0.16	97	-0.13	0.11
Regional analyses[7]						
1995	35	29	0.19	4659	0.06	0.04
1996	48	45	0.21	5022	-0.03	0.14

1 Average genotypic distance (see text).

2 Average Euclidean distance (m) between the isolates considered.

3 Correlation coefficient between Euclidean distance and genotypic distance according to the matrix comparisons (see text).

4 Potato fields with ≥ 9 genotypes were selected, except for the control Fcc.

5 As a control, 3 conventional fields (Fc5,7,11; Zwankhuizen *et al.*, 1998) were used (see text).

6 Censored samples from plots were considered. Results of analyses without censoring were not different in a qualitative sense.

7 1995: fields Fc2, Fo5, Fo6 (cluster 1) and Fo2, Fo3, Fo4 (cluster 2) were considered. 1996: fields Fo1, Fo2, Fo6 (cluster 1) and Fo3, Fo4, Fo5 (cluster 2) were considered. Per cluster, censored samples were used.

Discussion

The capriciousness of late blight

Late blight, caused by *Phytophthora infestans*, shows a capricious pattern of occurrence. The disease always challenges epidemiologists by its sudden appearance in sites not intended to be sampled. The relative unpredictability of the disease has consequences for the design of sampling surveys. Our statistical design was far from unbiased (Zwankhuizen *et al.*, 1998). Basically, the research area was stratified into 4 major strata: refuse piles, conventional fields, organic fields, and allotment gardens. Some strata received more attention than others, because of the objectives of the research. For example, the organic fields and the surrounding conventional fields were inspected intensively (Zwankhuizen *et al.*, 1998). However, the weight we attributed to the different strata was not equal over the years. Any field-oriented research is also confronted with dependency among the data obtained. The weather conditions and the progress of the epidemics influenced the statistical design, the selection of the data, and the statistical analyses. We used the data to test *a posteriori* hypotheses, rather than testing *a priori* hypotheses.

The foregoing considerations must be taken into account when interpreting the data of our study and the outcome of the statistical tests. Notwithstanding these objections, we have confidence in the general trend which shows up in our study. Results of the genotypic analyses were combined with field observations (Zwankhuizen *et al.*, 1998; *subm.(a)*). The combination allowed us to discriminate between possible inoculum sources for specific fields and enabled us to relate the genotypic data to the epidemiological situation, making some conclusions more plausible than others.

Large scale versus small scale population studies

In the central highlands of Mexico, the centre of origin of *P. infestans*, Goodwin *et al.* (1992b) detected very diverse populations and almost every isolate of *P. infestans* had a different genotype. The present study confirms earlier studies by Drenth *et al.* (1993, 1994) in The Netherlands and Sujkowski *et al.* (1994) in Poland that *P. infestans* populations outside Mexico can also be highly diverse. In Table 6, our study is compared with three large scale studies ($\geq$ 1000 km^2) in Europe and Northern America. Genotypic diversity in the North-American populations of 1992 and 1993 was relatively low (h'_0 = 0.31). The overall level of diversity at the large scale in Poland between 1985 and 1991 and in The Netherlands in 1989 was of the same order of magnitude as in our present three-year study at the small scale (150 km^2). Significant differences in diversity levels existed within our research area, indicating spatial and temporal effects.

Table 5. Annual distributions of the frequencies of sampling sites in Southern Flevoland and adjacent areas (The Netherlands), 1994 - 1996, with relatively low, intermediate and high genotypic distance (D) [1].

Year[2]	No. of sites			Total
	$D < 0.01$	$0.01 \leq D < 0.07$	$D \geq 0.07$	
1994	15	13	7	35
1995	12	10	9	31
1996	5	9	16	30
Total	32	32	32	96

1 The chi-square test indicated a slightly significant (P = 0.047) interaction between genotypic distance classes and years. Chi-square tests applied to pairs of years (2 x 3 tables) showed a significant interaction between genotypic distance classes and 1994 and 1996 (P = 0.012), but no interaction for the pairs of years 1994 and 1995 (P = 0.69) and 1995 and 1996 (P 0.087).

2 Average genotypic distances calculated over all sampling sites were 0.04, 0.05, and 0.08, for 1994, 1995, and 1996, respectively. The values for the analysis with censored samples were 0.07, 0.09, and 0.11, respectively.

Spatial and temporal aspects of genotypic diversity and genotypic distance

Comparison of diversities in space revealed significant differences among categories of sampling sites. Relatively high genotypic diversities were found in allotment gardens and organic fields. This is in agreement with our findings that the majority of isolates in these sites belonged to unique genotypes, suggesting oospore-initiated disease outbreaks (Zwankhuizen *et al., subm.(a)*). One special case was considered: the diversity along a regional disease gradient in 1994. The null-hypothesis, that diversity remains equal over distance, could not be rejected.

Temporal aspects of diversity were studied. Significant differences within and between seasons were found. Diversity increased over the three years, and was the highest in 1996, the year with the lowest disease levels (Zwankhuizen *et al.*, 1998). Within-season comparison was only possible in 1994 because of poor epidemic development in 1995 and 1996. In 1994, the level of diversity followed the epidemic pattern: at the beginning of the season a high level in the refuse piles, a lower level in the early infested fields, a higher

Table 6. Comparison of population studies of *P. infestans* at different scales of distance.

Research area (reference)	Sampling years	Approx. largest distance between sampling sites (km)	No. of markers	No. of isolates	No. of genotypes	No. of unique genotypes (%)	h'_o[1]
Netherlands (Drenth *et al.*, 1993)	1989	300	26[2]	153	35	21 (60)	0.45
Poland (Sujkowski *et al.*, 1994)	1985 - 1991	800	29[3]	175	81	69 (85)	0.65
Northern America (Goodwin *et al.*, 1995)	1992 - 1993	4000	28[4]	130	9	4 (44)	0.31
S. Flevoland[5] (This study)	1993 - 1996	50 (22)[6]	24[7]	1048	170	138 (81)	0.54

1 Normalised Shannon diversity (see text).

2 26 RG57 fingerprint loci.

3 26 RG57 fingerprint loci, mating type and two allozyme loci (*Gpi* and *Pep*).

4 25 RG57 fingerprint loci, mating type and two allozyme loci (*Gpi* and *Pep*).

5 See also Zwankhuizen *et al.* (1998) and Zwankhuizen *et al.* (*subm.(a)*).

6 If the refuse pile in Wezep (located outside the research area) is not included, the largest distance is 22 km.

7 23 RG57 fingerprint loci and mating type.

level in the organic fields after a hot and dry period, and towards the end of the season, a lower level in the infested conventional fields.

Genotypic distance was associated with the level of diversity. Lower genotypic distances were encountered in sites with higher diversities, as confirmed by significant (negative) rank correlation coefficients. For nearly all cases studied, no relation was found between the genotypic distance and Euclidean distance between isolates. This apparent lack of correlation might be attributed to the fact that genotypes of *P. infestans* can easily disperse over larger distances. Furthermore, influx of genotypes from other sites cannot be excluded.

The analysis of the genotypic distance distributions over the years revealed a significant population change. The *P. infestans* population in Southern Flevoland shifted from relatively low genotypic distances in 1994 to relatively high genotypic distances in 1996. So the *P. infestans* population became more diverse while the average genotypic distance of the isolates decreased during the three-year period. Additional sampling of the *P. infestans* population over consecutive years is needed to further elucidate changes in population structure.

Processes affecting population structure

Processes that affect the structure of populations of plant pathogens are mutation, gene flow, selection, random genetic drift, and mating systems (Milgroom & Fry, 1997).

Mutation. Mutation plays an important role in generating variation in *P. infestans*, as is well-known for virulence (Goodwin, 1997). Genotypic analyses of the strongly asexual populations of *P. infestans* in the US revealed a number of extra RG57 fingerprint bands (Goodwin, 1997). It was concluded that at least 4 mutations could have occurred within clonal lineages. In our study, with 154 different fingerprint patterns, it is difficult to assess how much variation can be attributed to mutation. The fact that not even one new fingerprint band was found indicates that mutation has had a limited effect, in comparison to other processes.

Gene flow. In 1994 and 1995, early disease development was definitely associated with refuse piles (Zwankhuizen *et al.*, 1998). Although immigration of genotypes from outside the research area seems limited in these years, the unsprayed organic potato fields (ca. 140 ha in total each year) could have acted as large spore traps and multipliers of inoculum. This could be one of the explanations for the occurrence of high numbers of genotypes in organic potato fields. Circumstantial evidence for the potential role of organic potato crops as spore traps comes from the identification of the rare genotype NL-66, first obtained in the allotment garden compound Ah and one month later in an organic field located 5 km downwind of that compound. In 1996, the organic fields in the research area belonged to the first infested fields reported in The Netherlands so that substantial influx of inoculum from other areas seems unlikely in that year. Gene flow between populations or

subpopulations within the research area was obvious and substructuring of the *P. infestans* population was observed. In 1994, isolates of *P. infestans* from the allotment gardens apparently belonged to the population present in the commercial fields. In contrast, most allotment gardens had populations quite distinct from those in the commercial fields in 1995.

Selection and genetic drift. We cannot distinguish between selection and genetic drift. Genetic drift confounds the effect of selection (Leung *et al.*, 1993). The biased sampling and the many bottlenecks through which populations pass make it difficult to attribute changes in proportions of genotypes to either selection or genetic drift. Selection for virulence is well-known in *P. infestans* (Turkensteen, 1973; Goodwin, 1997). The majority of potatoes grown in the research area were of the susceptible cultivar Bintje, so indirect selection via virulence may have been limited in our study. Our data do not allow to make inferences on selection for certain genotypes. The predominance of certain genotypes might be the result of a higher pathogenic fitness (Day and Shattock, 1997; Kato *et al.*, 1997) or better vegetative survival characteristics. An illustration of a supposedly serious bottleneck is the population development in 1996. The number of asexual infection sources was very low due to limited tuber infections in autumn 1995 and due to the cold winter of 1995/1996. The predominant genotypes were almost extinct in 1996.

Sexual recombination. The most likely explanation for the high level of diversity and for the continuous generation of new genotypes is the process of sexual recombination, during the development of oospores in infected tissue. The oospores can act as inoculum sources and can either overwinter in soil, or germinate directly after formation within the crop during the course of the growing season. The occurrence of sexual reproduction is probably the most important explanation for the relatively high diversity levels in the research area (150 km^2). The level of diversity even seems to increase. The effect of sexual recombination on the genotypic composition of populations will be most evident in dry years, such as 1996. In wet years, populations will be dominated by a few, rapidly spreading genotypes, of which 1994 was but a modest example.

Epidemiology and population structure

The results reported here show that the structure of the *P. infestans* population differed from year to year. Among several explanatory factors, weather effects and inoculum sources are most evident. Weather conditions substantially affected the development of the potato late blight epidemics in the research area (Zwankhuizen *et al.*, 1998). During the research period, the weather was often unfavourable to late blight development. At first sight, poor disease development might be perceived as a restraint to the general applicability of our findings. However, low infection levels allowed us to link genotypic analyses to epidemiological phenomena. Such a linkage will not easily be found in years

with severe late blight outbreaks due to favourable weather, when the earliest genotypes from refuse piles will rapidly predominate and thus mask the unique and rare genotypes.

The epidemic model of population structure described by Maynard Smith *et al.* (1993) is characterised by frequent recombination and the subsequent occurrence of a few, highly successful genotypes during the asexual phase of the epidemic. This model might be partially applicable to the *P. infestans* populations analysed in this study. The structure will be confounded by overwintering asexual inoculum, sexual propagation within the crop during the growing season (Zwankhuizen *et al.* (*subm.(a)*), and in dry years, by reduced spread of genotypes, resulting in diverse subpopulations across the area.

