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FULL RESEARCH PAPER

Effects of cropping history and origin of seed potatoes on population structure of *Phytophthora infestans*

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Abstract The effects of origin of seed potatoes and the cropping history on the phenotypic structure of *Phytophthora infestans* populations was studied in northern Hessia, central Germany, from 2000 to 2002. Populations originating from fields with a history of potato cropping with only short or no rotation (old fields) were compared with populations from new fields, i.e., where no potatoes had been grown for at least 30 years and seed potatoes were either imported from breeders or produced on-farm (certified). The main goal was to determine the importance of seed potato infection in the establishment of new *P. infestans* populations. Isolates were characterized for mating type, virulences and rep- (repetitive extragenic palindromic) PCR fingerprints. Among a total of 639 isolates sampled from 31 sites, mating types A1 and A2 co-existed in all three years in 60–92% of the sites. Over all three years, 53 pathotypes were detected in a subsample of 272 isolates. Isolates originating from the new fields had significantly higher frequencies of the virulences v1, v2, v3, v6 and v7, indicating general effects of seed introduction into a new region. Thirty-six fingerprints were detected in a subsample of 281 isolates of which 22 were unique while four occurred in all three years and in many sites. Pathogen populations from potato fields that were grown from seed tubers of geographically different origin differed significantly based on χ^2 tests. While the Nei genetic distances were less than .1 among the local populations, distances to the US lineages US-1, US-6, US-7 and US-8 ranged from .22 to .47; however, the bootstrap values were not significant. Populations from old fields were more diverse and 14 of the 22 rep-PCR types occurred there among 132 isolates tested in comparison to six in the new fields (n = 140 isolates) and two among six isolates from volunteers. The results also suggest that both sexual and asexual reproduction play a role.

Keywords Diversity · Late blight · Pathotypes · Potato · Rep-PCR · Virulence

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Introduction

Late blight, caused by *Phytophthora infestans* is one of the most important diseases affecting potato production worldwide. The first introduction of the pathogen into Europe in the 1840s via infected seed potatoes (Fry et al., 1993) lead to the great potato famine in Ireland in 1845–1850 (Bourke, 1964). Prior to the 1980s, only the A1

mating type occurred throughout the world, whereas both mating types co-existed in central Mexico, the centre of origin of the fungus (Niederhauser, 1999). In the early 1980s, however, a world-wide migration of *P. infestans* populations consisting of both mating types occurred replacing the old relatively homogeneous population (Fry et al., 1993). In several studies, the new populations have been characterized by more complex pathotypes (Drenth, Tas, & Govers, 1994; Goodwin, Sujkowski, & Fry, 1995), higher genetic diversity (Brurberg, Hannukkala, & Hermansen, 1999; Fry et al., 1993; Goodwin et al., 1994, among others) and also increased aggressiveness in comparison to the old clonal lineage (e.g., Day & Shattock, 1997; Medina, Platt, & Peters, 1999; Tooley, Sweigard, & Fry, 1986). This has lead to earlier and more severe late blight outbreaks (e.g. Drenth, Turkensteen, & Govers, 1993; Goodwin et al., 1998; Sujkowski, Goodwin, Dyer, & Fry, 1994) and an increase of costs for crop protection and of yield losses (Johnson, Cummings, & Hamm, 2000).

Seed potatoes have played a key role in the worldwide migrations of *P. infestans* in the 1840s as well as during the 1980s (Fry et al., 1993; Niederhauser, 1999) and just a few percent infected tubers could be responsible for the initiation of late blight epidemics in a potato field (Andrivon, 1994). However, other researchers have reported no transmission of *P. infestans* via infected tubers over several years (Platt, Peters, Medina, & Arsenault, 1998; Jenkins & Jones, 2003) suggesting that latent tuber infections either destroy their host (Miller, Cummings, Mikitzel, & Johnson, 2002) or the pathogen dies of starvation so that the sprouts do not emerge or a healthy potato plant results, respectively. However, PCR-based detection of *P. infestans* demonstrated more infection in tubers than previously thought (Adler, 2001).

The importance of latent tuber infections for *P. infestans* epidemics probably differs between organic and conventional production. While no seed-borne below-ground foci were detected in conventional potato fields in the Netherlands (Zwankhuizen, Govers, & Zadoks, 1998), the authors expected infected seed to be a more important inoculum source in organic potato production because no seed treatment with protective fungicides is practised there. On the other hand, it could also be that the generally faster death of organic potato canopies results in shorter epidemics compared to sprayed crops thus reducing the chances for tuber infections via the soil. Overall, however, the importance of seed-borne inoculum in the establishment of new epidemics is not clear and it is difficult to test as inoculum originating from neighbouring fields, the soil and from the seed tubers will have to be differentiated.

For the molecular characterization of *P. infestans* populations various methods have been employed such as allozymes (Tooley, Fry, & Villarreal-Gonzalez, 1985), RFLPs (Goodwin, Spielman, Matuszak, Bergeron, & Fry, 1992), AFLPs (Abu-El Samen, Secor, & Gudmestad, 2003), microsatellites (Knapova & Gisi, 2002) or RAPD-PCR (Maufrand, Archer, Buck, Shattock, & Shaw, 1995). The allozymes Glucose-6-phosphate-isomerase (Gpi) and peptidase (Pep) are used to define and compare different clonal (US)-lineages (Forbes et al., 1998; Goodwin et al., 1995). In addition, RFLP fingerprinting with probe RG57 resulting in about 25 DNA bands functions as an international standard fingerprinting method for use in the Global Marker Database on *P. infestans* (Forbes et al., 1998), allowing for worldwide comparison of isolates and the determination of migration patterns of the pathogen (e.g., Goodwin et al., 1992; Sujkowski et al., 1994). A higher number of identifiable polymorphisms can be obtained through AFLPs with 50 to more than 100 loci (Abu-El Samen et al., 2003; van der Lee, de Witte, Drenth, Alfonso, & Govers, 1997; Zwankhuizen et al., 1998). However, both RFLP and AFLP techniques are labour-intensive and expensive to generate because the fragments are detected by silver staining, fluorescent dye or radioactivity or require special sequencing equipment.

In contrast, RAPD-PCR technology requires only small amounts of DNA (15–25 ng) and is a non-radioactive and cost-effective assay that can be performed within a few hours. PCR using different RAPD primers each of 10-mer length produces about 10–30 polymorphic DNA fragments revealing genetic diversity among *P. infestans*

isolates (Carlisle, Cooke, & Brown, 2001; Mahuku, Peters, Platt, & Daayf, 2000) that can differentiate isolates belonging to the same clonal lineages (Ghimire, Hyde, Hodgkiss, Shaw, & Liew, 2002). Rep- (repetitive extragenic palindromic) PCR was developed originally to distinguish between different bacterial strains. The rep-PCR primers are sequences of 15–18 bp in length occurring throughout the genome and are highly conserved repetitive elements in many organisms (Versalovic, Schneider, de Bruijn, & Lupski, 1994). Rep-PCR has been used successfully to discriminate among closely related phytopathogenic bacterial species and to reveal diversity within species among isolates (Rademaker et al., 2000; Scortichini & Rossi, 2003). Initial applications of this technology to *P. infestans* using one pair of primers revealed about 15 polymorphic DNA fragments of a good reproducibility (Bouws-Beuermann and Finckh, unpubl.). These results, the cost-effectiveness, simple protocols and speed were the main motives to establish and optimize rep-PCR technology in our laboratory at the University of Kassel, west Germany.

