Genotypic Diversity of *Phytophthora infestans* in The Netherlands Revealed by DNA Polymorphisms

A. Drenth, S. B. Goodwin, W. E. Fry, and L. C. Davidse

First author: Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands, and Research Institute for Plant Protection, Binnenhaven 12, 6709 PD Wageningen. Second and third authors: Department of Plant Pathology, Cornell University, Ithaca, NY 14853. Fourth author: Biotech Center Royal Sluis, P.O. Box 22, 1600 AA Enkhuizen, The Netherlands.

Supported in part by The Netherlands Organization for Scientific Research (NWO); Dutch Potato Association (NAA); Department of Plant Pathology, Cornell University Hatch Project 153430, USDA Competitive Grant 89-37151-4477; and The Netherlands-America Fulbright Commission.

We thank F. Govers, J. C. Zadoks, and two anonymous reviewers for critical evaluation of the manuscript.

Accepted for publication 29 June 1993.

ABSTRACT

Drenth, A., Goodwin, S. B., Fry, W. E., and Davidse, L. C. 1993. Genotypic diversity of *Phytophthora infestans* in The Netherlands revealed by DNA polymorphisms. Phytopathology 83:1087-1092.

We used DNA fingerprinting to estimate genotypic diversity among 153 isolates of *Phytophthora infestans* collected from potato and tomato plants in 14 fields distributed over six regions in The Netherlands. The DNA fingerprint probe, RG57, hybridized to 21 fragments of genomic DNA, 16 of which were polymorphic. Thirty-five RG57 genotypes were identified among the 153 isolates. Half of the isolates had the most widely distributed genotype, which was found in 10 fields in five of the six regions sampled. However, 89% of the genotypes were detected in only one field, and 60% occurred only once. Two mitochondrial DNA types, designated A and B, were found. Type A occurred in 143 isolates and was found in all fields in every region. Type B, in contrast, was found in only 10 isolates, all collected in community gardens. Partitioning of the genotypic diversity into components with the Shannon diversity index revealed that 52% of the diversity was associated with differences occurring within fields, 8% was due to differences among fields within regions, and 40% was accounted for by differences among regions. Genotypic differentiation was observed between isolates collected in community gardens and in commercial potato fields. Canonical variate analysis grouped isolates from commercial potato fields together, regardless of the geographic distance between the fields. Isolates from community gardens differed among regions and differed from the isolates collected in commercial potato fields.

Additional keywords: Oomycetes, population genetics, potato late blight, restriction fragment length polymorphism (RFLP).

Phytophthora infestans (Mont.) de Bary, the fungus that causes late blight of potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.), is considered the most damaging pathogen of potatoes worldwide (15). This oomycete is heterothallic, with two mating types designated A1 and A2. Pairing between isolates of opposite mating type results in the production of oospores.

Until the early 1980s, only the A1 mating type was distributed throughout the world; the A2 mating type was detected only in central Mexico. The appearance of the A2 mating type in Europe in 1981 (14) was the first indication that *P. infestans* populations in Europe were changing. Subsequently, the A2 mating type was detected in many countries in Europe (18,22) and in Egypt (20), Japan (19), and the United States (5).

New allozyme genotypes were associated with the introduction of the A2 mating type into Europe, providing additional evidence for a major change in these populations (21). Because both mating types have been found within this "new" population, sexual reproduction of the pathogen is possible and can generate new genotypes with greater adaptability than in a strictly asexually reproducing population. Furthermore, functional oospores in soil can be an additional source of inoculum.

In a previous study (7), we used allozymes and mating types to characterize isolates of *P. infestans* collected in The Netherlands. Significant differences in genotype frequencies were detected not only among isolates collected in different regions of the country, but also between isolates of different mating types and between isolates collected from potato and tomato (7). Because we analyzed the diversity in only two allozymes, glucose phosphate isomerase (*Gpi*) and peptidase (*Pep*), and because the diversity for these allozymes is limited to two alleles, similarity of allozyme genotypes does not necessarily indicate a lack of genetic diversity among the isolates. The allozyme markers were simply not numerous enough to define an isolate-specific genotype. Hence, numerous polymorphic markers are necessary to distinguish *P. infestans* isolates unambiguously and to assess the level of genetic diversity accurately.