Since the structure of *P. infestans* populations may be quite variable, population studies of this pathogen need to be accompanied by epidemiological studies to better understand why a population has a certain structure. Both on a global and regional level, the geographic and temporal structuring of *P. infestans* populations is a dynamic process (Andrivon, 1994). Prediction of the direction in which the population structure will evolve in the future is nearly impossible, even in our small research area, because of the many interactions which can have an impact on the establishment of a *P. infestans* population. Potato crops will be continuously confronted with a 'shifty' pathogen, which will make potato growing increasingly dependent on disease control measures, such as the use of resistant cultivars and the application of fungicides.

Chapter 5

Epidemiology of potato late blight in The Netherlands from 1950 - 1996

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Submitted

Abstract

Potato late blight epidemics in The Netherlands, caused by *Phytophthora infestans*, were analysed using agronomic and meteorological variables. Severity of late blight epidemics was characterised by a disease index (DI) with a 0-4 scale (0 = absence of late blight, 4 = severe epidemic). DI fluctuated from year to year and, superimposed on the yearly pattern, a multi-year pattern was identified in which 3 periods were evident: I (1950-1968), II (1969-1978), and III (1979-1996). The average DI in these periods were 2.9, 0, and 2, respectively. Major changes of the DI between individual years, including the transitions from period I to II and from period II to III, corresponded with changes in meteorological variables. Major variables were disease enhancing, such as the number of days with precipitation during the growing season (DPRg) and the number of hours with a temperature between 10 and 27 °C and a relative humidity > 90 % (HF), as well as disease limiting, such as the number of hours per growing season with temperatures > 27 °C (HU) and the amount of global radiation (GR). The transition from period II, the blight-free period, to period III coincided with the occurrence of resistance against the fungicide metalaxyl and the introduction of the 'new' *P. infestans* population in The Netherlands. Chi-square analyses and Spearman's rank correlations identified the disease level in the previous season, DPRg, HF, and GR as significant variables ($P \leq 0.01$). Linear discriminant analysis was conducted using the blight status of the previous year (LBp, 0 = absence, 1 = presence of late blight), total values (per growing season) and monthly values for DPRg, HF, GR, HU, and other meteorological variables. Using monthly values, the number of years correctly classified was 40 (87.0 %). Five out of the six incorrectly classified years were in period III. For both the analyses (total and monthly), the blight status of the previous year (LBp), the number of days with precipitation (DPRg) and the number of hours with favourable conditions (HF) were the most important discriminating variables. The importance of agronomic factors seemed evident, but could not be inferred from our data. The effect of man on the polyetic pattern of disease outbreaks is not well understood. Polyetic studies of plant diseases, which may help to identify key factors for the development of epidemics, need more attention in crop protection.

Introduction

The development of plant diseases in agricultural crops during the course of the growing season and the carry-over of disease to the next growing season are affected by many factors. Zadoks and Schein (1979) distinguished four categories, interconnected by a disease *tetrahedron*. These are (*i*) the pathogen, (*ii*) the host, (*iii*) the environment, and (*iv*) man. The base of the *tetrahedron* symbolises the interaction between the first three categories. Each of these is influenced by man. Aspects of the pathogen include *e.g.* the

initial level of inoculum and pathogenicity. Important properties of the host are *e.g.* the general level of resistance and the density of the crop in an area. The environment, including biotic and abiotic elements, affects both the host and the pathogen (Bourke, 1970; Zadoks and Schein, 1979). Well-known examples of abiotic factors are (micro)climate (Harrison, 1992) and the application of pesticides.

For diseases commonly controlled by routine application of fungicides, the weather is regarded as a major variable which determines the epidemiological pattern of plant diseases (Jones, 1986). This is especially true for one of the most devastating plant diseases: potato late blight caused by *Phytophthora infestans* (Mont.) de Bary (Hooker, 1981). After its introduction in the 1840s in Europe, where it caused the Irish potato famine (Bourke, 1964), and subsequent migration to other continents (Goodwin *et al.*, 1994b), this fungus has been a serious threat to potato growing world-wide. Many efforts have been made to manage this disease, from breeding for resistance to chemical control (Dowley *et al.*, 1995). In some years or periods of years, late blight and its causal agent received more than usual attention. Events responsible were *e.g.* the detection of the A2 mating type in Europe, recent migrations and apparent population replacements all over the world, the detection of sexual reproduction in field populations, and severe outbreaks of disease due to resistance to the fungicide metalaxyl (Fry and Goodwin, 1997).

The identification of a 'new' *P. infestans* population in The Netherlands, most likely introduced in the late 1970s, and the evidence for sexual reproduction (Drenth *et al.*, 1993; 1994) required reassessment of the relative importance of the various inoculum sources. We examined the relative impact of these inoculum sources, including oospores arising from sexual reproduction, on the development of late blight epidemics by analysing the epidemiology and population structure of *P. infestans* in Southern Flevoland (The Netherlands) from 1994 to 1996 in chapter 2 (Zwankhuizen *et al.* 1998) and in chapters 3 and 4 (Zwankhuizen *et al.*, *subm.(a;b)*). Weather conditions and *P. infestans* epidemiology differed dramatically between these years. In 1994 and 1995, first late blight was observed in the research area before the end of June, whereas in 1996 the first symptoms were found in the end of July. During the 1996 growing season, only a very limited number of potato fields were infested throughout The Netherlands. A low carry-over of inoculum from the previous year and unfavourable weather conditions in spring 1996 contributed to this exceptional phenomenon (Zwankhuizen *et al.*, 1998).

The 1996 observations initiated the study described in this chapter. From literature it is known that the epidemic pattern can be highly variable and that late blight can be practically absent for several years (Croxall and Smith, 1976; Secor *et al.* 1995; Johnson *et al.*, 1996). We were interested to find out whether there may have been years or periods of years in The Netherlands without any significant occurrence of the late blight disease and, if so, which factors might have contributed to the epidemiological pattern. With regard to the establishment of the 'new' population of *P. infestans* in The Netherlands, it is of special importance to find out whether the severity of late blight epidemics has increased since the

late 1970s, a fact often mentioned in literature (Fry and Goodwin, 1997), or other conditions have changed in favour of late blight outbreaks.

In this chapter we analyse the polyetic (≈ multi-year; Zadoks, 1978) pattern of potato late blight disease outbreaks. Agricultural and meteorological data were used to identify key factors which had a major impact on the development of late blight epidemics in The Netherlands from 1950 to 1996.

Materials and methods

Characterisation of late blight epidemics

Late blight epidemics in The Netherlands from 1950 to 1996 were analysed. The years prior to 1950 were excluded from the analysis because adequate data from years prior to 1950 could not be found. Data on the severity of late blight epidemics were obtained from many reports and publications. The main sources were the annual reports of the Plant Protection Service (Anonymous, 1949 - 1987) and the Dutch journal Gewasbescherming (Anonymous, Barel, Van der Wal, Van Velde, 1979 - 1994). Additional data were the observations on late blight in The Netherlands by Van der Zaag (1956), Lohuis and Zadoks (*unpublished*), Davidse and Zadoks (*unpublished*), Davidse *et al.* (1989), Fry *et al.* (1991), and the first author (Zwankhuizen *et al.*, 1998).

This study considers late blight at a national scale. Years were rated for severity of the blight epidemics according to a late blight disease index (DI). This index needed to be very general, because detailed information about the disease levels, the number of infested fields, and regional differences were mostly lacking. The index accounts for any large difference in severity of the late blight epidemics between the different potato growing regions in The Netherlands. The scale of DI ranges from 0 to 4, with 0 = no blight observed or reported; 1 = late blight reported in a very limited number of fields in only a part of the country, with on average very low disease levels per field; 2 = late blight reported in most or all regions in a limited number of fields, with on average low or intermediate disease levels per field; 3 = late blight reported in many fields in all regions, with on average intermediate disease levels per field; 4 = late blight reported in most fields in all regions, with on average high disease levels per field.

Agronomic data

Agronomic factors affecting late blight development include the host and disease control activities. This information is sparse and often difficult to find. Nevertheless, some data were obtained which are supposed to be indicative for the change in agricultural practices over the research period (Table 1). The total hectareage of arable farming, the number of

farms with arable farming and the hectareage of potatoes in The Netherlands indicate the cropping density and the scale at which potatoes have been grown over the past decades. The total amount of nitrogen (from organic manure and from fertilisers) applied per ha of agricultural crops was included because the level of fertilisation (especially nitrogen) affects development of plant diseases by increasing the susceptibility of crops (Van Bruggen, 1995). Data on organic agriculture were also included. Organic agriculture has been increasingly stimulated by the Dutch government (Anonymous, 1992b). Since no fungicides are used in organic potato crops, high levels of disease can occur in these fields which can act as large mid-season infection sources (Zwankhuizen *et al.*, 1998).

No information on the amounts of fungicides used by growers for the control of late blight was available, nor were experimental results on the relative efficacy of the fungicides used. Compounds commonly used were (Egan *et al.*, 1995; anonymous sources of the Agricultural Advisory Service): copper compounds (1950s), dithiocarbamates and triphenyltin compounds (1960s), various combinations of dithiocarbamates and triphenyltin compounds (1970s), various combinations of dithiocarbamates and triphenyltin compounds and cyanoacetamide-oximes and phenylamides (metalaxyl) (1980s). In the 1990s, some new compounds became available, of which fluazinam (a pyridineamine) is frequently used. Considering the use of these compounds in the successive decades, the efficacy of the control by fungicides was rated as shown in Table 1, according to 'expert judgement' by H. Schepers (H. Schepers, *pers. com.*). The number of sprayers was also included.

Meteorological data

Meteorological data collected by the Royal Dutch Meteorological Institute (KNMI) at De Bilt (located in the centre of The Netherlands) were used. The variables used in this study were divided into 4 categories (Table 2): those affecting the formation of inoculum in the year prior to the year studied (A), those affecting the overwintering of inoculum and the development of disease on refuse piles (B) and those enhancing (C) and limiting (D) disease development during the growing season.

A. Formation of inoculum. Variables belonging to this category were the number of precipitation days and the total amount of precipitation (mm) during August through October. In this period, potato tubers can be infected by spores, washed down by rain from diseased foliage, resulting in diseased seed tubers and diseased tubers on refuse piles. (Van der Zaag, 1956). Of course, the disease level in the previous growing season (DIp), which is a major variable determining the formation of inoculum, was used in the analyses.

B. Overwintering of inoculum. Total precipitation (mm) during the period between two successive cropping seasons (November through April) was included because, supposedly, a high amount of winter precipitation will lead to decreased numbers of infected tubers surviving the winter period (either groundkeepers or tubers discarded on refuse piles) due to rotting. The Hellmann value (Hellmann, 1917), calculated as the

Table 1. Agronomic data (The Netherlands, 1950 to 1996).

Period	Hectareage of arable farming (x 10^5)[1,2]	No. of farms with arable farming (x 10^3)[1]	Hectareage of potatoes (x 10^3)[3]	Nitrogen application (kg N/ha)[1,4]	Efficacy of fungicides[5]	No. of sprayers (x 10^3)[1,6]
1950-1960	9.1	200	156	190	1	6
1960-1970	7.9	150	151	315	1.2	11
1970-1980	7.0	110	163	444	1.4	19
1980-1990	7.5	88	174	442	1.4 (1.9)[7]	24
1990-1996	8.0	80	180	403	1.4 (1.9)[7]	-[8]

1 Source: Statistics Netherlands (Centraal Bureau voor de Statistiek).

2 The total acreage with organic agriculture and horticulture in The Netherlands increased from 2724 ha in 1986 to 12789 ha in 1995. Approximately 43 % of this area is grown with arable crops and grasses (Anonymous, 1996).