When in 1999, potato production was taken up on the organic 300 ha experimental farm of the University of Kassel in central west Germany in fields where no potatoes had been grown for over 30 years and in a region where potatoes are a minor crop only, this offered a unique opportunity to study the establishment and evolution of *P. infestans* populations. All inoculum in the 'new fields' on the farm had to originate either from the seed potatoes or from the surrounding region. The underlying hypothesis for the work was that if seed-borne inoculum played a role in starting epidemics, then *P. infestans* populations from fields with a different origin of seed potatoes should differ. To test this, the pathogen population structures in newly established fields of potatoes on the farm from different seed tuber sources but also from the relevant surrounding fields had to be studied.

Previous observations on the spatial dynamics of the disease occurrence across the farm, the main wind direction and spore catches in 1999 suggested that the inoculum arrived from potato sites situated west of the farm which were infected about 10 days earlier (Bouws-Beuermann and Finckh, unpubl.). From 2000 on, *P. infestans* isolates were sampled in fields and experimental plots of the farm with seed potatoes originating either from northern German breeders or from the certified seed production on the experimental farm. Isolates were also collected from the surrounding potato crops where farmers and gardeners traditionally grow potatoes with short or no rotation, commonly from their own propagation and for their own consumption. During the years 2000 to 2002, a total of 639 isolates were characterized for mating type. Subsets of isolates were tested for virulences and molecular rep-PCR fingerprints in order to determine the phenotypic and haplotypic diversity among the *P. infestans* populations addressing the following questions: (i) Do *P. infestans* populations on the experimental farm originate from the surrounding area or from the seed potatoes? (ii) Are there differences between *P. infestans* populations due to the cultivar of origin and/or seed source? (iii) Do *P. infestans* populations change in the transition from the seed potato crop to the main crop?

Materials and methods

Collection of isolates

Totals of 95, 315 and 229 *P. infestans* isolates were collected during the years 2000, 2001 and 2002, respectively, from a 15×20 km region, situated in northern Hessia, central Germany, including the experimental farm of the University of Kassel (Fig. 1). About a third of the isolates was sampled from so-called 'old fields' (mostly up to 1 ha) from the western surroundings of the experimental farm. The majority of the isolates originated from potato fields and experimental plots which were planted on the experimental farm. During 2000, isolates were collected from nine, 9×10 m experimental plots distributed within a 150×300 m field. Experimental plots were separated with other crops by at least 9 m. Isolates were collected from six plots of cv. Secura (susceptible) and three plots of Simone (moderately resistant). Seed potatoes of both cultivars originated from growers in northern Germany.

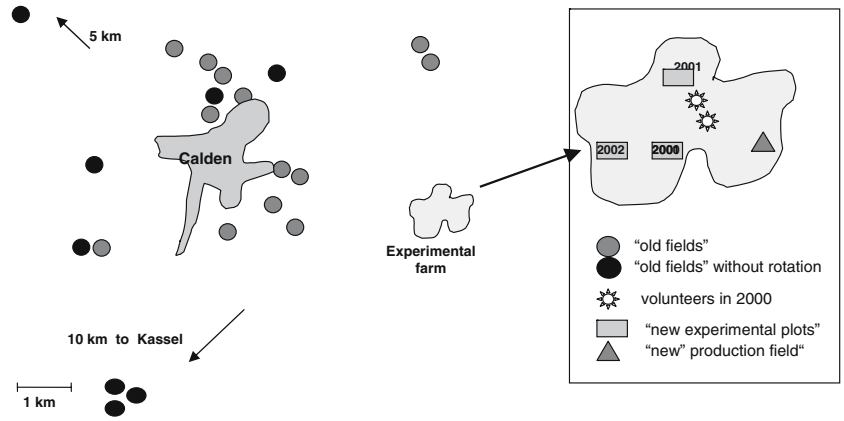

Fig. 1 Schematic map of a 15×20 km^2 region in northern Hessia near the town of Calden, central Germany where *Phytophthora infestans* isolates were sampled from old and new fields (i.e., with and without a potato cropping history, respectively) from 2000 to 2002. Fields without rotation indicate that potatoes were grown in two or three consecutive years

During 2001 and 2002, two experimental plots of 18×36 m (24 rows $\times$ 36 m) planted with cvs. Linda (susceptible) and Agria (moderately resistant) were sampled intensively (116 and 110 isolates, respectively). Certified seed potatoes produced on-farm were used in these experimental plots. During 2001, isolates also were sampled from larger seed potato production fields (>2 ha) of the same two cultivars on-farm. Seed tubers for these fields came from the respective breeders. Isolates were sampled from mid-June to mid-August during 2000 and 2001 and from mid-July to mid-August 2002, respectively. They were collected in diagonal transects from potato fields or by regular sampling every 3 m (in 2000) or 6 m (in 2001 and 2002) along two separate rows within the experimental plots. For comparison, isolates of the four most investigated clonal lineages US-1, US-6, US-7 and US-8 (kindly provided by W.E. Fry, Cornell University, Ithaca, New York) were included in the molecular analysis with rep-PCR.

Isolation and culturing of the pathogen

For the isolation of the pathogen, tubers of potato cv. Atica (early maturing, without any known R gene) were surface-sterilized, cut into 1 cm-thick slices and pressed dry on filter paper. Sandwiches with two tuber slices and a 1 cm^2 piece of the sporulating leaflet in between were prepared. The potato sandwiches were placed on filter paper in plastic boxes with transparent lids and kept at 17°C with 16 h daylength, starting with 16 h darkness. After five to seven days, sporulating mycelia emerging on top were harvested with sterile tweezers and plated on 1.5% pea agar (125 g of frozen peas l^{-1} of H_2O) containing 100 mg l^{-1} Ampicillin, 30 mg l^{-1} Rifamycin, 10 mg l^{-1} Benomyl and .4 mg l^{-1} Pimaricin. Pure cultures of *P. infestans* were transferred onto 1.5% pea agar without antibiotics after 5–7 days. The agar plates were stored at 17°C in the dark and examined weekly. Success rates with this method were about 85, 94 and 75% during the years 2000–2002, respectively. For long-term storage, the isolates were transferred into test tubes containing 1.5% pea agar, overlaid with sterilized mineral oil, and stored at 17°C in darkness.

Mating type testing

For mating type determination each isolate was paired on pea agar with known A1 and A2 tester strains, which were kindly provided by D. Andrivon (INRA, Rennes, France). The Petri dishes were incubated for 10–18 days at 17°C in the dark and examined microscopically for the presence of oospores. An isolate was considered to belong to mating type A1 if oospores were found when paired with a known A2 strain and vice-versa. Test isolates producing oospores with both A1 and A2 were scored as A1A2, probably self-fertile, but this was not confirmed.