The availability of DNA fingerprint probes that detect numerous restriction fragment length polymorphisms within the genome of *P. infestans* (12) enables more detailed analysis of *P. infestans* isolates. Probe RG57, which hybridizes to 25 different nuclear DNA fragments, is derived from a *P. infestans* genomic library and represents a moderately repetitive nuclear DNA sequence (12). Of the hybridizing fragments, 13 are known to segregate independently (12). The fragment patterns are somatically stable and are transmitted to sexual progeny in a Mendelian fashion. Therefore, probe RG57 provides genetic markers that probably span a large part of the *P. infestans* genome (12), and RG57 DNA fingerprinting is a powerful tool for analyzing the genetic structure of *P. infestans* populations (13). For example, RG57 DNA fingerprinting of *P. infestans* isolates from northern Mexico revealed much greater diversity than was detected with *Gpi* and *Pep* allozyme markers (13).

In addition to nuclear DNA polymorphisms, the mitochondrial DNA of *P. infestans,* which is a circular DNA molecule of 36.2 kb (16), is polymorphic. Analysis of 14 *P. infestans* isolates from The Netherlands identified two mitochondrial DNA types, A and B (10). Type A is widely distributed throughout the world, whereas type B is restricted to places where the A2 mating type is found (10). Mitochondrial DNA is a useful marker for migration events because it has maternal inheritance only (10).

In the experiments described here, we attempted to determine the genotypic diversity within *P. infestans* populations in The Netherlands as revealed by DNA fingerprint probe RG57, the number of *P. infestans* genotypes, the geographic distribution

of these genotypes, and the types of mitochondrial DNA present in Netherland *P. infestans* isolates. These data will enhance our understanding of the population genetics of *P. infestans* and may be useful for disease management strategies in the future.

MATERIALS AND METHODS

Collection and culture of *P. infestans*. Isolates of *P. infestans* were collected in The Netherlands during the summer of 1989 as described by Fry et al (7) (Fig. 1). A two-level hierarchical sampling scheme (14 fields within six regions) was used to allow genetic diversity to be partitioned within and among regions (Table 1). Single-lesion samples were collected randomly from potato and tomato plants in community gardens and commercial potato fields. Community garden isolates from potato and tomato were collected from contiguous gardens. Fields within 40 km of each other were considered to be in the same region. The minimum and maximum distances between regions were 75 and 300 km, respectively.

Southern analysis. Mycelium for DNA extraction was grown in liquid Rye A medium (4) in the dark at 18 C for about 14 days. Mycelium was ground to a fine powder in liquid nitrogen and mixed for 4 min at 55 C with 2.5 ml of extraction buffer containing three parts of water-saturated phenol, two parts of triisopropylnaphthalene sulfonic acid (20 mg/ml), two parts of 4-aminosalicylic acid (120 mg/ml), and one part of 5× RNB (1 M Tris-HCL, 1.25 M NaCl, 0.25 M ethylene glycol-bis-(2-aminoethyl ether) N,N,N′,N′-tetraacetic acid). The mixture was shaken vigorously for 2 min, and 0.25 vol of chloroform-isoamyl alcohol (24:1, v/v) was added. The aqueous phase was then extracted once with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v) and twice with an equal volume of chloroform-isoamyl alcohol (24:1, v/v). Nucleic acids were precipitated with 0.6 vol of 2-propanol, dissolved in $T_{10}E_1$ (10 mM Tris-HCl [pH 8], 1 mM EDTA), and treated for 30 min with RNase A (20 μg/ml) at 37 C. This method yields 250–500 μg of DNA per gram (wet weight) of mycelium. For Southern blot analysis, approximately 5 μg of genomic DNA was digested with the restriction enzyme *Eco*RI according to the manufacturer's instructions and size-fractionated on a 0.8% agarose gel for 14–16 h at 40 V (600 Vh) before alkaline transfer to Hybond N^+ (Amersham, Buckinghamshire, England) hybridization membrane.