3 Dutch Descriptive List of Varieties of Field Crops (1955, 1968, 1991, 1995). The percentage of the total hectareage planted with very susceptible cultivars (susceptibility figures according to the Descriptive List between 2 and 5; a high value means low susceptibility) was always more than 50 % of the total hectareage in the ware and seed potato regions in The Netherlands. The levels of susceptibility of the cultivars planted in the starch potato production area (in the north-east of the Netherlands) generally have higher susceptibility figures (4 - 7).

4 Total amount of nitrogen (organic manure and fertiliser) applied per ha of agricultural crops.

5 See text for details.

6 The percentage of sprayers owned by contractors decreased from 16 % in the 1950s to 4 % in the 1990s.

7 Efficacy 1.9 in case of no resistance of the fungus against metalaxyl.

8 No data available.

negative sum of the average daily temperatures below 0 °C from November through March, was included as a measure for the coldness of the winter. The number of hours with temperature ≤ -2 °C at 5 cm below the soil surface, from November through April, was used to indicate the freezing of potato tubers, and therewith the disappearance of tuber-borne inoculum. Potato tubers will freeze when they are exposed to 50 frost-degree hours, with a threshold temperature of -2 °C (Lumkes, 1974). A variable which might indicate the

Table 2. Meteorological variables included in this study. Four categories of variables were distinguished: A = affecting the formation of overwintering inoculum, B = affecting the overwintering of inoculum and the development of disease on refuse piles, C = enhancing disease development during the growing season, D = limiting disease development during the growing season. Data were obtained from the Royal Netherlands Meteorological Institute (KNMI) at De Bilt (The Netherlands) [1]. See text for details.

Variable category	Variable	Description	Unit
A	DPRh	No. of days with precipitation (≥ 0.1 mm) during harvest, August - October (3 months)	d
	APRh	Amount of precipitation during harvest, August - October	mm
B	APRw	Amount of precipitation during winter, November - April	mm
	HLL	Hellmann value (Σ average daily temperatures < 0 °C, November - March	-
	FDH	Hours with temperature ≤ -2 °C at - 5 cm, November - April	frost-degree hours
	TS	Temperature sum of average daily temperature > 0 °C at - 5 cm, January - May	degree-days
C	DPRg	No. of days with precipitation, May - September, per month or over total period	d
	APRg	Amount of precipitation, May - September, per month or over total period	mm
	HF	No. of hours with favourable conditions (10 °C ≤ T_h ≤ 27 °C and RLH_h ≥ 90 %)[2], May - September, per month or over total period	h
D	GR	Total global radiation, May - September, per month or over total period	J/cm^2
	HU	No. of hours with unfavourable conditions (T_h ≥ 27 °C)[2], May - September, per month or over total period	

1 Meteorological variables measured at 1.5 m height, unless stated otherwise.

2 T_h (hourly temperature), RLH_h (hourly relative humidity).

number of potato plants on refuse piles and volunteer plants in the field is the soil temperature sum on May 31. The temperature sum, reflecting the warming-up status of the soil prior to and during the beginning of the growing season, was calculated as the sum of the average daily temperatures (≥ 0 °C) at 5 cm below the soil surface, from January through May.

C. Favouring disease development. Variables enhancing the development of late blight during the growing season (taken from May through September) were the total and monthly numbers of days with precipitation, the total and monthly amounts of precipitation, and the total and monthly numbers of hours with favourable conditions for late blight development (Harrison, 1992). Hours favourable for late blight development were those hours when the temperature and the relative humidity met the following criteria: 10 °C ≤ T_h ≤ 27 °C and RLH_h ≥ 90 %, where T_h is the hourly temperature and RLH_h is the hourly relative humidity.

D. Limiting disease development. The total and monthly numbers of hours with unfavourable temperature (> 27 °C) and the total and monthly sum of the total global radiation from May through September were thought to be indicative for constraints to epidemic development of the disease.

Data analysis

The response variable in this study is the disease index DI. To analyse the fluctuations in severity of late blight epidemics, consecutive years having a disease index differing by at least 2 scale points on the 0-4 scale were considered. For those years, the variables were compared with the variables of the preceding year and the following year. Groups of years were compared using Tukey's multiple-comparisons procedure and chi-square contingency table analysis (Sokal and Rohlf, 1981).

The relation between DI and each of the (weather) variables (totals) separately was analysed using chi-square analysis of contingency tables and Spearman's rank correlations. For the contingency table analysis, the independent variable was divided into 2 classes: low (with values ≤ median value) and high (with values > median value). The values of the dependent variable DI was grouped into 3 classes: (*i*) years with DI = 0, (*ii*) years with DI = 1 or 2, and (*iii*) years with DI = 3 or 4. In addition, the relation (linear correlation) between the current year's disease level with the disease level in the previous year was investigated.

Stepwise linear discriminant analysis, using Wilk's method of selecting variables (Backhaus *et al.*, 1994) was performed to identify the meteorological variables with the highest contribution to the prediction of late blight severity. Linear discriminant functions have the following form: $Y = b_0 + b_1X_1 + b_2X_2 + ... + b_nX_n$, where Y = grouping or discriminant variable (which is DI), X_j = predictor variable (j=1,2,...n), b_j = discriminant coefficient for variable X_j, and b_0 = a constant.

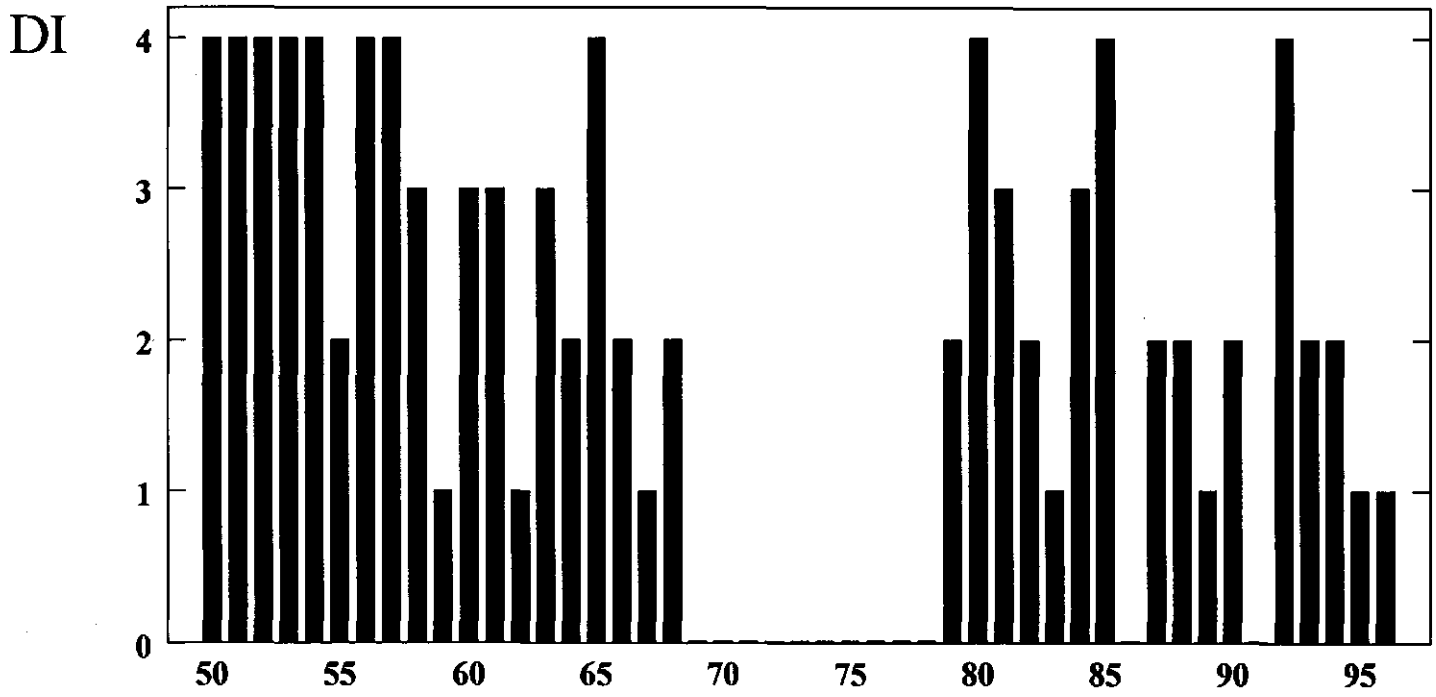

Figure 1. Severity of potato late blight epidemics in The Netherlands from 1950 through 1996. DI = disease index (see text). The periods 1950-1968, 1969-1978, and 1979-1996 were designated I, II, and III, respectively.

To estimate the effect of the blight status in the year prior to the current year, the presence (LBp = 1, *i.e.* for the years with DI = 1-4) or absence (LBp = 0, *i.e.* for the years with DI = 0) was included in the analysis. Discriminant analysis was carried out for several data sets. First, all meteorological variables (Table 2) were used as input for the analysis, for both the total values (for precipitation during the growing season, global radiation, hours favourable for late blight development and hours with unfavourable temperatures), and the monthly values. Each variable was tested for normality. Correlation between variables was computed using Spearman's rank correlations. After exclusion of variables with non-normal distributions, and of those with a significant ($P < 0.05$) correlation > 0.50 with the highest correlating variables, discriminant analysis was repeated.

Results

Late blight in The Netherlands from 1950 - 1996

The severity of late blight epidemics, expressed by the disease index (DI), over the years 1950 through 1996 is shown in Fig. 1. The values of the disease index assigned to the years under study were verified by interviews with several people who had been involved in late blight research and in the agricultural advisory service in The Netherlands in the past, and by comparing newspaper reports and (anonymous and mostly unpublished) reports of 6 experimental farms distributed over the country, covering the entire research period or a part of it. Late blight severity shows a fluctuating pattern and the severity of the epidemics generally changed from year to year. Two patterns can be identified: a pattern of year-to-year fluctuation and another pattern, superimposed on the yearly pattern, consisting of three different periods: 1950 through 1968 (period I), 1969 through 1978 (period II) and 1979 through 1996 (period III). Most remarkable is period II. Late blight was practically absent for 10 consecutive years.

Table 1 shows some agronomic data. The hectareage of potatoes has increased over time (15 %), whereas the number of farms with arable crops has decreased by more than 50 % during the same period. So the area of potatoes per farm has increased over time (approximately 3-fold). The input of nitrogen increased 2-fold. Over the entire period the effectiveness of the control of late blight by fungicide spraying has substantially increased. The efficacy of fungicides has increased by at least 40 %, and the number of sprayers owned by growers in the 1990s was 4-fold the numbers in the 1950s. Supposedly, timeliness of fungicide treatment also increased.

Meteorological data for the years under study are summarised in Table 3. In 30 out of 47 (64 %) winter periods, no hours with temperature ≤ - 2 °C (FDH) were recorded. The number of hours with temperature > 27 °C was low in several years, and even 0 in the growing seasons of 1962, 1965, and 1974.

Table 3. Severity of late blight epidemics (DI) and values (over the total periods) of the meteorological variables, The Netherlands, 1950 - 1996. See Table 2 and text for description of variables.