Virulence tests

Virulence tests were conducted with Black's single R-gene differential set (Black, Mastenbroek,

Mills, & Peterson, 1953; Malcolmson & Black, 1966), each possessing one of the race-specific resistance genes R1, R2, R3, R4, R5, R6, R7, R8, R10, R11 plus one line without a known R-gene R0 (kindly provided by D. Andrivon, INRA, Rennes, France). Potato plants were grown in 13 cm diam plastic containers in a sand-peat-compost mixture (each 33%) in the glasshouse at 18°C/15°C day/night, with 14 h day-length. The virulence tests were carried out with plant material about seven weeks old. Only fully expanded leaves were used for the tests. The leaflets were detached with a scalpel and deposited abaxial face up on moist filter paper in square Petri dishes (14 × 14 cm), and each differential host set was replicated twice. For the inoculation suspension, two week-old cultures on pea agar were flushed with 4 ml of sterilized water and sporangia removed by gently scraping the surface of the culture. Sporangia were counted with a 'Fuchs Rosendahl' haemocytometer and the suspensions were then stored at about 8°C for 3–4 h, to allow release of the zoospores. All sporangial suspensions were adjusted to a concentration of 5×10^4 sporangia ml^{-1}. Each leaflet was inoculated on the abaxial side with two separate 20 µl drops of sporangial suspension. The Petri dishes were incubated for 7 days at 18°C/15°C day/night, the first day in the dark and then with a 16 h daylength. To avoid drying of the leaflets, the lids of the Petri dishes were moistened with distilled water every two days. After an incubation period of 7 days, an isolate was considered virulent to a given differential host if at least two of the four inoculations resulted in sporulating lesions and if the R0-line was infected in the same way.

Genomic DNA extraction

To obtain pure mycelium of *P. infestans*, the pathogen was grown in 50 ml Erlenmeyer flasks containing sterilized liquid pea medium (125 g of frozen fresh peas l^{-1} of distilled H_2O, filtered and autoclaved twice at 120°C for 20 min, with 100 mg l^{-1} Ampicillin added). Mycelium of 10–14 day-old cultures was rinsed with sterilized water and harvested by vacuum filtration. The mycelium was frozen at –20°C and freeze dried in 2.0 ml micro centrifuge tubes. For DNA extraction, 15–20 mg of the freeze-dried mycelium was ground with a small amount of sterile quartz sand; 700 µl of extraction buffer (1 M Tris–HCl, .5 M EDTA, 10% SDS, pH 8.0) was added and the tubes were incubated for 1 h in a water bath at 65°C. After adding 50 µl of RNase A (1 mg ml^{-1}) and 50 µl of Proteinase K (10 mg ml^{-1}), the tubes were incubated for 1 h in a water bath at 37°C. The DNA was purified twice with phenol–chloroform–isoamyl-alcohol (25:24:1) followed by centrifugation at 15,000 and 13,000 r.p.m. for 15 and 10 min, respectively. A tenth of the supernatant quantity was added as 3 M NaOAc (usually 20–50 µl) plus 1 ml of isopropanol (100%) and the tube was centrifuged again at 13,000 r.p.m. for 6 min. Finally, the supernatant was mixed with 500 µl of ethanol (70%) and centrifuged at 13,000 r.p.m. for 5 min. The DNA pellet in the micro centrifuge tubes was dried in a desiccator and the DNA was dissolved in 100 µl of .1 M TE buffer (1 M Tris–HCl, pH 7.6; .5 M EDTA). DNA samples were stored at 4°C until dilutions were made, then stock solutions were stored at –20°C. For estimation of DNA concentration, 5 µl of each DNA sample, mixed with 5 µl of ultra pure water and 2 µl of loading buffer, was loaded on a .8% agarose gel. As DNA standard, 10 µl of phage λ DNA digested with *Hin*dIII and *Eco*RI was loaded with 2 µl of loading buffer per lane. The agarose gel was run at room temperature for 45 min at 100 V. After staining in a water bath containing ethidium bromide (100 µl of 10 mg ml^{-1} ethidium bromide added to 800 ml of distilled water) for about 20 min, the gel was photographed on a UV transilluminator (INTAS, Germany). The DNA content was quantified visually by comparing each DNA sample with the fragments of the DNA standard.

Rep-PCR analysis

For rep-PCR, amplification reactions were carried out in .2 ml microtubes with volumes of 25 µl of mix, containing 3 ng of DNA, 200 µM each dNTP, 1 U of *Taq* polymerase (Peqlab, Germany), 50 pM primer rep-I 5′-III ICG ICG ICA TCI GGC-3′ and 50 pM primer rep-II 5′-ICG ICT TAT CIG GCC TAC-3′, 2.5 µl of 10-fold

concentrated reaction buffer (200 mM Tris–HCl, pH 8.55, 160 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$) and 13.3 µl of sterile ultra pure water.

Amplifications were performed in a Biometra T 1 Thermocycler (Biometra, Germany) with one cycle at 95°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 1 min and extension at 65°C for 5 min, a final cycle at 65°C for 15 min, and then maintained at 4°C. Fifteen µl of the amplification products with 3 µl of loading buffer per lane were separated by gel electrophoresis in 1.5% agarose gels in 1× TBE buffer. As DNA standards, a 1 KB and/or a 100-bp ladder (Peqlab) were added to the gel, which was run at room temperature for about 6 h at 70 V. The gels were stained and photographed as described above.

Of the 281 isolates that were analyzed successfully, 218 were subjected to rep PCR more than once (110 twice, 118 three or more times). If there were faint bands in the first analysis, a second was performed. If there were contradictions, a third and the repeatable results were used.

Data analysis

Rep-PCR fingerprint patterns were analyzed with INTAS PCI Gel-Imager software (INTAS, Germany) with manual correction for faint bands and exclusion of ambiguous bands. A binary matrix for the presence (1) or absence (0) of reproducible bands was constructed and used for further analysis. Only the polymorphic bands were included in the analysis. POPGENE and TFPGA software (http://www.ualberta.ca/~fyeh/ and http://bioweb.usu.edu/mpmbio/index.htm, respectively) were used. For further analysis of molecular data, polymorphic bands plus mating type were treated as one locus each in a haploid dominant data set. Dendrograms were constructed from Nei's genetic standard distance (Nei, 1972) with the unweighted pair-group method of averages (UPGMA) with 1,000 permutations of bootstrapping using TFPGA software. Population differentiation was estimated with an exact test (Raymond & Rousset, 1995) on in our case haplotypes instead of genotypes, see Eq. 1, according to Goudet, Raymond, de Meeus, and Rousset (1996):

$$\Pr(s) = \frac{\prod_{i=1}^{r} N_i! \prod_{j=1}^{N_g} N_{.j}!}{N_{..}! \prod_{i=1}^{r} \prod_{j=1}^{N_g} N_{ij}!} \quad (1)$$

where Pr(S) = population probability, N_{ij} = number of individuals of haplotype j in population i, N_i = sample size of population i, $N_{.j}$ = total number of individuals with haplotype j, $N_{..}$ = total number of individuals sampled, r = number of populations and N_g = number of haplotypes.

Within an exact test, all possible tables given the marginal haplotype counts according to the value of a particular statistic are ranked and the probabilities Pr(S) of the tables with more extreme ranks are summed, the resulting sum being the P-value of the test. Under the null hypothesis of absence of population differentiation, defined contrasts of *P. infestans* populations (e.g., seed origin or cropping history of fields from which isolates originated) were contrasted with 10 batches, 2,000 permutations per batch and 1,000 dememorization steps as described in the TFPGA manual.

Statistical analysis of virulence frequencies was performed with the FREQ procedure of SAS software (SAS Institute, Inc.) using the two-tailed Fisher's exact test. The cumulative frequency of virulences, i.e., the mean complexity per isolate, was compared pair-wise with t-tests (GLM procedure). The Shannon diversity index H_o was calculated for defined populations using the following equation:

$$H_o = -\sum_{i=1}^{s} (P_i) * \ln(P_i) \quad (2)$$

where P_i = the frequency of isolates of the ith phenotype within a sample or population and s = the number of different phenotypes within a sample.