Membranes were prehybridized for 2 h at 65 C in 10 ml of hybridization solution (5× SSC, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate [SDS], 100 μg of salmon sperm DNA per milliliter). Probes were labeled with [α-^{32}P]dATP according to the random-primer labeling method of Feinberg and Vogelstein (6) and allowed to hybridize overnight. Filters were then washed three times for 20 min each at 65 C in 2× SSC, 0.5% SDS, and three times for 20 min each in 0.5× SSC, 0.1% SDS, and exposed for 1–3 days at −80 C to Kodak Xomat S film backed

Fig. 1. The six regions in The Netherlands where isolates of *Phytophthora infestans* were collected in 1989: northeast (NE), north (N), northwest (NW), west (W), central (C), and southwest (SW). One to four fields were sampled in each region.

TABLE 1. Regions sampled for *Phytophthora infestans* in The Netherlands in 1989 and the occurrence of RG57 genotypes by field

Region[a]	Field	No. of isolates	RG57 genotypes
Commercial potato fields			
NE	1	7	28
NE	2	24	3,10,13,28,29,32
NE	3	2	22,28
NE	4	2	28
N	5	8	28
N	6	8	2,28,32
NW	7	15	28
NW	8	18	28
SW	14	14	9,20,24–30,33–35
Community potato gardens			
C	10[b]	15	6,8,12,15,16,18,19,23,28
W	11	12	22,31
W	12[c]	6	17,21
Community tomato gardens			
C	9[b]	15	1,4–7,11,14
W	13[c]	7	17

[a] NE = northeast, N = north, NW = northwest, C = central, W = west, SW = southwest.
[b] Fields 9 and 10 were contiguous.
[c] Fields 12 and 13 were contiguous.

TABLE 2. Number of RG57 genotypes, RG57 hybridizing fragments, and polymorphic fragments and their relative frequencies in *Phytophthora infestans* isolates collected in six regions in The Netherlands

	Region[a]					
	NE	N	NW	C	W	SW
Different RG57 genotypes	7	3	1	15	4	12
RG57 hybridizing fragments	19	20	18	21	18	20
RG57 polymorphic fragments	8	10	0	15	4	10
Fragment no.						
1	1	1	1	1	1	1
2	0.11	0.25	0	0.27	0.56	0.36
3	0.97	0.94	1	0.73	1	0.93
4	1	1	1	1	1	0.50
5	1	1	1	0.83	1	1
6	0.86	0.94	1	0.13	0	0.93
7	0.97	0.94	1	0.73	1	0.93
8	0.91	0.94	1	0.93	0.92	0.64
9	0.86	0.94	1	0.40	0.44	0.79
10	1	1	1	0.83	1	1
13	1	1	1	1	1	1
14	1	1	1	0.97	1	1
14a	1	0.94	1	0.40	1	1
16	0.97	0.94	1	0.20	0.56	0.86
17	0	0.06	0	0.30	0	0.64
18	0	0	0	0.03	0	0
19	0.91	0.94	1	0.50	1	0.93
20	1	1	1	1	1	1
21	1	1	1	1	1	1
24	1	1	1	0.97	1	1
25	1	1	1	1	1	1

[a] NE = northeast, N = north, NW = northwest, C = central, W = west, SW = southwest.

with an intensifier screen. Probe RG57 was removed from the membrane by incubation for 30 min in 0.4 N NaOH at 42 C. Membranes were then hybridized with 25 ng of total purified mitochondrial DNA (10) to reveal polymorphisms in the mitochondrial DNA.

Data analysis. A multicharacter genotype was derived for each isolate based on the DNA fingerprint pattern. The RG57 genotypes were classified on the basis of the presence or absence of fragments, and each fragment was assumed to represent a single genetic locus (12). Isolates with the same RG57 fingerprint pattern were considered to be identical genotypes.