Year	DI	DPRh	APRh	APRw	HLL	FDH	TS	DPRg	APRg	HF	GR	HU
1950	4	63	243	474	53	184	1137	95	442	-	232301	-
1951	4	51	197	539	62	0	976	84	312	1054	232772	3
1952	4	60	229	393	20	0	1085	80	323	923	237056	34
1953	4	44	176	346	70	0	987	73	368	956	239696	44
1954	4	65	298	218	113	1032	900	99	444	477	209342	17
1955	2	56	237	281	85	59	766	77	282	1114	235222	17
1956	4	63	247	293	211	1515	853	91	388	680	205692	1
1957	4	71	409	300	19	0	1156	94	565	929	224571	63
1958	3	62	241	367	50	0	934	88	379	1245	219616	3
1959	1	32	99	352	52	0	1089	50	123	560	273648	83
1960	3	71	357	287	42	0	1106	93	372	1063	212812	3
1961	3	61	234	465	25	0	1209	89	358	1188	221149	33
1962	1	53	208	480	83	93	856	86	282	501	231400	0
1963	3	62	325	275	346	4169	758	85	446	1025	214887	8
1964	2	55	284	256	109	42	960	77	331	738	240042	36
1965	4	64	211	440	60	0	971	104	535	897	223919	0
1966	2	51	191	611	99	48	1073	82	496	771	227784	29
1967	1	68	271	508	30	0	1107	90	304	853	236924	23
1968	2	61	319	435	45	0	989	92	515	945	206658	17
1969	0	54	231	293	86	0	959	78	383	655	232557	87
1970	0	48	217	443	131	49	847	66	326	673	244536	29
1971	0	38	84	324	101	51	1038	69	271	776	240924	23
1972	0	37	133	272	40	0	1041	84	349	787	219123	28
1973	0	47	242	297	24	0	972	71	348	669	250247	55
1974	0	62	361	304	24	0	1138	78	442	1018	232866	0
1975	0	38	119	485	3	0	1060	55	251	752	258696	103
1976	0	38	123	295	73	0	1057	54	201	732	261452	197
1977	0	44	161	396	19	0	1047	73	269	1012	216915	9
1978	0	46	137	431	44	19	999	70	253	706	215924	27
1979	2	41	128	431	206	0	757	75	332	764	218900	5
1980	4	46	190	457	47	0	-	75	357	872	222674	17
1981	3	47	270	468	40	0	1135	77	348	896	220329	21
1982	2	52	187	305	127	453	983	71	230	744	250723	101
1983	1	46	196	479	24	0	1055	69	345	827	234529	106
1984	3	49	273	476	36	0	885	85	383	1010	202865	42
1985	4	51	167	249	194	90	876	85	357	1072	217606	28
1986	0	38	257	411	149	273	819	60	264	610	250695	59
1987	2	50	251	420	152	615	822	88	497	922	210720	13
1988	2	42	212	538	13	0	1202	75	366	1003	211157	2
1989	1	49	214	405	2	0	1280	58	234	642	262448	60
1990	2	44	199	394	8	0	1380	59	269	685	239995	49
1991	0	36	130	301	77	0	-	70	320	780	221259	61
1992	4	54	358	429	34	0	1166	67	404	764	250823	57

1993	2	51	219	388	41	42	1212	79	436	1109	214534	10
1994	2	51	400	528	63	0	1102	67	448	1145	230505	135
1995	1	37	145	512	22	0	1207	66	348	1068	249651	169
1996	1	45	184	181	151	349	837	67	239	898	221361	45
Median	2	51	217	396	52	0	1038	77	348	872	23505	29

Analysis of specific years and periods

In general, a decrease of DI from one year to the next corresponded with a decrease of the number of days or the amount of precipitation (DPRg and APRg, respectively) and the number of hours favourable for late blight (HF), and with an increase of the global radiation (GR) and the number of unfavourable hours (HU) (Table 4). On average, the proportional change (+/-) of these variables was 23, 43, 44, 11 and 244 %, respectively, for the years which differed by at least 2 DI points. Exceptions were the transitions from 1954 to 1955 (decrease of DI, increase of HF), 1955 to 1956 (increase of DI, decrease of HF) and 1990 to 1991 (decrease of DI, increase of DPRg, APRg, and HF). High amounts of precipitation in the end of the growing seasons might be responsible for the exception, *e.g.* in 1955.

The change in the meteorological variables (DPRg, APRg, HF, GR, HU) corresponded with the change in DI for the transition years from period I to period II (1986, 1969) and for II to III (1978, 1979). The weather conditions were apparently unfavourable for late blight development in 1969 when compared to 1968. Weather conditions were continuously less favourable for late blight development in period II following the transition 1968 - 1969, according to the mean values of the variables in Table 4. Only DPRg and DPRh were significantly lower in period II and III than in period I (Table 4).

Relation between late blight severity and individual variables

Chi-square analyses of 2 x 3 contingency tables (categorial variables x DI groups) and Spearman's rank correlations, applied to the same data sets, show a positive relation between DI and DIp (the disease index in the previous season), DPRg, APRg, and to a lesser extent for HF (Table 5). DI appears to be negatively correlated with GR, but the chi-square analysis revealed no association between DI and GR category.

Table 4. Analysis of the fluctuation of the severity of the late blight epidemics for individual years and for periods I, II, and III. The individual years differed ≥ 2 on the 0-4 scale of the disease index DI. Each column shows the proportional change (%) of the respective variable [1] (except for DI) at the transition from one year to the next. For the periods I, II, and III, the mean values are shown.

Transition	DI	DPRh	APRh	APRw	HLL	FDH	TS	DPRg	APRg	HF	GR	HU
1954-1955	-2	48	69	29	-24	-94	-15	-22	-37	134	12	0
1955-1956	2	-14	-21	4	147	2472	11	18	38	-39	-13	-94
1958-1959	-2	-13	-41	-4	5	0	17	-43	-68	-55	25	2667
1959-1960	2	-48	-59	-18	-19	0	2	86	203	90	-22	-96
1961-1962	-2	-14	-35	3	234	0	-29	-3	-21	-58	5	-100
1962-1963	2	-13	-11	-43	316	4363	-11	-1	58	105	-7	0
1964-1965	2	-11	-13	72	-45	-100	1	35	62	22	-7	-100
1965-1966	-2	16	-26	39	67	0	10	-21	-7	-14	2	0
1968-1969	-2	-10	18	-33	89	0	-3	-15	-26	-31	13	412
1978-1979	2	5	-15	0	363	-100	-24	7	31	8	1	-81
1979-1980	2	-11	-6	6	-77	0	-	0	7	14	2	240
1983-1984	2	-12	5	-1	52	0	-16	23	11	22	-14	-60
1985-1986	-4	4	-39	65	-23	204	-7	-29	-26	-43	15	111
1986-1987	2	-25	54	2	1	125	0	47	88	51	-16	-78
1990-1991	-2	-10	-7	-24	820	0	-	19	19	14	-8	24
1991-1992	4	-18	-35	43	-57	0	-	-4	26	-2	13	-7
1992-1993	2	50	175	-9	23	0	4	18	8	45	-14	-82

Mean values for the three periods[2]

Period I	2.9	58	247	385	83	376	995	86	382	900	227657	25
Period II	0	47	199	354	54	12	1016	70	309	778	237324	56
Period III	2	46	218	409	77	101	1045	72	343	878	229487	54

[1] See Table 2 for description of the variables.

[2] Analysis using Tukey's multiple-comparisons test and chi-square analysis of contingency tables revealed no significant differences between the mean values of the three periods, except for DPRg (I significantly higher than II and III, $P < 0.001$) and for DPRh (I significantly higher than II and III, $P < 0.001$).

Discriminant analysis

Preliminary discriminant analysis showed that DPRg (number of precipitation days during the growing season) gave slightly better results than APRg (amount of precipitation during the growing season) and therefore, we continued the analyses using DPRg. Four categories of analyses were carried out, indicated as (*i*) yearly+, (*ii*) yearly-, (*iii*) monthly+, and (*iv*) monthly- (Table 6). In the first and third category, all meteorological variables (with total and monthly values for DPRg, HF, GR, and HU, respectively) were used, including LBp, the blight status of the previous year. In the second and fourth category, variables HLL, FDH, GR, and HU were excluded from the analysis, because of their correlations with other variables. HLL and FDH were correlated with TS (r = -0.74 and r = 0.75, respectively) and GR and HU were correlated with DPRg (r = -0.75 and r = -0.61, respectively).

Discriminant analysis using monthly values for the specified meteorological variables gave the best results, in terms of the number of years correctly classified (Table 6). For both the + and - analyses, the number of correctly classified years was at least 10 higher. Monthly+ analysis resulted in 40 correctly classified years out of 46 years. The years not correctly classified were 1956 (period I), 1980, 1982, 1986, 1991, 1992 (period III). For the years 1956, 1980, 1982, and 1992, the estimated DI was lower than the actual DI, for 1986 and 1991, the model overestimated the DI. For the other discriminant analyses (for yearly+, yearly-, monthly-), the majority of years not correctly classified also belonged to period III. Most important discriminating variables were LBp (variable category A), DPRg, and HF (both variable category C). These variables appear in all classification functions and have the highest standardised discriminant coefficients. The yearly- and monthly- analyses, with correlated variables excluded, resulted in substantially lower numbers of correctly classified years.

The variables HLL, FDH and HU and DIp had non-normal distributions. After logarithmic transformation, variables HLL and HU were normally distributed but the result of the discriminant analyses was slightly worse when the log-transformed values were included in the yearly and monthly analyses (results not shown). Excluding LBp from the analyses also gave worse results, *e.g.* the number of correctly classified years for the yearly analyses was 24 instead of 27.

Discussion

Levels of aggregation

Severity of late blight epidemics was characterised by the disease index (DI) on a 0-4 scale, covering epidemic development in The Netherlands as a whole. Although our indexing considered regional differences, the country-wide aggregation of disease severity levels is

Table 5. Relation between DI and DI in the previous year (DIp) and meteorological variables, using chi-square analysis and Spearman's rank correlations (r)[1,2].

Variable ($N = 47$)[3]	2 (rows)[4] x 3 (columns)[5] contingency tables				Chi-square P	Spearman r	P
		c1	c2	c3			
DIp	r1	9	5	4	0.012	0.53	< 0.001
	r2	3	13	12			
DPRg	r1	9	12	4	0.008	0.57	< 0.001
	r2	3	6	13			
APRg	r1	9	12	3	0.002	0.52	< 0.001
	r2	3	6	14			
HF	r1	10	10	3	0.003	0.37	0.01
	r2	2	8	13			
GR	r1	4	8	12	0.11	-0.38	< 0.01
	r2	8	10	5			

1 See Table 2 for description of the variables.

2 The relation between DI and DPRh, APRh, APRw, HLL, FDH, TS, HU, was not significant for both chi-square and correlation analyses.

3 $N = 46$ for DIp, HF, HU, DPRh, APRh; $N = 45$ for TS.

4 Rows represent the two classes of the independent variable. Low (r1) = values ≤ median value, high (r2) = with values > median value.

5 Columns represent three classes of disease severity. Low (c1): DI = 0, intermediate (c2): DI = 1 or 2, high (c3): DI = 3 or 4.

certainly open to criticism. An important objection concerns the differences between potato growing regions in The Netherlands. Major differences exist between the starch potato growing area on reclaimed peat soil in the north-east of the country and the other ware and seed potato growing areas on the northern, central and south-western marine clay soils. However, the scarcity of detailed data on severity of late blight epidemics enforced us to use a rough estimate of the disease level per year. The fact that the disease re-occurred at relatively high intensity at the transition from period II to III suggests that late blight might have been present at very low levels. These levels were so low that this important disease was not reported by the Agricultual Advisory Service and the Plant Protection Service.

Table 6. Coefficients of the linear classification functions of the discriminant analysis and the classification results. Results are shown for four analyses: yearly+, yearly-, monthly+, monthly-. For explanation see text.