From these, normalized Shannon statistics (Sheldon, 1969) were calculated. As this statistic takes into account the total number of tested isolates the normalized Shannon indices H′ present the fraction of the maximum diversity per sample thus ranging from 0 to 1. The normalized Shannon index was considered adequate, as the potential number of genotypes was much larger than the sample sizes (Grünwald, Goodwin,

Milgroom, & Fry, 2003). Diversity indices were calculated based on pathotypes and also for multilocus haplotypic data (mating type plus binary data of polymorphic rep-PCR bands) of isolates of different populations or sampling sites.

Results

Late blight epidemics from 2000 to 2002

During 2000, the first lesions were observed on June 20th in three old potato fields about 2–4 km northwest of the experimental farm, as well as on volunteer plants within a pea field located downwind of the plots on the experimental farm. Following heavy rainfall during the last days of June, the epidemic started in mid-July in the plots at the experimental farm, while the production fields on the farm were already up to 15% infected, sometimes with foci of 90% disease severity. Due to the high disease pressure the susceptible crop in the experiment was destroyed within two weeks.

During 2001, the first symptoms were found on potato plants in a refuse pile adjacent to the experimental plots on 20th of June, and these plants were destroyed immediately. The first surrounding potato fields were infected on 10th of July, and two days later, late blight started in the susceptible potato experimental plots. With prolonged hot and dry weather until the first week of August, disease development was slow and plants died in mid-August. During 2002, late blight in the experimental plots started about two weeks earlier, on the 28th of June but with a moderate epidemic increase. Due to continuous heavy rainfall during mid-July, the susceptible plants in the plots died within three weeks.

Mating types

Mating types were determined successfully for all 639 isolates of *P. infestans*. Both mating types were detected in all three years, with A2 frequencies of .46, .13 and .31, in 2000–2002, respectively. During 2001, the 116 isolates originating from the experimental plots were uniformly A1. If these are excluded, the frequency of mating type A2 during 2001 amounts to .21. Both mating types co-existed within the same site in 65, 60 and 92% of the different sites sampled from 2000 to 2002, respectively. In 2000, an interesting pattern was observed in the experimental plots. Within an area of about 150 by 300 m the A2 types were concentrated in the north to northwest and the A1 in the southeast region, with an area in the centre where both mating types co-existed within the plots. In 2001, both mating types were found in the large new fields that had not been planted to potatoes for at least 30 years, while only A1 was found in the experimental plots also located in a new field. Finally, in 2002 both mating types occurred side by side throughout the examined experimental plots with both potato cultivars.

Pathotypes

In total, 79, 114 and 79 isolates of the collections of 2000 to 2002 were tested successfully for virulence. Over all three years, 53 different pathotypes were detected with 26, 36 and 22 pathotypes in 2000, 2001 and 2002, respectively (Table 1). Sixteen pathotypes were found at least four times with eight of these occurring in all three years. Four isolates originating from a volunteer during 2000 ($n = 1$) and the new experimental plots during 2002 ($n = 3$) were able to overcome all tested resistance genes. No general association of pathotype and either A1 or A2 mating type was observed. However, the majority of the isolates of the two most common pathotypes 1.3.4.7.10.11 and 1.3.4.7.8.10.11 were of A1 mating type (82 and 92%, respectively).

The number of virulences per isolate varied between 2 and 10 with 91 out of the 272 tested isolates possessing six virulences, 95 fewer and 86 more than six (data not shown). The mean number of virulences per tested isolate was 6.2, 5.4 and 5.9 from 2000 to 2002, respectively. During 2001, but not 2000 or 2002, the mean complexity of isolates increased significantly within the growing season (Table 1) with an increase of 4.3–5.7 in the old fields and 5.2–6.2 in the new fields. Virulences v2, v5 and v6 were rarest, however, with significantly higher frequencies among isolates belonging to the A2 mating

Table 1 Pathotypes of 249 out of 272 isolates *Phytophthora infestans* that were tested for their virulence and found more than once, mean complexity and normalized Shannon diversity of populations collected early and late in late blight epidemics during 2000–2002

Pathotype	vir[a]	*n*	Year			A2[b]
			2000	2001	2002	
4.11	2	4		4		
3.4.7.11	4	4		4		
1.4.7.11	4	4		2	2	2
1.3.4.7.8	5	4		2	2	1
1.2.3.4.5.6.7.8.10.11	10	4	1		3	4
1.3.4.7.8.11	6	4	1	2	1	
1.3.4.5.7.8.10.11	8	4	1	1	2	1
3.4.7	3	5	1	4		3
1.2.3.4.6.7	6	5	1	2	2	3
1.3.4.6.7.8.10.11	8	7	4	3		1
1.3.4.6.7.10.11	7	8	7	1		5
3.4.7.10.11	5	8	1	4	3	2
1.3.4.7	4	15	7	3	5	7
1.3.4.7.11	5	17	3	9	5	5
1.3.4.7.8.10.11	7	48	7	22	19	4
1.3.4.7.10.11	6	75	27	25	23	13
Unique pathotypes[c]		23	7	11	5	10
In total		272	79	114	79	
Number of pathotypes		53	26	36	22	
Mean complexity[d]		Early	6.1	5.0	6.0	
		Late	6.3	5.9**	5.9	
H′			.59	.62	.54	

[a] Number of virulences per pathotype

[b] Number of isolates that were of A2 mating type

[c] The unique pathotypes are published in Bouws-Beuermann (2005) and can be obtained from the authors

[d] Mean complexity, i.e., mean number of virulences per isolate from populations sampled early and late during the epidemics

**Indicate that differences between early and late sampled isolates were highly significant at $P < .001$ (*t*-test)

type (Fig. 2). On the other hand, v8, v10 (both with $P = .057$) and v11 ($P < .01$), were more frequent among A1 isolates. The virulence v2 was significantly more frequent among isolates which were collected early during the three late blight epidemics ($P = .02$). Also, virulences v5 and v6 occurred more frequently among the early collected isolates; however, the differences were not significant. Interestingly, v2 occurred only in combination with either v5 or v6 or both, whereas v5 and v6 also occurred alone. All three virulences were present only in isolates with at least 5 or more virulences.

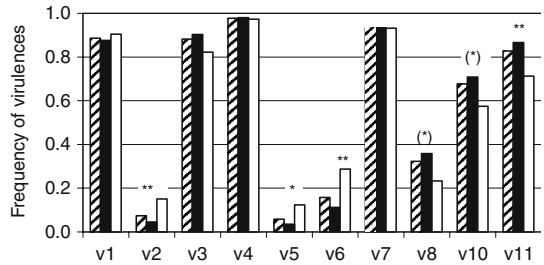

Fig. 2 Frequency of virulences for all tested isolates of *Phytophthora infestans* ($n = 272$, hatched) and separately for mating type A1 ($n = 195$, black) and A2 ($n = 73$, white). (*), * and ** indicate that virulence frequencies of A1 and A2 isolates differed significantly at ($P = .057$); $P < .05$ and $P < .01$, respectively (two-tailed Fisher's exact test)

The frequencies of v1, v5 and v10 were significantly lower within the collection of 2001 than in those from 2000 and 2002. Virulences v2 and especially v6 ($P < .001$) were most frequent among isolates of the collection from 2000 (Fig. 3). With respect to the cropping history of potato fields, i.e., the comparison of new fields ($n = 159$) versus old fields ($n = 113$), isolates from the new fields had significantly higher frequencies of the virulences v1 (.92/.83), v2 (.11/.03), v3 (.94/.81), v6 (.20/.10) and v7 (.97/.88) (all comparisons with $P < .05$). Within the new fields and experimental plots on the farm, *P. infestans* virulence frequencies of isolates originating from sites