The Shannon diversity index (1) was used to measure genotypic diversity as described previously (11). Genotypic diversity in each field was calculated as $h_o = -\Sigma_{i=1}^{k} p_i \ln p_i$, where p_i is the frequency of isolates with the i^{th} genotype in the field and k is the number of genotypes in the field.

Because a two-level hierarchical sampling scheme (fields within regions) was used when isolates were collected, the total genotypic diversity can be partitioned into components based on the amount of diversity within and among subpopulations. The relative magnitude of each component was assessed following methods developed by Lewontin (17), Zhang et al (25), and Goodwin et al (11). For each region, h_{field} was calculated as the mean of h_o for all fields in the region, and h_{region} was calculated as the mean frequency of all genotypes within the region. The total diversity, h_{total}, was determined from the mean frequencies of all genotypes in the entire sample. The mean within-field and among-field within-region diversity values, $\bar{h}_{field}$ and $\bar{h}_{region}$, were the average h_{field} and h_{region} values, weighted by the number of fields in each region. The total diversity was allocated to hierarchical components as follows: $\bar{h}_{field}/h_{total}$ is the proportion of total diversity that is due to differences within fields; $(h_{region} - \bar{h}_{field})/h_{total}$ is the proportion of total diversity due to differences among fields within regions; and $(h_{total} - \bar{h}_{region})/h_{total}$ is the proportion of total diversity due to differences among regions.

The data obtained with probe RG57 (presence or absence of fragments) and mitochondrial DNA types (A or B) were also analyzed by canonical variate analysis (CVA) with the GENSTAT