Variable	Variable category[1]	DI					No. of years correctly classified (%)
Yearly+							27 (57.5)
		0	1	2	3	4	
LBp	A	-8.867	-1.271	-2.798	-1.211	-2.666	
DPRg	C	3.441	3.46	3.439	3.514	3.621	
GR	D	0.003	0.003	0.003	0.003	0.003	
HF	C	0.051	0.052	0.056	0.061	0.055	
constant		-477.917	-492.043	-472.765	-485.671	-507.081	
Yearly -							24 (51.1)
LBp	A	0.578	8.28	6.479	8.064	6.91	
DPRg	C	0.591	0.577	0.639	0.715	0.731	
HF	C	0.024	0.024	0.029	0.034	0.027	
constant		-31.019	-34.98	-41.638	-54.728	-47.651	
Monthly+							40 (87.0)[2]
LBp	A	-4.548	13.196	5.711	10.095	7.209	
APRw	B	0.104	0.142	0.122	0.146	0.132	
HLL	B	0.142	0.174	0.204	0.157	0.193	
FDH	B	0	-0.002	-0.004	0.004	-0.002	
DPRjune	C	6.577	6.684	6.291	7.078	6.942	
DPRseptember	C	2.233	2.557	2.72	2.482	2.967	
GRmay	D	0.003	0.002	0.003	0.003	0.003	

GR*june*	D	0.004	0.004	0.004	0.004	0.004	
GRjuly	D	-1.4E-05	8.2E-05	4.0E-05	-0.000591	-0.000254	
HFmay	C	0.378	0.308	0.339	0.447	0.389	
HFaugust	C	0.146	0.18	0.166	0.212	0.194	
HFseptember	C	-0.029	-0.036	-0.016	-0.005	-0.037	
HUmay	D	0.626	0.633	0.641	1.075	1.053	
HUseptember	D	1.209	2.77	2.105	3.489	2.529	
DPRaugustP[3]	A	-0.109	-0.865	-0.106	-0.544	-0.296	
DPRseptemberP	A	-0.12	-0.744	-0.397	-0.718	-0.383	
constant		-290.852	-310.968	-294.135	-322.189	-327.84	
Monthly -							34 (74.0)[2]
LBp	A	-4.903	8.945	4.708	8.008	5.271	
APRw	B	0.031	0.052	0.039	0.037	0.041	
DPRjuly	C	0.137	0.087	0.344	0.896	0.292	
DPRaugust	C	1.401	1.415	1.461	1.196	1.771	
DPRseptember	C	1.092	1.132	1.044	0.773	1.437	
HFmay	C	0.069	0.018	0.04	0.08	0.04	
HFjune	C	0.075	0.067	0.126	0.119	0.113	
HFjuly	C	0.036	0.04	0.021	-0.022	0.035	
HFaugust	C	-0.014	0.011	-0.015	0.031	-0.009	
HFseptember	C	-0.025	-0.027	-0.015	0.004	-0.037	
DPRaugustP	A	0.477	0.052	0.507	0.533	0.285	
DPRseptemberP	A	0.756	0.354	0.542	0.251	0.655	
constant		-41.689	-46.305	-53.832	-63.01	-61.777	

1 See Table 2 for description of variables. LBp is the blight status of the previous year (1 = late blight, 0 = no late blight).

2 For these analyses, data of 46 years were used because of missing values.

3 P indicates precipitation in the previous year at harvest in August or September.

The agronomic data were also at the country-wide level. Data relevant for this study were scarce and only very general data were presented. Meteorological variables collected at De Bilt in the centre of The Netherlands were used in the country-wide analysis. Although the weather conditions can vary between the different growing regions within the season, we estimate the effect on the results of our study to be limited because the variation in weather data among years was much higher than the variation in weather among the regions within the years (Anonymous, 1949 -1987).

The effect of weather conditions on the severity of late blight epidemics was analysed by discriminant analysis, using meteorological variables at the seasonal level and at the monthly level (for some variables). Analysis with the monthly values gave substantially better classification results. Other studies also show that interesting relations can be found between the level of disease and certain mean monthly weather variables (Daamen *et al.*, 1992; Johnson *et al.*, 1996). However, the interpretation of such relations and their usefulness for disease management purposes is less clear (Zadoks, 1994).

Polyetic patterns of late blight development

Two superimposed patterns of late blight development may be discerned. A short-term pattern, with annual fluctuations of the severity of late blight epidemics, and a long-term pattern, with two periods of mostly moderately to severe disease levels, separated by a period practically without late blight. Similar patterns have been described in the literature. Late blight disease was absent between 1942 and the 1980s in the Red River Valley in North-Dakota (USA) (Secor *et al.*, 1995). In south-central Washington (USA), late blight was first identified in 1947 and was reported again in 1974 (Johnson *et al.*, 1996). Between 1974 and 1994, periods of consecutive years with late blight presence alternated with periods of consecutive years in which no late blight was recorded. The study of Croxall and Smith (1976) shows yearly fluctuations of the late blight level in the East Midlands, UK, from 1923 to 1967. This period was followed by a period of growing seasons with minor late blight development from 1968 to 1974, the onset of this period being the same as for period II in The Netherlands. Kluge and Gutsche (1985) found no period of absence of late blight in Eastern Germany from 1959 through 1982.

The range of years studied in this chapter does not allow us to discriminate between a multi-year pattern, consisting of periods of consecutive years with relatively low levels of disease alternated with periods of years with high levels, and the hypothesis that late blight has been almost continuously present at significant high levels since the introduction of *P. infestans* in The Netherlands in the 1840s, with only one gap between 1969 and 1979. However, interviews with several people who were involved in late blight research and control in the past and unpublished reports of experimental farms indicate that there have been periods of consecutive years with minor late blight development. Long-term studies with rusts of wheat (Nagarajan and Joshi, 1975; Coakley *et al.*, 1988) and potato viruses

(Bagnall, 1988; 1992) also show alternating periods. The epidemics of potato leaf roll virus seemed to be linked to drought and sunspot cycles (Bagnall, 1988).

The fluctuations in late blight severity and the (practical) absence of the disease in some years have significant consequences for the genetic composition of the *P. infestans* populations (Zwankhuizen et al., *subm.(a;b)*) due to population processes like selection, genetic drift, and gene flow.

Factors affecting polyetic late blight development

All statistical analyses revealed that the meteorological variables precipitation during the growing season (number of days; DPRg) and the number of hours with favourable temperature and relative humidity during the growing season (HF) were closely related with late blight severity, as expressed by DI. These variables belonged to the category of variables enhancing disease development during the growing season (category C). The presence (and level) of late blight in the previous year, indicated as LBp and DIp, respectively, were important variables, which belonged to category A. A high level of late blight in the previous year will affect late blight development in the next year via the infection and survival in potato tubers, providing inoculum for the next season. Johnson *et al.* (1996) also found monthly precipitations and the blight status of the previous year to be the most important ones. Other variables in category B (such as the precipitation during the winter period) and D (variables limiting late blight development) appeared to be less important.

The fact that some variables did not show a significant relation with DI does not mean that these variables are unimportant. Such variables might contribute significantly in specific years. Many factors may interact and the effect of each variable in these complex interactions is unknown. Especially, the effect of the agronomic variables and the influence of man (see below) is not well understood. For example, from the 1950s to the 1990s, the number of farms decreased but the hectareage of potatoes increased. This might have had a positive effect on the development of the disease because of the larger fields with susceptible crops (monocultures) and a narrowing of the crop rotations. However, the scaling-up of the potato growing may also have had a negative effect on late blight. Potatoes are nowadays grown by well-educated professionals and many of the growers have their own sprayer. Also the effect of the host, through susceptibility and input of fertiliser, did not seem evident in this study but this might have arisen by a lack of data rather than a lack of causality.

Analysis of the specific periods and years generally showed that major changes in late blight development corresponded with changes of the meteorological variables. Not all changes were statistically significant when compared for periods I to III. Only the number of days with precipitation was significantly higher in period I than in periods II and III. The combination of all the factors might have had such an impact that late blight disappeared

Polyetic studies of plant disease epidemics allow us to infer key factors which determine the overwintering and the development of plant diseases during the growing season. The results of long-term studies might help to interpret the results of short-term experiments and may provide information for policy makers. A Multi-Year Crop Protection Plan has been introduced in The Netherlands (Anonymous, 1991). This plan aims at a reduction of the volume of pesticides used within a certain period of time. However, such targets might be not achievable in certain periods of years with weather conditions favourable to late blight development. Implementation of such plans, with high impact for growers, without considering the long-term 'behaviour' of pests and diseases will result in a conflict between growers, whose priority is a healthy, marketable crop, on one hand, and on the other policy-makers, whose priority is achieving their planning targets, and consumers, who want a product which is produced in a perceived environmentally safe manner. Long-term studies require adequate documentation of disease data, based on epidemiological observations and genetic studies, and relevant agronomic data, to be collected by the actors in the area of crop protection.

Chapter 6

General discussion

The shifty appearance and nature of the late blight pathogen Phytophthora infestans

Stakman (1947) excellently qualified plant diseases as 'shifty enemies'. A good example of such a shifty enemy of plants is potato late blight. Since the first dramatic appearance of potato late blight in Europe in 1845, potato growers have been experiencing *Phytophthora infestans* as 'the devastating plant destroyer'. Although fungicides for chemical control have been available since the late 1880s (Egan *et al.*, 1995), growers always have to be alert to protect their crops against destruction by the disease. The late blight pathogen appears to be shifty both in its appearance and in its nature.

With respect to its shifty appearance, the disease may occur for several consecutive years, but may also disappear for a period of years, as seen in The Netherlands between 1969 and 1978 (Anonymous, 1949-1987). After such a period of near-absence, the disease suddenly can re-appear at alarming high levels (Chapter 5).

The shifty nature of the late blight pathogen is clearly illustrated by changes in genotypic composition of *P. infestans* populations and by the alternating importance of different inoculum sources. Changes in race composition of the old *P. infestans* populations due to the use of cultivars with R-gene resistance are well-known (Goodwin, 1997). Major population changes in Europe and the rest of the world have been observed since the 1980s (Fry and Goodwin, 1997), dwarfing the changes in race spectrum of *P. infestans* populations observed earlier. The global population changes, as revealed by characterisation of isolates with biochemical and molecular markers, appear to be crucial for potato late blight development in the future (Fry and Goodwin, 1997). *P. infestans* populations appeared to be highly diverse in space and time at the small scale (Chapter 3 and 4).

The importance of infection sources, refuse piles and foci originating from infected seed tubers, may vary among regions and among years in The Netherlands (Van der Zaag, 1956; Lohuis *et al.*, 1967; Davidse *et al.*, 1968; 1989; Zwankhuizen *et al.*, 1998). Since the 1980s, oospores can serve as an important inoculum source in The Netherlands, in addition to tuber-borne inoculum. Previously, this inoculum source was exclusively present in the highlands of central Mexico, the only area where sexual reproduction was known to occur (Niederhauser, 1956; Gallegly and Galindo, 1958; Tooley *et al.*, 1985; Goodwin *et al.*, 1992b).

Overwintering and spread of P. infestans *in Southern Flevoland and adjacent areas*

Oospores vs. tuber-borne inoculum (Chapters 2, 3 and 4). Before the start of the research described in this thesis, the epidemiological evidence that oospores play a role in the development of late blight in The Netherlands was scarce and indirect. From the results described in this thesis, it can be concluded that in the present situation, oospores do play a role in the epidemiology of late blight in The Netherlands.