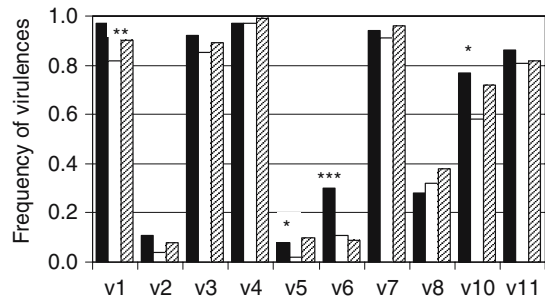

Fig. 3 Frequency of virulences of isolates of *Phytophthora infestans* collected during 2000 ($n = 79$, black), 2001 ($n = 114$, white) and 2002 ($n = 79$, hatched), respectively. *, ** and *** indicate that virulence frequencies of isolates differed significantly over years at $P < .05$, $P < .01$ and $P < .001$, respectively (two-tailed Fisher's exact test)

planted with introduced seed (n = 82) or on-farm certified seed (n = 70) did not differ significantly except for v1 (P = .02) which was more common for isolates originating from sites with introduced seed. Generally, no association of virulence frequency with potato cultivar or host population size (e.g., experimental plots versus large fields from which the isolates originated) was found.

Rep-PCR fingerprints

Overall, 44, 137 and 100 different isolates of the populations from 2000 to 2002 plus one isolate each of the US-lineages US-1, US-6, US-7 and US-8 were analyzed successfully for their rep-PCR fingerprints. Seventeen and 15 out of 23 reproducible DNA fragments were polymorphic among all (i.e., US-isolates included) or only the north Hessian isolates, respectively. Rep-PCR of isolates belonging to the US lineages revealed somewhat different fingerprint patterns from the north Hessian collections (Fig. 4). Excluding the US isolates, 36 rep-PCR fingerprints were identified of which 22 were unique and another six were found only during one of the three years, albeit several times (Table 2). Most of the unique rep-PCR types occurred in the old fields (n = 14), followed by the experimental plots (n = 5), the volunteers during 2000 (n = 2) and the seed production field during 2001 (n = 1). Combination of mating type with DNA fingerprinting pattern resulted in 51 multilocus phenotypes. Four rep-PCR fingerprints occurred over all three years with fingerprints rep-1 and rep-2 dominating the populations with 32 and 30%, respectively. These most common rep-PCR types were of both mating types and they were found in all categories of origin. Moreover, within each of these common rep-PCR types several pathotypes were found (Table 2) revealing no obvious association of molecular type with virulence.

Overall, the sampled *P. infestans* populations were highly similar with genetic distances (Nei, 1972) of .05 (Fig. 5). While the genetic distances to the four US-lineages were much greater ranging from a Nei distance of .22–.47, the bootstrap values were low, however, and only US-7 and US-8 were clearly separated from the others at a Nei distance of about .40. Generally, the *P. infestans* populations sampled from old fields were highly diverse with normalized Shannon diversity indices of H′ = .91, .60 and .65 during 2000, 2001 and 2002, respectively (Table 3). Overall, the populations originating from fields with and without rotation could not be differentiated. However, most of the unique rep-PCR types from the old fields came from fields where potatoes had been cultivated in successive years. Some isolates from the new fields were also unique except for the populations originating

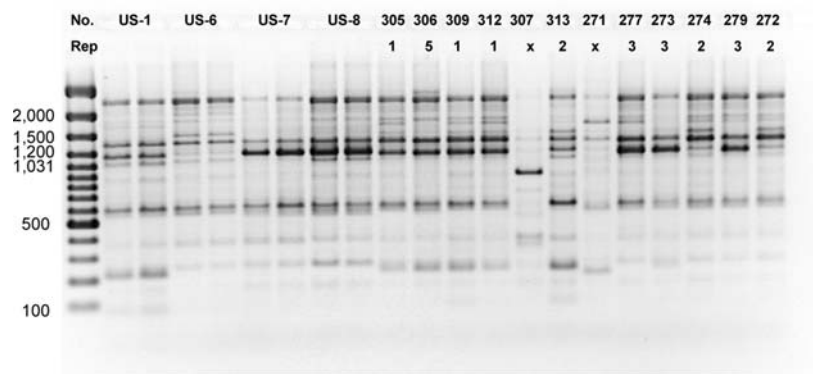

Fig. 4 Rep-PCR fingerprints each of two *Phytophthora infestans* isolates belonging to clonal US-lineages in comparison with 11 north Hessian isolates originating from an old field during 2002. The rep-PCR types of the isolates are given (× indicates that isolate could not be typed properly in this gel). Isolates of lineages US-7, US-8 and isolate 307, 273, 274, 279 and 272, respectively, were A2 mating type. Isolate 313 produced oospores with both mating types

Table 2 Banding patterns of rep-PCR fingerprints of 259 out of the 281 *Phytophthora infestans* isolates collected in northern Hessia from 2000 to 2002 that occurred more than once plus one isolate each of the clonal lineages[a] US-1, US-6, US-7 and US-8

No.[b]	rep-PCR fingerprint[c]	2000	2001	2002	Total	A2	Pathotypes (*n*)[d]
1	0 1 1 1 0 1 0 1 0 0 1 0 0 0 0 1	14	52	24	90	24	21 (60)
2	0 1 1 1 0 1 0 1 0 0 1 0 0 0 1 0	10	52	23	85	26	20 (46)
3	0 1 1 1 0 1 1 1 0 0 1 0 0 0 1 0	3	6	20	29	15	10 (18)
4	0 1 1 1 0 1 1 1 0 0 1 0 0 0 0 1	2	5	11	18	6	6 (10)
5	1 1 1 1 0 1 0 1 0 0 1 0 0 0 0 1		6	8	14	5	5 (6)
6	0 1 1 1 1 1 0 1 0 0 1 0 0 1 0 1	4			4		2 (4)
7	1 1 1 1 0 1 0 1 0 0 1 0 0 0 1 0		1	3	4	3	2 (2)
8	0 1 1 1 0 1 0 1 0 0 1 0 1 0 1 0		3		3		n.d.
9	0 0 1 1 0 1 0 1 0 0 1 0 0 0 0 1		2		2	1	n.d.
10	0 1 0 1 0 1 0 1 0 0 1 0 0 0 1 0		1	1	2	1	1
11	0 1 1 1 0 1 0 1 0 0 1 0 1 0 0 1		2		2		2 (2)
12	0 1 1 1 0 1 0 1 0 0 1 0 0 0 1 0		1	1	2	1	1
13	1 1 1 1 0 1 1 1 0 0 1 0 0 0 0 1			2	2	1	n.d.
14	1 1 1 1 0 1 1 1 0 0 1 0 0 0 1 0			2	2	1	1
Unique fingerprints[e]		11	6	5	22		
US-1	0 1 1 1 0 1 0 1 1 0 0 1 0 0 1 1			(1)[a]	1		
US-8	0 1 1 1 0 1 1 0 1 0 1 0 1 0 1 0			(1)	1		
US-7	0 1 1 1 0 1 1 1 0 1 1 0 1 0 1 0			(1)	1		
US-6	1 1 1 1 0 1 0 1 1 1 0 1 0 0 1 0			(1)	1		
Isolates tested in total[f]		44	137	100	281		
Different rep-PCR types[f]		16	17	15	36		
polymorphic bands[f]		12	11	11	17		
H′[f]		.40	.27	.33			