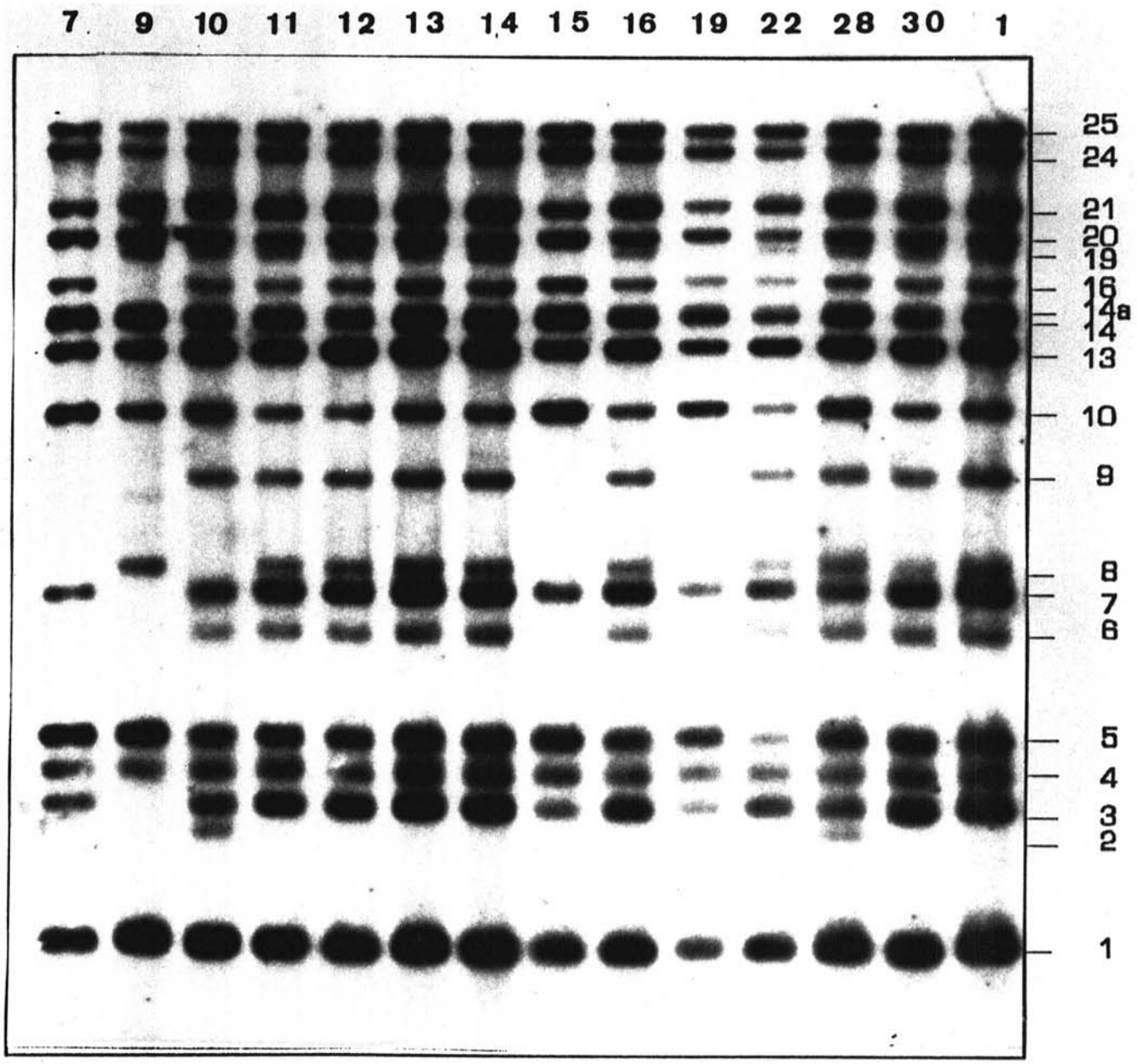

Fig. 2. Autoradiograph of a Southern blot after hybridization with probe RG57, showing DNA fingerprint patterns of representative isolates of *Phytophthora infestans* from the northeast region in The Netherlands. The numbers along the top are isolate numbers. Isolate 1 is from field 3; the other isolates shown are from field 2. The fragment numbers indicated at the right correspond to those used by Goodwin et al (12). Six genotypes can be observed on this autoradiograph (see also Table 1): isolate 9 is genotype 3, isolate 7 is genotype 10, isolate 10 is genotype 29, isolate 28 is genotype 32, isolates 15 and 19 are genotype 13, and isolates 1, 11–14, 16, 22, and 30 are genotype 28 (see Table 3 for genotypes).

statistical package (Genstat 5, release 2.1; Rothamsted Experimental Station, Harpenden, United Kingdom). CVA is a multivariate technique that does not require any underlying distributional assumptions (9). This analysis allows the detection of genotypic differentiation among groups of isolates—for example, isolates from different fields—rather than among the individual isolates. In the multidimensional space of the original variables, CVA selects directions such that the ratio of among-field diversity to within-field diversity exhibited in each direction is maximized, before reducing the number of dimensions. Thus, a large part of the diversity can be represented by the first two latent vectors, which can be plotted on two axes. In this way, genotypic differentiation among preassigned groups can be visualized. Groups were formed by field (14 groups) to gain insight into the genotypic differentiation among fields.

RESULTS

Identification and geographic distribution of genotypes. DNA fingerprinting patterns of 153 isolates collected in six geographic regions in The Netherlands (Table 1) showed that probe RG57 hybridized to 21 fragments of nuclear genomic DNA, 16 of which were polymorphic (Table 2). Fragments 1, 13, 20, 21, and 25 were invariant (Fig. 2). Thirty-five genotypes were identified among the 153 isolates (Table 3). Most of the genotypes (60%) were unique.

Fragment frequencies among isolates collected in the northeast and the north were fairly similar (Table 2). Of the fragments found in isolates collected in the north, only fragment 17, which was found at a low frequency in the north, was not found in isolates collected in the northeast. The isolates collected in the northwest did not show any diversity.

Isolates from the central region contained all the fragments found in the 1989 population (Table 2). Polymorphism of fragments 5, 10, 14, 18, and 24 was found only among these isolates, and they were the only isolates that contained fragment 18. Fragments 6, 9, 14a, 16, and 19 occurred at a much lower frequency in the isolates collected in the central region than in isolates collected from commercial potato fields in the northeast, north, northwest, and southwest.