Indications for the importance of oospores as overwintering inoculum in allotment gardens come from an experiment using composted tomato waste from allotment gardens. It was shown that the waste material contained oospores which were infectious. Field observations on early disease foci and early diseased commercial potato fields sometimes suggest a role for oospores in the development of disease, *e.g.* in the absence of another distinct infection source. However, the results of the characterisation of isolates collected throughout the research area clearly demonstrated the importance of oospores in the development of late blight in several situations. In allotment gardens, the relatively high numbers of unique genotypes and the high levels of genotypic diversity suggest that oospores had initiated the disease in most of the plots. In commercial potato fields, oospores also play a role in the development of disease, but the relative importance was lower than in the allotment gardens. In fact, there was only one conventional field, infested early in the season in 1995, for which both the field observations and the results of the isolate characterisation point towards an obvious role for oospores as the initiating inoculum. No infection sources were found in this field nor in a vast area around it. Characterisation of 28 isolates revealed 10 unique or rare genotypes, with the A1 and A2 mating types present in a 1 : 1 ratio. The high numbers of unique genotypes found every year and the increasing overall level of genotypic diversity and genotypic distance clearly suggest that sexual reproduction occurred and that oospores were involved in the development of disease in commercial potato fields in the research area, even though the oospores could not be found.

The relatively high numbers of unique genotypes in the organic potato fields in 1996 and the apparent absence of infection sources outside those fields at the time of sampling suggest that the *P. infestans* isolates collected in those organic fields originated from oospores formed during the growing season. Initially, disease might have developed from infected seed tubers. After mating of a few genotypes, re-infection of the crop by germinated oospores could have generated the observed variation. The results of the study are in agreement with those of Drenth *et al.* (1993; 1995), Turkensteen *et al.* (1996), Sujkowski *et al.* (1994), and Goodwin *et al.* (1995), who found that sexual reproduction now also occurs outside central Mexico.

Tuber-borne inoculum was evident in refuse piles. No foci were found with diseased seed tubers. Most foci apparently had developed from asexual inoculum coming from infested refuse piles.

Relative importance of infection sources (Chapters 2, 3 and 4). Combined epidemiological and genotypic analyses identified infested refuse piles as the most important infection source for the development of late blight epidemics in 1994 and 1995. Approximately 70 % of the fields with early disease foci were located near an infested refuse pile. Genotyping of isolates collected from refuse piles and conventional and organic fields confirmed the association. The results are in agreement with studies from Bonde and Schultz (1943), Boyd (1974), Lohuis *et al.* (1967), and Davidse *et al.* (1968).

No foci with infected seed tubers were found. Isolate characterisations indicate that some genotypes might have survived in infected seed tubers and initiated disease foci in some cases, resulting in disease spread over the field. For other regions and other years, infected seed tubers were shown to be more important (Van der Zaag, 1956; Davidse *et al.*, 1989). No infected volunteer potato plants were found early in the season.

Disease gradient analysis and genotyping of isolates identified organic potato fields in 1994 as the major mid-season infection sources for the surrounding potato fields on an area of at least 25 km^2. In 1995 and 1996, the fields were potential infection sources, but no actual disease spread was observed due to weather conditions unfavourable to late blight development.

In 1994, the disease in allotment gardens was mainly initiated by inoculum coming from the potato fields. There are some sparse indications, derived from the isolate characterisations, that inoculum from allotment gardens also might have initiated disease in potato fields. The disease spread from the allotment gardens to the commercial potato fields was found to be limited. However, the effect on disease development in the commercial fields might have been substantial because of the possibly enhanced pathogenic fitness of the isolates which originated from the mainly sexual populations in the allotment gardens.

Populations of P. infestans *and their genotypic dynamics*

Genotyping of isolates allowed to identify populations and subpopulations (Chapters 3 and 4). Population substructuring is a very dynamic process. Population (sub)structure differed between years and was influenced by the epidemic development of the disease. In 1994, a year with weather relatively favourable to late blight development, the population was characterised by a relatively low level of diversity and a low level of genetic distance, whereas in 1996, a year with minor epidemic development, the genotypic diversity and genotypic distance were relatively high.

How the population structure will develop in the future cannot be inferred from this study. One can reasonably argue what the effect of *e.g.* genetic drift will be on the population structure of *P. infestans*, but it is unknown how all the population shaping processes together affect the structure of the population. More research at supra-regional level is needed to find out whether a meta-population model applies to the *P. infestans* populations in The Netherlands, or even across Europe (Andrivon, 1994). A meta-population structure would mean an additional dimension to the dynamic pattern of population development.

Epidemic development of potato late blight in perspective

Polyetic patterns of late blight epidemics. The long-term study on the severity of late blight epidemics in The Netherlands from 1950 through 1996 (Chapter 5) shows that the level of disease in the previous year, the number of days with precipitation during the growing season, and the number of hours with favourable temperature and relative humidity during the growing season were the most important factors determining the level of disease in any given year (Chapter 5). The results suggest the yearly fluctuation in severity to be part of a multi-year cycle. More than one and a half century after the first epidemic of late blight, the disease is still rather uncontrollable when the weather conditions are really favourable. The pattern of disease seems to become even more unpredictable, probably due to population changes which took place around the 1980s.

The effect of man. It is obvious that man has a substantial effect on the development of *P. infestans* epidemics. Many decisions taken by growers, from the choice of less susceptible cultivars to supervised application of fungicides via decision support systems, affect the severity of late blight in a certain year. However, the ultimate effect is largely unknown because actions taken by man interact with many known and unknown variables. In addition to this, people act according to their perceptions (Mumford, 1982) and these are not necessarily based on logical considerations. For example, during the surveys in Southern Flevoland, it was observed that many growers spent much time to control volunteer potato plants using a herbicide stick. Each time they went to their fields, they passed a refuse pile on their farmyard. The majority of the refuse piles were not covered of incompletely covered by plastic foil. Refuse piles were identified as the most important infection source (Chapter 2), whereas volunteer plants were not found to be very important for late blight epidemiology in this study. But apparently, growers perceived volunteer plants as the more hazardous (potential) infection source.

Generalisation of the results. Two major facts may reduce the generalisation value of our findings. First, the weather was rather adverse to late blight during the three-year research period, influencing the epidemic development and the population structure (Chapters 3 and 4). However, the low infection levels in 1995 and 1996 proved to be an advantage for this study. It allowed to link genotypic to epidemiological phenomena which would have been overruled by 'blanket infection' in years with severe late blight outbreaks due to favourable weather. During severe outbreaks, as seen in 1994, the earliest genotypes from refuse piles will rapidly predominate so that unique and rare genotypes, though still present, will hardly be detected by current sampling procedures. In conclusion, the substantial differences between the years and the minor disease development provided insight into the shifty nature and variable character of *P. infestans*, demonstrating the dangerous nature of this pathogen.

Second, the research was carried out in a relatively small area (150 km^2) in one of the polders in The Netherlands. Nevertheless, the results are generally applicable to the ware and seed potato growing areas, because potato growing practices are largely similar in

the potato growing areas on the marine clay soils in The Netherlands. For other regions, especially the starch potato growing region on reclaimed peat soils in the north-east of The Netherlands, the relative importance of inoculum sources and infection sources might be different, because of different potato growing practices, including soil type, crop rotation, intensity of fungicide applications, and cultivars.

Phytophthora infestans*: a threatening and challenging pathogen*

Potato growing and P. infestans *in the future.* Potato late blight is still the most important disease on potatoes (Hooker, 1981). *P. infestans* is able to destroy an apparently healthy crop within two weeks time. The threat of this fungus will increase in the future, because oospores will gradually become a significant inoculum source in commercial potato growing areas. Massive infections originating from oospores can lead to numerous outbreaks of a potentially more aggressive disease. A wide-spread presence of infectious oospores in soils will evidently increase the importance of volunteer plants as (primary) infection sources. Moreover, in-the-green-crop sexual reproduction and re-infection by germinated oospores may create even more diverse populations. The results of the research described in this thesis demonstrate that organic potato fields can act as large infection sources. Since the area of organic agriculture is likely to increase in the near future, more problems due to severe mid-season disease outbreaks can be expected.

Future research. Shaw (1983) called the *Peronosporales*, the taxonomic order which includes *P. infestans*, 'a fungal geneticist's nightmare'. Historical and present studies show that *P. infestans* is not only a nightmare for fungal geneticists. *P. infestans* is also a nightmare for epidemiologists and statisticians. The pathogen challenges sampling protocols and statistical methodology because the development of disease is extremely dependent on weather conditions and the fungus often does not occur in those sites intended to be sampled. Spatial and temporal dependency of data is inevitable. An *a priori* survey protocol, statistically well-designed, often cannot be implemented. Notwithstanding these objections, the results of this study show that field-oriented research is needed to further understand the consequences of the population displacements of *P. infestans*. Field-oriented research needs to be complemented by well-designed experiments to statistically test the hypotheses generated by the field studies.

Epidemiological experiments are needed to study *i.a.* the longevity of oospores in different soil types, the effect of crop rotation, and the possibly increased pathogenic fitness of the *P. infestans* population (Day and Shattock, 1997; Kato *et al.*, 1997). The results need to be incorporated in decision support systems. Genetic experiments using selection-neutral and selected markers are needed to quantify the population shaping processes, among which are selection and mating system (McDonald, 1997; Milgroom and Fry, 1997).

Epidemiological and population studies, based on large-scale sampling of potato growing regions, in combination with detailed sampling of fields, should address the question how the population structure evolves and how the importance of sexual reproduction and oospores develops over time relative to other infection sources.

Policy and educational aspects. The appearance of a 'new' *P. infestans* population and the apparent increase of the disease pressure due to organic potato growing also challenges policy makers. The increased risk of severe damage by a potentially more hazardous disease needs consolidation of existing regulations as well as new regulations. The regulation concerning the control of refuse piles (Anonymous, 1969) needs much more attention, especially with regard to the enforcement of the regulation, although the fact that refuse piles need to be eliminated or covered has been known for more than 30 years already. The longer an epidemic is retarded, the lower the probability that sexual reproduction can occur and oospores can be formed. The fact that high levels of disease can occur in organic potato crops needs additional regulation of the timely destruction of diseased foliage. Regulation should consider the levels of disease which can be tolerated with respect to the spread of the disease, as well as the risk of tuber infection. New regulation with regard to crop rotation may be necessary before oospores become a common inoculum source in many areas.

The apparent population shift of the pathogen needs to be followed by a shift of attitude of growers and pesticide suppliers with respect to late blight. Many growers are not aware of the details of the issues regarding the new late blight situation. Currently, the only questions many growers ask are: 'Which chemical compound do I have to apply and in which amount?' Growers will increasingly be confronted with many other questions, related to prevention (control of refuse piles, volunteer plants, possible role of oospores, choice of cultivar, crop rotation, fertilisation) and control (time and way of application of fungicides) of late blight. Consequently, the contents of crop protection courses and study programs of agricultural schools have to be critically evaluated.

Concluding remarks

Epidemiological and molecular-genetic analyses revealed that infested refuse piles were the most important infection source for the development of late blight epidemics in Southern Flevoland in 1994 and 1995. It was demonstrated that oospores play a role in the development of late blight in allotment gardens and, to a minor extent, in commercial potato crops. Infested organic potato crops served as important mid-season infection sources. A long-term study on the polyetic development of late blight epidemics in The Netherlands from 1950 through 1996 identified a multi-year pattern. The disease level in the previous year and the number of days with precipitation during the growing season were the most important factors determining the current year's disease level.

This study shows that *P. infestans* will continue to be a nightmare for potato growers. The disease pressure will probably increase in the future, due to the presence of oospores and the increasing hectareage of organically grown potatoes. Both issues need additional or new regulation. More research is needed to further understand the epidemiology and population structure of *P. infestans*. Fungicides will remain indispensable for the control of late blight, especially if the level of resistance of the cultivars used in the future is not substantially increased.