[a] Isolates of the four US lineages were tested with the collection from 2002 resulting in a total of 104 tested isolates. Each US strain revealed one of the overall unique rep-PCR types resulting in a total of 40 unique rep-PCR patterns

[b] Rep-PCR fingerprints were sorted by frequency and then assigned a number

[c] Polymorphic DNA fragments of 2,900; 2,000; 1,650; 1,500; 1,250; 1,150; 1,100; 1,050; 1,020; 870; 850; 800; 520; 450; 250; 220 bp size are listed

[d] Number of different pathotypes within rep-PCR type. The number of isolates tested for virulences is given in parenthesis. n.d. = pathotype was not determined

[e] The patterns of the unique types are published in Bouws-Beuermann (2005) and can be obtained from the author

[f] Only data of the north Hessian collections were considered. For estimation of the normalized Shannon diversity H′ only the polymorphic bands per collection were considered

from the experimental plots during 2001. There, the isolates all shared the most common rep-PCR fingerprints rep-1 and rep-2 representing only one and six pathotypes, respectively, setting the populations clearly apart from the old field populations (Table 3).

The seed potatoes for Simone and Secura in the experimental plots during 2000 and for the seed production fields of Agria and Linda during 2001 all originated from different growers in northern Germany. This different origin affected the *P. infestans* populations as reflected by the UPGMA dendrogram (Fig. 6) with a significant population differentiation between Agria and Linda fields in 2001 ($P = .04$, Table 3). In contrast, the populations from the experimental plots during 2001 and 2002 with the seed originating from the experimental farm clearly paired together (Fig. 6). The effects of origin of seed potatoes also became clear when comparing populations derived from experimental plots which were planted with on-farm produced certified seed potatoes during 2001 with the populations in the larger fields grown with breeders seed ($P < .001$, Table 3). While there were clear effects of origin of seed potatoes, no prediction as to the population composition over time could be made. Thus, the populations in the experimental plots during 2002 grown with potatoes produced on-farm in 2001 were again

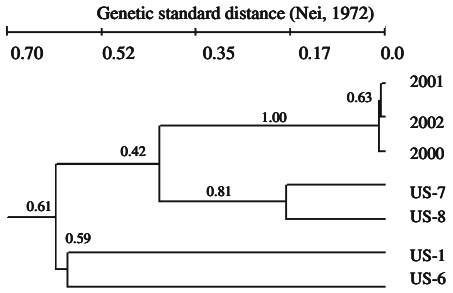

Fig. 5 UPGMA dendrogram of multilocus haplotypes (Rep-PCR fingerprints plus mating type) of *Phytophthora infestans* isolates collected from different sites in northern Hessia, 2000 to 2002, compared to single isolates of four clonal US lineages. Cluster analysis was based on a genetic standard distance matrix (Nei, 1972) with 1,000 permutations of bootstrapping and proportions of >.50 similar replicates are marked at the nodes

highly dissimilar from the 2001 populations (Table 3).

Discussion

Overall, the *P. infestans* populations studied were highly diverse with respect to pathotypes and rep-PCR fingerprints. While two rep-PCR fingerprints dominated the population in all three years, representing on average 62% of the isolates, these were clearly not clones as isolates with the same fingerprint differed for mating type and pathotype. There were clear effects of the origin of seed potatoes and year on the population composition and/or virulence frequencies while effects of potato cultivars and cropping history of fields were ambiguous.

The rep-PCR fingerprints were well reproducible and readily detected polymorphisms among the *P. infestans* isolates. The results for the reference isolates from the US were similar to SSR microsatellite analysis (Knapova & Gisi, 2002) and to RFLP results using probe RG-57 (Forbes et al., 1998). In these studies, lineage US-7 and US-8 always grouped closest together and clearly separated from US-1 (Knapova & Gisi, 2002) or US-1 and US-6 lineages (Forbes et al., 1998). In contrast, RAPD-PCR and AFLP analysis either grouped US-1 with US-7 or with US-8, respectively (Ghimire et al., 2002; Knapova & Gisi, 2002).

The apparently greater similarity of the north Hessian *P. infestans* populations to the reference isolate US-1 in comparison to isolates of the other US lineages does not allow for any conclusions

Table 3 Population differentiation of *Phytophthora infestans* isolates (χ^2-statistics, Raymond & Rousset, 1995) with different origin based on multilocus haplotypes

Population 1	Population 2	n^{a}_{loci}	n^{b}_{test}	χ^2	*P*-value	H′[c]
Potato cropping history						
Old fields with rotation	No rotation (2000–2002)	15	42|90	21	.87	.66|.78
Old fields (2000)	New plots (2000)	11	14|27	13	.93	.91|.57
Old fields (2001)	New plots (2001)	11	68|33	51	<.001	.60|.16
Old fields (2001)	New large fields (2001)	11	68|30	29	.12	.60|.48
Old fields (2002)	New plots (2002)	11	50|50	18	.25	.65|.61
Origin of seed potatoes in new fields[d]						
Simone	Secura (plots 2000)	10	16|11	14	.82	.59|.59
Agria	Linda (plots 2001)	2	18|15	7	.14	.09|.13
Agria	Linda (large fields 2001)	6	14|16	22	.04	.40|.60
Own certified (plots 2001)	Breeders seed (fields 2001)	6	33|30	63	<.001	.16|.48
Agria	Linda (plots 2002)	7	25|25	15	.39	.76|.62
Seed production fields (2001)	Plots (2002)	8	30|50	47	<.001	.48|.61

[a] n_{loci} = number of polymorphic loci (i.e., rep-PCR bands plus mating type) between populations. The resulting degrees of freedom are df = n_{loci} * 2

[b] n_{test} = number of isolates tested per population 1 and 2, separated with a vertical line

[c] Normalized Shannon diversity H′ calculated on multilocus haplotypes per population

[d] Potato plots during 2000 and seed production fields during 2001 were planted with two cultivars of introduced seed potatoes, while the plots from 2001 to 2002 were planted with certified seed potatoes produced on farm

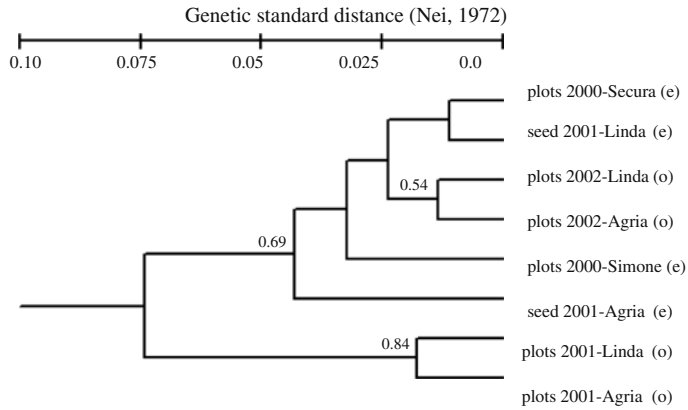

Fig. 6 UPGMA dendrogram of multilocus haplotypes of *Phytophthora infestans* isolates collected from new plots and seed production fields planted with two different potato cultivars from 2000 to 2002. The origin of the seed potatoes was either external (e) from various growers in northern Germany or from certified seed production on farm (o). Cluster analysis was based on a genetic standard distance matrix (Nei, 1972) with 1,000 permutations of bootstrapping and proportions of >.50 similar replicates are marked at the nodes

about the persistence of members of the pre-1980 population as the bootstrap values were low. Isolates of the old German populations would be necessary to allow for clear conclusions. Other recently characterized German populations were dominated by individuals belonging to the new populations (Rullich & Schöber, 2000). Generally, the similarity of our isolates with the US lineages was low as in the study of Knapova and Gisi (2002) comparing French and Swiss isolates with isolates of the US lineages, supporting the hypothesis of Goodwin, Cohen, Deahl, and Fry (1994) that the European populations may have evolved independently from the populations in the US. This is supported by results of characterization studies on composition and diversity of *P. infestans* populations in different European countries (Day, Wattier, Shaw, & Shattock, 2004; Drenth et al., 1994; Fry, Drenth, Mantel, Davidse, & Goodwin, 1991; Sujkowski, Goodwin, & Fry, 1996).