The isolates collected in the west lacked fragment 6 and had a relatively low frequency of fragments 9 and 16 compared to the isolates collected in commercial potato fields. Polymorphism of fragment 4 was restricted to isolates collected in the southwest, and these isolates had fragment 17 at a higher frequency than all other isolates.

Genotypic diversity varied among regions. In the central and southwest regions, genotypic diversity was high: Of the 21 RG57 fragments identified in isolates collected in the central region, 15 were polymorphic, resulting in 15 different genotypes (Table 2); in the southwest, 12 genotypes were found. In the north and west, genotypic diversity was limited to three and four genotypes, respectively. The northwest had no diversity at all; all isolates had the same genotype.

Most of the genotypic diversity in the northeast was in field 2, in which six genotypes were found (Table 1). Genotype 28 was present in all four fields in the northeast. Genotype 28 was prevalent in the north; only two other genotypes, 2 and 32, were found there. In the northwest, all of the isolates from both of the fields sampled were genotype 28. In contrast, the isolates collected in the central region were very diverse. Field 9 contained seven genotypes, six of which were unique; the seventh (genotype 6) was also found in contiguous field 10. Seven of the nine genotypes found in field 10 were unique to this field. Field 11

TABLE 3. DNA fingerprint patterns (1 = presence, 0 = absence of each fragment) revealed by probe RG57, the mitochondrial types identified in each RG57 genotype, and the frequency of each genotype among 153 isolates of *Phytophthora infestans* from The Netherlands

RG57 genotype	RG57 fragment[a]																					Mitochondrial DNA type	Frequency
	1	2	3	4	5	6	7	8	9	10	13	14	14a	16	17	18	19	20	21	24	25		
1	1	0	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	1	1	1	1	A	1
2	1	1	0	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	A	1
3	1	0	0	1	1	0	0	1	0	1	1	1	1	0	0	0	1	1	1	1	1	A	1
4	1	0	0	1	1	0	0	1	0	1	1	1	0	0	1	0	1	1	1	1	1	B	1
5	1	0	1	1	1	0	1	1	0	0	1	1	0	0	0	0	1	1	1	1	1	A	4
6	1	0	1	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1	B	5
7	1	1	0	1	1	0	0	1	0	1	1	1	0	1	0	0	0	1	1	1	1	A	1
8	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	A	4
9	1	1	0	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	1	A	1
10	1	0	1	1	1	0	1	0	0	1	1	1	1	1	0	0	0	1	1	1	1	A	2
11	1	0	1	1	1	0	1	1	0	1	1	1	0	0	1	0	0	1	1	1	1	A	3
12	1	0	0	1	1	0	0	1	1	1	1	1	1	0	1	0	1	1	1	0	1	B	1
13	1	0	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	A	1
14	1	0	1	1	1	0	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	B	1
15	1	0	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	A	1
16	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	1	1	A	1
17	1	0	1	1	1	0	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	A	11
18	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	0	1	1	1	1	1	A	2
19	1	0	1	1	1	0	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	A	1
20	1	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	A	1
21	1	1	1	1	1	0	1	0	0	1	1	1	1	1	0	0	1	1	1	1	1	B	2
22	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	A	2
23	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	A	1
24	1	0	1	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	A	1
25	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	A	1
26	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	A	1
27	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	A	1
28	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	A	77
29	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	A	2
30	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	A	2
31	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	A	11
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	A	5
33	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	A	1
34	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	A	1
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	A	1

[a] Fragment numbers correspond to those used by Goodwin et al (12,13).

in the west contained two genotypes; one (genotype 31) was unique, and the other (genotype 22) was also found in field 3 in the northeast. Fields 12 and 13 contained genotypes that were only found in the west. Field 14 in the southwest was infested by 12 genotypes, 10 of them unique to this field. The other two were the prevalent genotype 28 and genotype 29, which was also identified in field 2 in the northeast.