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Summary

Chapter 1

Late blight is the most devastating fungal disease of potatoes. The causal agent *Phytophthora infestans* was first introduced in Europe in the mid-nineteenth century. The diseased caused the well-known Irish potato famine. At that time, only A1 mating type isolates were present, and hence, the pathogen could only propagate by asexual reproduction. Analyses of *P. infestans* populations revealed that around 1980, 'new' *P. infestans* isolates had been introduced in The Netherlands. In the 'new' populations, isolates with the A2 mating type were detected. As a result of the presence of both mating types, the pathogen can now reproduce sexually and form oospores. These oospores can overwinter in the absence of the host and can serve as soil-borne inoculum. The presence of soil-borne inoculum additional to tuber-borne inoculum may affect the epidemiology of the disease. Another factor which influences the epidemiology is organic agriculture. The presence of high levels of disease in unsprayed organic potato crops may substantially affect the overwintering and spread of the pathogen. Three major research questions were addressed in this thesis: (*i*) What is the importance of the oospores as overwintering inoculum relative to tuber-borne inoculum? (*ii*) What is the relative impact of the various infection sources on epidemic development of *P. infestans*? (*iii*) What are the key factors determining the multi-year pattern of potato late blight epidemics in The Netherlands?

Chapter 2

Natural potato late blight epidemics were studied to assess the relative impact of various infection sources of *Phytophthora infestans* in Southern Flevoland (The Netherlands) from 1994 through 1996. Disease surveys were combined with characterization of isolates for mating type and DNA fingerprint pattern using probe RG57. Seventy-four percent of the commercial potato fields with early foci were clearly associated with nearby infested refuse piles. Characterization of isolates from refuse piles and fields confirmed the association. Infected seed tubers, volunteer plants, and infested allotment gardens appeared to be of minor importance for late blight development in potato fields. Several foci in refuse piles, potato fields, and allotment gardens contained more than one genotype. Due to favorable weather in August 1994, infested organic potato fields became major inoculum sources, resulting in the spread of *P. infestans* to adjacent conventional potato fields. Analyses of disease gradients, both at field and regional levels, confirmed the role of the organic fields as mid-season infection sources. The mean slope of field gradients downwind of refuse piles (point sources) was significantly steeper (100-fold difference) than the mean slope of field gradients downwind of organic fields (area sources). The genotypic composition of

the *P. infestans* populations along the gradient and of the source populations in the organic potato crops did not differ significantly. Analysis of the region gradient revealed genotype-specific disease gradients. Control measures are recommended.

Chapter 3

Genotypic changes in populations of *Phytophthora infestans* in Southern Flevoland (150 km^2) were analysed by characterising isolates from potato refuse piles, conventional and organic potato fields, and potatoes and tomatoes in allotment gardens for mating type (1712 isolates) and DNA fingerprint pattern using probe RG57 (1048 isolates). The overall percentages of isolates that were A2 varied from 4 % in 1994 with weather favourable for late blight to 56 % in 1996 with unfavourable weather. The percentages of genotypes that were A2 ranged from 32 % in 1994 to 45 % in 1996. Among 981 isolates collected on potato, 146 genotypes were identified, of which 116 (79 %) were unique. Most unique genotypes were encountered in organic potato fields and in allotment gardens. Four genotypes predominated during 1994 and 1995. Highest percentages of isolates with these genotypes were encountered in refuse piles and conventional potato fields. Refuse piles were identified as the most important infection source in 1994 and 1995. The predominant genotypes from these years were nearly extinct in 1996, suggesting that the population had passed through a bottleneck at the transition from 1995 to 1996. The high numbers of unique genotypes detected every year indicate a role of oospores in commercial potato fields. In 1996, disease in organic fields was probably initiated by a few genotypes, originating from seed tubers. Subsequently, in-the-green crop sexual recombination occurred, followed by multiplication and spread of recombinants. In allotment gardens, oospores appeared the most important infection source. Evidence for potato-tomato specialisation was not convincing. Probe RG57 was used to identify (sub)populations and to directly track individual genotypes. AFLP fingerprinting was conducted to further distinguish between isolates with identical RG57 fingerprints from unrelated sites within and outside the research area, found during or prior to the present study. Evidently, sexual reproduction plays a role in the development of *P. infestans* populations in The Netherlands.

Chapter 4

Genotypic diversities and genotypic distances among populations of *Phytophthora infestans* on potato and tomato were analysed in space and time. Over the period 1994 through 1996, *P. infestans* isolates were collected from 99 sites, including refuse piles, conventional potato fields, organic potato fields, and allotment gardens in Southern Flevoland and adjacent areas (The Netherlands). Genotypes were determined by mating

type assessment and DNA fingerpinting. Genotypic diversity was high in allotment gardens and in organic potato fields. Significant differences in diversity levels existed between compounds of allotment gardens. The diversity changed both within the season and among seasons. Within one season (1994), diversity was highest in refuse piles and organic fields, and lowest in early infested conventional potato fields. Diversity increased over the years. For the total populations, the normalised Shannon index ranged from 0.34 in 1994, with weather relatively favourable to late blight development, to 0.61 in 1996, a year with relatively unfavourable weather conditions. Accordingly, genotypic distances increased over the years. In 1994, only 20 % of the sampling sites had an average genotypic distance $D \geq 0.07$. For 1995 and 1996, the percentages of sites with an average $D \geq 0.07$ was 29 % and 35 %, respectively, indicating a population shift. No relation was found between the Euclidean distance and the genotypic distance of isolates. The genotypic distance between isolates was negatively correlated with the level of genotypic diversity ($r = -0.91$). Analysis of the *P. infestans* populations in Southern Flevoland suggests an annual supply of new genotypes originating from sexual reproduction, resulting in diversification between sites and generation of unique genotypes. The structure of a *P. infestans* population developed during a severe epidemic may differ significantly from that of a population developed under unfavourable conditions.

Chapter 5

Potato late blight epidemics in The Netherlands (1950 - 1996), caused by *Phytophthora infestans*, were analysed using agronomic and meteorological variables. Severity of late blight epidemics was characterised by a disease index (DI) with a 0-4 scale (0 = absence of late blight, 4 = severe epidemic). DI fluctuated from year to year and, superimposed on the yearly pattern, a multi-year pattern was identified in which 3 periods were evident: I (1950-1968), II (1969-1978), and III (1979-1996). The average DI in these periods were 2.9, 0, and 2, respectively. Major changes of the DI between subsequent years, including the transitions from period I to II and from period II to III, corresponded with changes in meteorological variables. Major variables were disease enhancing, such as the number of days with precipitation during the growing season (DPRg) and the number of hours per growing season with a temperature between 10 and 27 °C and a relative humidity > 90 % (HF), as well as disease limiting, such as the number of hours per growing season with temperatures > 27 °C (HU) and the amount of global radiation (GR). The transition from period II, the blight-free period, to period III coincided with the occurrence of resistance against the fungicide metalaxyl and the introduction of the 'new' *P. infestans* population in The Netherlands. Chi-square analyses and Spearman's rank correlations identified the disease level in the previous season, DPRg, HF, and GR as significant variables ($P \leq 0.01$). Linear discriminant analysis was conducted using the blight status of the previous year (LBp, 0 = absence, 1 = presence of late blight), total values (per growing season) and

monthly values for DPRg, HF, GR, HU, and other meteorological variables. Using monthly values, the number of years correctly classified was 40 (87.0 %). Five out of the six incorrectly classified years were in period III. For both the analyses (total and monthly), the blight status of the previous year (LBp), the number of days with precipitation (DPRg) and the number of hours with favourable conditions (HF) were the most important discriminating variables. The importance of agronomic factors seemed evident, but could not be inferred from our data. The effect of man on the polyetic pattern of disease outbreaks is not well understood. Polyetic studies of plant diseases, which may help to identify key factors for the development of epidemics, need more attention in crop protection.

Chapter 6

Results of chapters 2-5 are integrated and discussed. Infested refuse piles were the most important infection sources early in the season. Infested organic potato fields appeared major mid-season infection sources. The results of this research show that oospores play a role in the development of late blight disease in commercial potato fields and allotment gardens in Southern Flevoland and adjacent areas. The results are applicable to other potato growing areas in The Netherlands. Epidemiological and population genetic studies are needed to further study the role of oospores in the epidemiology of *P. infestans*, and to investigate how the *P. infestans* population will evolve in the future. Field-oriented research needs to be followed by experiments to test hypotheses generated in field studies. The presence of a variable *P. infestans* population, the fact that oospores now play a role in the development of epidemics, and the increasing area of organic potato crops has important consequences for potato growing in the future, especially in periods of years with weather conditions favourable to late blight.

Samenvatting

Hoofdstuk 1

De aardappelziekte is de meest verwoestende schimmelziekte van de aardappel. De ziekte, veroorzaakt door *Phytophthora infestans*, werd halverwege de negentiende eeuw voor het eerst in Europa geïntroduceerd en veroorzaakte de bekende hongersnood in Ierland. In die periode waren er alleen isolaten met het A1 paringstype aanwezig. Aldus kon het pathogeen zich alleen vermeerderen via ongeslachtelijke voortplanting. Analyse van populaties van *P. infestans* toonden aan dat er rond 1980 'nieuwe' isolaten in Nederland geïntroduceerd waren, waaronder ook isolaten met het A2 paringstype. Als gevolg van de aanwezigheid van beide paringstypen kan de ziekteverwekker nu ook geslachtelijk voortplanten waarbij oösporen gevormd worden. Deze oösporen kunnen in de grond overwinteren in afwezigheid van de waardplant en als bodemgebonden inoculum fungeren. De aanwezigheid van deze vorm van inoculum, naast het inoculum in de aardappelknollen, beïnvloedt de epidemiologie van de ziekte. Een andere factor die de epidemiologie beïnvloedt is de biologische landbouw. De aanwezigheid van hoge ziekteniveaus in onbespoten biologische aardappelpercelen zal effect hebben op de overwintering en verspreiding van de ziekteverwekker. In dit proefschrift werden drie hoofdvragen onderzocht: (*i*) Wat is het belang van oösporen als overwinterend inoculum, vergeleken met zieke aardappelknollen ? (*ii*) Wat is het relatieve belang van de diverse infectiebronnen voor de ontwikkeling van epidemieën van *P. infestans* ? (*iii*) Wat zijn de belangrijkste factoren die het meerjarige patroon van aardappelziekte-epidemieën in Nederland bepalen ?

Hoofdstuk 2

Van 1994 tot en met 1996 werden in Zuidelijk Flevoland natuurlijke epidemieën van de aardappelziekte bestudeerd om het relatieve belang van de verschillende infectiebronnen van *P. infestans* vast te stellen. Surveys werden gecombineerd met het verzamelen van isolaten die gekarakteriseerd werden door bepaling van het paringstype en de DNA-vingerafdruk met probe RG57. Vierenzeventig procent van de aardappelpercelen met vroege haarden waren duidelijk geassocieerd met nabijgelegen afvalhopen met zieke planten. Karakterisering van isolaten van afvalhopen en aardappelpercelen bevestigden de associatie. Geïnfecteerd pootgoed, aardappelopslagplanten en zieke aardappelveldjes in volkstuinen bleken van gering belang voor de ziekte-ontwikkeling in aardappelpercelen. Verscheidene haarden op afvalhopen, in percelen en in volkstuinen bevatten meer dan één genotype. Als gevolg van het gunstige weer in augustus 1994 bleken de biologische aardappelpercelen belangrijke infectiebronnen te zijn voor de verspreiding van de ziekte naar nabijgelegen gangbare aardappelpercelen. Gradiënt-analyse, uitgevoerd op veldniveau

en op regioniveau, bevestigde dat de aangetaste biologische percelen halverwege het groeiseizoen belangrijke infectiebronnen waren. De gemiddelde helling van de ziekte-gradiënten van percelen benedenwinds van afvalhopen (puntbronnen) was significant steiler (een factor 100 verschil) dan de gemiddelde helling van ziekte-gradiënten van percelen benedenwinds van biologische percelen (oppervlaktebronnen). De genotypische samenstelling van de *P. infestans* populaties langs de gradiënt en van de bronpopulatie in de biologische percelen was niet significant verschillend. Analyse van de regiogradiënt liet genotype-specifieke gradiënten zien. Maatregelen ter voorkoming van de aardappelziekte werden besproken.