The overall diversity among the 281 isolates that were analyzed with rep-PCR and two primers ($H' = .64$) was similar to a Canadian study employing RAPD analysis. There, with six RAPD primers, 77 polymorphic bands were detected, resulting in high Shannon diversity ($H' = .76$) within the *P. infestans* populations (Mahuku et al., 2000). However, the results are highly variable as 11 primers resulted in only 26 polymorphic bands in a study in Nepal (Ghimire et al., 2002). While diversity indices $H' > .70$ have been reported for *P. infestans* in other European regions (Brurberg et al., 1999; Sujkowski et al., 1994) it is important to keep in mind the scale at which the collections were sampled. For example, the normalized Shannon diversity of populations from Norway and Finland was .75 and .83 (Brurberg et al., 1999). In contrast, our samples originated from a relatively small 15 by 20 km region in northern Hessia in central Germany. If sampling had been done in a larger area with a similar population structure, an even higher diversity could be expected.

With increasing sampling intensity more rare phenotypes will be picked up (Day et al., 2004). Thus, if there were no differences, a lower diversity should be expected in the old fields where sampling intensity was lower than in the new fields. In contrast, the number of different and unique rep-PCR types and the Shannon diversity indices among the isolates from the old fields were considerably higher than those from the new fields. This might be a result of the growing practices at those sites. Very similar to allotment gardens, potatoes were commonly grown with short or no rotation and the farmers usually planted their own potato seed. Other

researchers also reported higher genetic diversity of *P. infestans* populations derived from allotments than from conventional potato fields (Cooke et al., 2003; Zwankhuizen et al., 1998). Because of early infections, foci of severe infection, and the common co-existence of both mating types within most of the old fields, oospores may have provided some inoculum in these sites. However, the sampling intensity in the old fields was much lower than in the new areas. The association of the rarer virulences v2, v5 and v6 with mating type A2 and the significant differences in virulence frequencies between new and old fields on the other hand suggest that asexual reproduction and migration through seed potatoes were also important in shaping the *P. infestans* populations studied.

The effects of the origin of the seed potatoes were clearly evident in the large fields of Agria and Linda during 2001 and also when comparing with the experimental plots grown from the certified seed produced on the farm. In contrast, the lack of significant differentiation between the populations on Simone and Secura during 2000 might be due to differences in initial population size and also experimental plot arrangement. The Agria and Linda seed production fields (grown from basic seed tubers obtained from growers elsewhere) in 2001 were about 2 ha each while the size of the Simone and Secura experimental plots was only 700 m^2 per cultivar combined over all plots. Thus, most likely, the initial *P. infestans* population sizes from the introduced seed potatoes were much smaller in 2000 than in 2001.

The changes of the *P. infestans* populations from the seed potato fields in 2001 to the experimental plots in 2002 that were grown from these seed potatoes may be due to genetic bottlenecks through which a predominantly asexually reproducing population has to go through each winter (Drenth et al., 1994; Fry et al., 1991). These may have been very narrow for our studied populations due to low field size and also low tuber infections due to faster death of the unsprayed potato canopy and relatively unfavourable weather. The occurrence of only a few genotypes in successive years is also suggestive of bottlenecks which have been detected and inferred in many other cases (e.g., Drenth et al., 1994; Goodwin et al., 1995; Zwankhuizen, Govers, & Zadoks, 2000). It has been suggested that selective pressure in storage differs from that in the field as isolates of moderate aggressiveness will be advantageous for survival in stored tubers while highly aggressive isolates might kill their host and isolates of low aggressiveness might fail to establish infections in the field (Montarry, Corbiere, Lesueur, Glais, & Andrivon, 2005). Selection for a few advantageous genotypes during the winter would explain why the populations on Agria and Linda experimental plots were more similar to each other than to the populations in the original seed potato fields. A more detailed study of the isolates originating from the experimental plots during 2002 indicated cultivar-specific adaptation which was already apparent at the beginning of the epidemic (Bouws-Beuermann, 2005).

It is unclear if the results of this study are influenced by the organic growing practices at the experimental farm. In organic farming, seed potatoes are often pre-sprouted, a process during which most infected tubers should become visible and consequently discarded. This will contribute to late blight control. As the frequency of latent infected tubers and also the transmission rates via seed-borne inoculum are not finally clarified, more intensive screenings of seed tuber lots are needed when studying field populations. It would be interesting to compare the influence of migration via seed potatoes in organic versus conventional farming, when tubers are treated. Therefore, *P. infestans* populations from different locations/fields grown from the same lots of seed potatoes (and cultivars) should be characterized to better understand the role of seed potatoes on the inter- and intraregional population dynamics over time.

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References

Abu-El Samen, F. M., Secor, G. A., & Gudmestad, N. C. (2003). Genetic variation among asexual progeny of *Phytophthora infestans* detected with RAPD and AFLP markers. *Plant Pathology, 52*, 314–325.

Adler, N. (2001). Untersuchungen zum Befall von Kartoffel mit Phytophthora infestans (Mont.) de Bary mittels visueller Bonituren und PCR-Methoden. PhD Thesis. Germany: Agricultural University of Weihenstephan.

Andrivon, D. (1994). Dynamics of the survival and infectivity to potato tubers of sporangia of *Phytophthora infestans* in three different soils. *Soil Biology and Biochemistry, 26*, 945–952.

Black, W., Mastenbroek, C., Mills, W. R., & Peterson, L. C. (1953). Screening of *Solanum* species for resistance to physiologic races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivates. *Euphytica, 2*, 173–240.

Bourke, P. M. A. (1964). Emergence of potato blight, 1843–46. *Nature, 203*, 805–808.

Bouws-Beuermann, H. (2005). Effects of strip intercropping on late blight severity, yields of potatoes (Solanum tuberosum Lindl.) and on population structure of *P. infestans*. PhD Thesis. Germany: University of Kassel, Faculty of Organic Agricultural Sciences.

Brurberg, M. B., Hannukkala, A., & Hermansen, A. (1999). Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research, 103*, 1609–1615.

Carlisle, D. J., Cooke, L. R., & Brown, A. E. (2001). Phenotypic and genotypic characterisation of Northern Ireland isolates of *Phytophthora infestans*. *European Journal of Plant Pathology, 107*, 291–303.

Cooke, D. E. L., Young, V., Birch, P. R. J., Toth, R., Gourlay, F., Day, J. P., Carnegie, S. F., & Duncan, J. M. (2003). Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995–1997). *Plant Pathology, 52*, 181–192.