Seventy-seven of the 153 isolates had genotype 28 (Table 3). This genotype was found in 10 fields and in every region except the west. Eleven isolates had genotype 17, which occurred only in fields 12 and 13 in the west (Table 1). Genotype 31 was identified in 11 isolates, all from field 11 in the west. Two isolates from field 2 (northeast) and three from field 6 (north) had genotype 32. In field 9 (central), five isolates had genotype 6. Genotypes 22 and 29 were each found twice, genotype 22 in fields 3 (northeast) and 11 (west) and genotype 29 in fields 2 (northeast) and 14 (southwest). Four genotypes (10, 18, 21, and 30) were each found twice in the same field. Twenty-one genotypes were found in only one isolate each (Table 3).

Occurrence of mitochondrial DNA types. Two mitochondrial DNA types were observed among the 153 isolates analyzed. They are identical to the A and B types described by Goodwin et al (10) that correspond to types I and II described by Carter et al (2,3). Isolates that belonged to the same RG57 genotype always had the same type of mitochondrial DNA. Type A, found in 30 genotypes among 143 isolates, was most common and was found in isolates from all 14 fields. Type B was found in 10 isolates with five genotypes. These isolates were collected in gardens in the central and west regions, where the frequency of type B was 0.27 and 0.08, respectively.

Hierarchical components of genotypic diversity. When total genotypic diversity, estimated by the Shannon diversity index based on data obtained with probe RG57, was partitioned into hierarchical components by field within regions, the within-field component of diversity was 52%, the among-fields within-regions component was 8%, and the among-regions component was 40%.

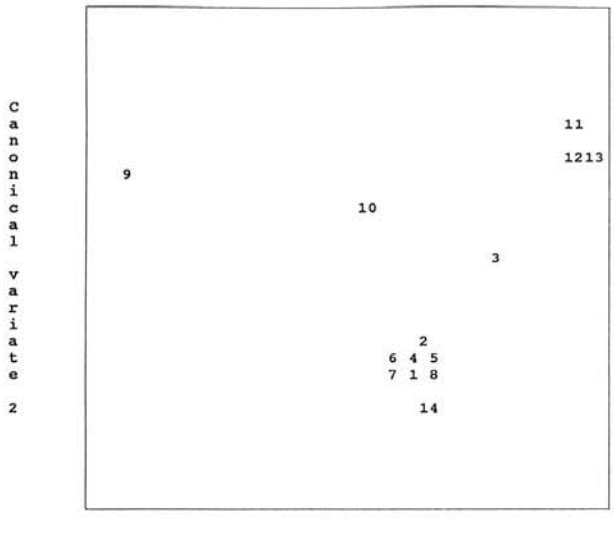

Fig. 3. Plot of the canonical variate means in the first two dimensions that represent the 14 fields (1–14) in which isolates of *Phytophthora infestans* were collected. Mitochondrial DNA type (A or B) and the 16 polymorphic RG57 bands were used as input in the canonical variate analysis. The *x*-axis shows the first canonical vector and represents 40.5% of the total diversity. The *y*-axis shows the second canonical vector and represents 31.3% of the diversity. The first and second canonical variate means showed ratios of among-field to within-field variation in each dimension of 8.2 and 6.3, respectively. Clustering can be observed among isolates collected in fields 11–13 (community gardens) from the west region and among isolates collected in fields 1, 2, 4–8, and 14 (commercial potato fields) in the northeast, north, northwest, and southwest regions (see Table 1). Genotypic differentiation is clearly present among isolates collected in fields 9 and 10, fields 11–13, and fields 1, 2, 4–8, and 14.

Genotypic differentiation among fields. The 14 fields were used as groups in a CVA to search for genotypic differentiation among fields (Fig. 3). The first canonical variate accounted for 40.5% of the diversity and the second for 31.3% of the diversity. It is evident from Figure 3 that fields 1, 2, 4–8, and 14 are clustered. This cluster comprises commercial potato fields located in four regions. Fields 11–13, all in the west, form another cluster. Isolates from field 9, collected from tomato plants in a community garden complex (Table 1), were the most distinct from other isolates. Isolates from field 13 were also collected from tomato plants but were very similar to the isolates collected from potato in the neighboring field 12. Field 3, which contained many different genotypes (Table 1), is situated in the CVA plot somewhat away from the cluster of other commercial potato fields. The CVA plot clearly shows that the isolates from community gardens (fields 9–13 from the central and west regions) form different clusters than the isolates from commercial potato fields. All the commercial potato fields except field 3 form one cluster regardless of their geographic locations.