Hoofdstuk 3

Veranderingen in de genotypische samenstellingen van populaties van *Phytophthora infestans* in Zuidelijk Flevoland (150 km^2) werden geanalyseerd door het paringstype (van 1712 isolaten) en het DNA-vingerafdrukpatroon (van 1048 isolaten) te bepalen. De isolaten waren afkomstig van aardappelafvalhopen, van gangbare en biologische aardappelpercelen en van aardappel- en tomatenveldjes in volkstuinen. Het percentage isolaten met het A2 paringstype varieerde van 4% in 1994, een jaar met gunstig weer voor de aardappelziekte, tot 56 % in 1996, een jaar met ongunstig weer. Het percentage genotypen met het A2 paringstype varieerde van 32 % in 1994 tot 45 % in 1996. In 981 isolaten afkomstig van aardappelplanten werden 146 genotypen geïdentificeerd. Van deze genotypen waren er 116 uniek (79 %). De meeste unieke genotypen werden gevonden in biologische percelen en in de volkstuinen. Vier genotypen domineerden gedurende 1994 en 1995. De hoogste percentages isolaten met deze genotypen werden gevonden op afvalhopen en in gangbare percelen. In 1994 en 1995 werden afvalhopen als belangrijkste besmettingsbron geïdentificeerd. De genotypen die in 1994 en 1995 domineerden waren in 1996 zo goed als verdwenen. Blijkbaar was de overgang van 1995 naar 1996 een belangrijke hindernis ('bottleneck') voor de populatie. Het grote aantal unieke genotypen dat elk jaar gevonden wordt geeft aan dat oösporen een rol spelen in commerciële aardappelpercelen. In 1996 werd de ziekte op de biologische percelen vermoedelijk veroorzaakt door enkele genotypen afkomstig uit geïnfecteerd pootgoed. Vervolgens is er waarschijnlijk in het gewas geslachtelijke voortplanting opgetreden, gevolgd door asexuele vermeerdering en verspreiding van recombinanten. Oösporen bleken de belangrijkste inoculumbron voor het ontstaan van de ziekte in volkstuinen. Er zijn geen duidelijke aanwijzingen verkregen voor aardappel-tomaat specialisatie. DNA vingerafdrukprobe RG57 werd gebruikt om (sub)populaties te identificeren en om individuele genotypen te volgen. AFLP-vingerafdrukken werden gebruikt om verschillen te bepalen tussen isolaten met een identieke RG57 DNA-vingerafdruk. Daartoe werden isolaten, afkomstig van ongerelateerde plaatsen binnen en buiten het onderzoeksgebied, en isolaten verzameld gedurende deze onderzoeksperiode en daarvoor, onderling vergeleken. De resultaten van deze studie geven

aan dat sexuele voortplanting een rol speelt bij de ontwikkeling van populaties van *P. infestans* in Nederland.

Hoofdstuk 4

De genotypische diversiteit en genotypische afstanden van en tussen populaties van *Phytophthora infestans* op aardappel en tomaat werden geanalyseerd in ruimte en tijd. Van 1994 tot en met 1996 werden isolaten van *P. infestans* van 99 objecten verzameld, te weten van afvalhopen, gangbare aardappelpercelen, biologische aardappelpercelen en volkstuinen in Zuidelijk Flevoland en aangrenzend gebied. Isolaten werden gekarakteriseerd door het paringstype en het DNA-vingerafdrukpatroon te bepalen. De genotypische diversiteit was hoog in volkstuinen en in biologische aardappelpercelen. Significante verschillen in diversiteit werden gevonden tussen volkstuincomplexen. De diversiteit veranderde binnen één seizoen en tussen de seizoenen. In 1994 was de diversiteit het hoogst in de afvalhopen en de biologische percelen en het laagst in de vroeg aangetaste gangbare percelen. De diversiteit nam toe over de jaren. De genormaliseerde Shannon index nam toe van 0.34 in 1994, een jaar met relatief gunstig weer voor de ziekte-ontwikkeling, tot 0.61 in 1996, een jaar met relatief ongunstige weersomstandigheden. De genotypische afstand nam overeenkomstig toe. In 1994 had slechts 20 % van de bemonsterde objecten een genotypische afstand $D \geq 0.07$. In 1995 en 1996 was het percentage bemonsterde objecten met een gemiddelde $D \geq 0.07$ respectievelijk 29 % en 35 %. Deze cijfers duiden op een verschuiving in genotypische afstanden binnen populaties over de tijd. Er werd geen relatie gevonden tussen de geografische afstand en de genotypische afstand van isolaten. De genotypische afstand tussen isolaten was negatief gecorreleerd met het diversiteitsniveau (r = -0.91). De analyse van de *P. infestans* populaties in Zuidelijk Flevoland doet vermoeden dat ieder jaar nieuwe genotypen ontstaan door geslachtelijke voortplanting. Die geslachtelijke voortplanting is verantwoordelijk voor het ontstaan van verschillen in genotypische samenstelling van aangetaste objecten en zorgt voor het ontstaan van unieke genotypen. De structuur van een *P. infestans* populatie die zich gedurende een hevige epidemie ontwikkelt kan sterk afwijken van de structuur van een populatie die zich ontwikkelt onder ongunstige omstandigheden.

Hoofdstuk 5

Epidemieën van de aardappelziekte in Nederland (1950 - 1996), veroorzaakt door *Phytophthora infestans*, werden geanalyseerd met gebruikmaking van landbouwkundige en meteorologische variabelen. De ernst van de epidemieën werd gekarakteriseerd door middel van een ziekte-index (DI) met een schaal van 0 tot 4 (0 = afwezigheid van de

aardappelziekte, 4 = ernstige epidemie). DI fluctueerde van jaar tot jaar. Een meerjarig patroon met 3 perioden werd zichtbaar: I (1950-1968), II (1969-1978), and III (1979-1996). De gemiddelde DI in deze perioden was respectievelijk 2.9, 0, and 2. Grote veranderingen in DI tussen opeenvolgende jaren, inclusief de overgangsjaren van periode I naar II en van periode II naar III, kwamen overeen met veranderingen in meteorologische variabelen. De belangrijkste variabelen behoorden tot de categorie van variabelen die de ontwikkeling van de epidemie stimuleren, zoals het aantal dagen met neerslag gedurende het groeiseizoen (DPRg) en het aantal uren per groeiseizoen met een temperatuur tussen de 10 en 27 °C en een relatieve luchtvochtigheid > 90 % (HF), en van variabelen die de ziekte-ontwikkeling beperken, zoals het aantal uren per groeiseizoen met temperaturen > 27 °C (HU) en de hoeveelheid globale straling per groeiseizoen (GR). De overgang van periode II, de periode met nauwelijks of geen aardappelziekte, naar periode III viel samen met het optreden van resistentie tegen het fungicide metalaxyl en de introductie van de 'nieuwe' *P. infestans*-populatie in Nederland. Chi-kwadraat toetsen en Spearman rangcorrelaties wezen op een significant verband tussen het ziekteniveau in een zeker jaar en het ziekteniveau in het voorgaande jaar, DPRg, HF en GR ($P \leq 0.01$). Lineaire discriminant-analyse werd uitgevoerd met de volgende groepen variabelen: de aardappelziekte-status van het vorige jaar (LBp, 0 = afwezigheid van aardappelziekte, 1 = aardappelziekte waargenomen), totale waarden (per groeiseizoen) en maandelijkse waarden voor DPRg, HF, GR en HU; en andere meteorologische variabelen. Met gebruikmaking van maandelijkse variabelen was het aantal juist geclassificeerde jaren 40 (87 %). Vijf van de zes onjuist geclassificeerde jaren behoorden tot periode III. Voor zowel de analyse met totale waarden als met maandelijkse waarden waren de aardappelziekte-status van het vorige jaar (LBp), het aantal dagen met neerslag en het aantal uren met gunstige omstandigheden (HF) de belangrijkste discriminerende variabelen. Het belang van landbouwkundige factoren lijkt evident maar kon niet afgeleid worden uit onze gegevens. Het effect van menselijk gedrag op het meerjarig patroon van uitbraken van de aardappelziekte is onduidelijk. Meerjarige studies van plantenziekten, waarmee belangrijke sturende variabelen geïdentificeerd kunnen worden, verdienen meer aandacht in de gewasbescherming.

Hoofdstuk 6

De resultaten van de hoofdstukken 2-5 worden geïntegreerd en besproken. Zieke afvalhopen waren de belangrijkste infectiebronnen vroeg in het seizoen. Aangetaste biologische percelen bleken belangrijke infectiebronnen halverwege het groeiseizoen. De resultaten van dit onderzoek geven aan dat oösporen een rol spelen in het jaarlijkse ontstaan van de ziekte in commerciële aardappelpercelen en in volkstuinen in Zuidelijk Flevoland en aangrenzend gebied. Waarschijnlijk spelen oösporen ook in andere aardappelteeltgebieden een rol. Meer epidemiologische en populatie-genetische studies zijn nodig om het belang van oösporen voor de epidemiologie van *P. infestans* vast te stellen en

om na te gaan hoe de populatie-structuur zich ontwikkelt in de toekomst. Beschrijvend veldonderzoek dient te worden gevolgd door veldproeven om hypothesen te toetsen die naar aanleiding van het beschrijvend veldonderzoek opgesteld zijn. De aanwezigheid van een variabele *P. infestans* populatie, de rol van oösporen in het ontstaan van epidemieën en het toenemende areaal aan biologisch geteelde aardappelen hebben belangrijke gevolgen voor de aardappelteelt, vooral in perioden van jaren met gunstige weersomstandigheden voor de ontwikkeling van de schimmel.

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Curriculum vitae

Maarten Jacob Zwankhuizen werd geboren op 31 oktober 1968 te Veenendaal. Na het behalen van het HAVO-diploma aan de christelijke scholengemeenschap 'Prins Maurits' te Middelharnis in 1986, begon hij met de studie Landbouw aan de Agrarische Hogeschool van het KNLC te Dordrecht (later Delft). In juni 1990 werd het diploma behaald; de hoofdvakken waren Plantenteelt, Gewasbescherming en Marketing. Een stage werd verricht voor de Stichting Proefbedrijven Flevoland en het Proefstation voor de Akkerbouw en Groenteteelt in de Vollegrond te Lelystad. In September 1990 begon hij met de studie Plantenziektenkunde (doorstroomprogramma) aan de Landbouwuniversiteit te Wageningen. De doctoraalstudie omvatte twee afstudeervakken: Ecologische Fytopathologie en Theoretische Productie Ecologie. In augustus 1993 behaalde hij het ingenieursdiploma met lof. Per september 1993 werd hij aangesteld als Assistent In Opleiding bij de vakgroep Fytopathologie van de Landbouwuniversiteit. Het onderzoek uitgevoerd tijdens dit vierjarig dienstverband staat beschreven in dit proefschrift. Sinds 1 november 1997 is hij in dienst bij Baan Company N.V.