Day, J. P., & Shattock, R. C. (1997). Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European Journal of Plant Pathology, 103*, 379–391.

Day, J. P., Wattier, R. A. M., Shaw, D. S., & Shattock, R. C. (2004). Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995–98. *Plant Pathology, 53*, 303–315.

Drenth, A., Turkensteen, L. J., & Govers, F. (1993). The occurrence of the A2 mating type of *Phytophthora infestans* in the Netherlands; significance and consequences. *Netherlands Journal of Plant Pathology, 99*, 57–67.

Drenth, A., Tas, I. C. Q., & Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology, 100*, 97–107.

Forbes, G. A., Goodwin, S. B., Drenth, A., Oyarzun, P., Ordonez, M. E., & Fry, W. E. (1998). A global marker database for *Phythopthora infestans*. *Plant Disease, 82*, 811–818.

Fry, W. E., Drenth, A. T., Mantel, B. C., Davidse, L. C., & Goodwin, S. B. (1991). Population genetic structure of *Phytophthora infestans* in the Netherlands. *Phytopathology, 81*, 1330–1336.

Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Spielman, L. J., Deahl, K. L., Inglis, D. A., & Sandian, K. (1993). Historical and recent migrations of *Phytophthora infestans*. Chronology, pathways, and implications. *Plant Disease, 77*, 653–661.

Ghimire, S. R., Hyde, K. D., Hodgkiss, I. J., Shaw, D. S., & Liew, E. C. Y. (2002). Variations in the *Phytophthora infestans* population in Nepal as revealed by nuclear and mitochondrial DNA polymorphisms. *Phytopathology, 93*, 236–243.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., & Fry, W. E. (1992). Clonal diversity and genetic differentiation of populations in Northern and Central Mexico. *Phytopathology, 82*, 955–961.

Goodwin, S. B., Cohen, B. A., Deahl, K. L., & Fry, W. E. (1994). Migration from Northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology, 84*, 553–558.

Goodwin, S. B., Sujkowski, L. S., & Fry, W. E. (1995). Rapid evolution of phytopathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology, 85*, 669–676.

Goodwin, S. B., Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K., & Fry, W. E. (1998). Genetic changes within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology, 88*, 939–949.

Goudet, J., Raymond, M., de Meeus, T., & Rousset, F. (1996). Testing differentiation in diploid populations. *Genetics, 144*, 1933–1940.

Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., & Fry, W. E. (2003). Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology, 93*, 738–746.

Jenkins, J. C., & Jones, R. K. (2003). Classifying the relative host reaction in potato cultivars and breeding lines to the US-8 strain of *Phytophthora infestans* in Minnesota. *Plant Disease, 87*, 983–990.

Johnson, D. A., Cummings, T. F., & Hamm, P. B. (2000). Cost of fungicides used to manage potato late blight in the Columbia Basin: 1996 to 1998. *Plant Disease, 84*, 399–402.

Knapova, G., & Gisi, U. (2002). Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology, 51*, 641–653.

Mahuku, G., Peters, R. D., Platt, D. W., & Daayf, F. (2000). Random amplified polymorphic DNA (RAPD)

analysis of *Phytophthora infestans* isolates collected in Canada during 1994 to 1996. *Plant Pathology, 49*, 252–260.

Malcolmson, J. F., & Black, W. (1966). New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica, 15*, 199–203.

Maufrand, R., Archer, S. A., Buck, K. W., Shattock, R. C., & Shaw, D. S. (1995). The use of RAPDs in genetic studies of *Phytophthora infestans*. In L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, & E. O'Sullivan (Eds.), *Phytophthora* 150 (pp. 55–63). Dublin, Ireland: Boole Press Ltd.

Medina, M. V., Platt, H. W., & Peters, R. D. (1999). Severity of late blight tuber infection caused by US-1 and US-8 genotypes of *Phytophthora infestans* in 12 potato cultivars. *Canadian Journal of Plant Pathology, 21*, 399–390.

Miller, J. S., Cummings, T. F., Mikitzel, L. J., & Johnson, D. A. (2002). Influence of timing of harvest in relation to haulm killing and planting date on potato tuber rot caused by *Phytophthora infestans*. *Plant Disease, 86*, 264–268.

Montarry, J., Corbiere, R., Lesueur, S., Glais, I., & Andrivon, D. (2005). Does selection by resistant hosts trigger local adaptation in plant-pathogen systems? *Journal of Evolutionary Biology, 19*, 522–531.

Nei, M. (1972). Genetic distance between populations. *American Naturalist, 106*, 283–292.

Niederhauser, J. S. (1999). *Phytophthora infestans*: The Mexican connection. In J. A. Lucas, R. C. Shattock, D. S. Shaw, & L. R. Cooke (Eds.), *Phytophthora* (pp. 22–45). Cambridge: Cambridge University Press.

Platt, H. W., Peters, R. D., Medina, M., & Arsenault, W. (1998). Impact of seed potatoes infected with *Phytophthora infestans* (US-1 or US-8 genotypes) on crop growth and disease risk. *American Journal of Potato Research, 75*, 67–73.

Rademaker, J. L., Hoste, W. B., Louws, F. J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P., & de Bruin, F. J. (2000). Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studes: *Xanthomonas* as a model system. *International Journal of Systemic Evolutionary Microbiology, 50*, 665–677.

Raymond, M. L., & Rousset, F. (1995). An exact test for population differentiation. *Evolution, 49*, 1280–1283.

Rullich, G., & Schöber-Butin, B. (2000). Old and new populations of *Phytophthora infestans* in Germany. In K. Westerdijk & H. T. A. M. Schepers (Eds.), *Proceedings of the 5th Workshop on the European network for development of an Integrated Control Strategy of potato late blight* (pp. 233–238). Germany: 6–10 September 2000 in Munich.

Scortichini, M., & Rossi, M. P. (2003). Genetic diversity of *Xanthomonas arboricola* pv. *fragariae* strains and comparison with some other *X. arboricola* pathovars using repetitive PCR genomic fingerprinting. *Journal of Phytopathology, 151*, 113–119 .

Sheldon, A. L. (1969). Equitability indices: dependence on the species count. *Ecology, 50*, 466–467.

Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., & Fry, W. E. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology, 84*, 201–207.

Sujkowski, L. S., Goodwin, S. B., & Fry, W. E. (1996). Changes in specific virulence in Polish populations of *Phytophthora infestans* 1985–1991. *European Journal of Plant Pathology, 102*, 555–561.

Tooley, P. W., Fry, W. E., & Villarreal-Gonzalez, M. J. (1985). Isozyme characterization of sexual and asexual *Phytophthora infestans*. *The Journal of Heredity, 76*, 431–435.

Tooley, P. W., Sweigard, J. A., & Fry, W. E. (1986). Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. *Phytopathology, 76*, 1209–1212.

van der Lee, T., de Witte, I., Drenth, A., Alfonso, C., & Govers, F. (1997). AFLP linkage map of the oomycete *Phytophthora infestans*. *Fungal Genetics and Biology, 21*, 278–291.

Versalovic, J., Schneider, M., de Bruijn, F. J., & Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence based PCR (rep-PCR). *Methods of Cell Molecular Biology, 5*, 25–40.

Zwankhuizen, M. J., Govers, F., & Zadoks, J. C. (1998). Development of potato blight epidemics. Disease foci, disease gradients, and infection sources. *Phytopathology, 88*, 754–763.

Zwankhuizen, M. J., Govers, F., & Zadoks, J. C. (2000). Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology, 106*, 667–680.