DISCUSSION

DNA fingerprinting of isolates with probe RG57 revealed significantly more diversity in the *P. infestans* population in The Netherlands than had been detected previously. Population analysis based on allozymes, mating type, and metalaxyl resistance identified 17 genotypes among 205 isolates (7), compared to 35 genotypes revealed among 153 isolates by RG57 DNA fingerprinting. Combining all markers distinguishes 45 genotypes among the 153 isolates.

Several populations that showed limited or no diversity in previous studies (7) were demonstrated to consist of different RG57 genotypes. For example, the combination of allozymes, mating type, and metalaxyl resistance differentiated three genotypes among 17 isolates from field 14 in the southwest, whereas probe RG57 revealed 12 genotypes among 14 isolates analyzed. Thus, allozyme uniformity does not necessarily imply lack of genetic diversity in *P. infestans* populations. This is especially true in The Netherlands, where only two alleles for *Gpi* and two for *Pep* have been found (7). Similar findings were reported recently by Goodwin et al (13), who used RG57 DNA fingerprinting to analyze the Mexican *P. infestans* population structure.

In the hierarchical sampling scheme, most of the overall genotypic diversity occurred at the lowest level, within the fields. This high within-field component of diversity was largely due to the many unique genotypes in fields 2, 9, 10, and 14. The low level of genotypic diversity among fields within regions is indicative of high levels of gene flow within regions, either from migration of airborne sporangia or from common sources of seed potatoes. Migration probably occurs freely over large parts of The Netherlands, which would explain why genotype 28 was found in five of the six regions. In the west, the only region where genotype 28 was absent, the two genotypes identified (17 and 21) were found nowhere else. This region has very little commercial potato production, which may limit migration from the rest of the country.

The genotypic differentiation among regions is primarily due to the west and central regions, which differed from the other regions, as can be seen in the CVA plot (Fig. 3). The clustering of commercial potato field isolates in the CVA suggests a common source of inoculum for these fields. Multivariate analysis, in this case CVA, is more sensitive in revealing genotypic differentiation associated with geography than single-locus methods (24). Further investigations are needed to determine why isolates collected in community gardens in the west and central regions are different from each other as well as different from isolates collected in commercial potato fields in other regions.

Partial host substructuring was observed within the *P. infestans* population in The Netherlands (Fig. 3). The isolates in fields 9 (central) and 13 (west) were collected from tomato. Six of the seven genotypes found in field 9 were unique to this field, but the remaining genotype was also present in potatoes in the neigh-

boring field 10 (Table 1). And the single genotype identified in isolates collected from tomatoes in field 13 was also found in potatoes in the neighboring field 12. Evidently, some genotypes can occur on both hosts in The Netherlands. Hence, population substructuring by host, as reported in the literature (7,8,23), was not complete.

The occurrence of two types of mitochondrial DNA in *P. infestans* isolates from The Netherlands is comparable to the situation in the United Kingdom (2). The low frequency of isolates with type B can be explained by limited introduction of this type or by selection against this type. Why isolates with type B were restricted to the community gardens is unclear. Type B was found on both potato and tomato in the central region but only on potato in the west. Type B was found in A1 as well as A2 mating type isolates. Type A is most common throughout the world (10). Type B has been found in northern Mexico and in the countries where A2 mating type isolates have been introduced, such as Poland, The Netherlands, Japan, Brazil, Egypt, the United Kingdom, and the United States (2,10). Most likely, type B was introduced with the "new" *P. infestans* population.

The introduction of A2 mating type isolates into Europe has opened the possibility of sexual reproduction of *P. infestans*. Pairing of A1 and A2 mating type isolates from The Netherlands in vitro resulted in viable progeny (12). The pattern of diversity we found in The Netherlands is consistent with the hypothesis that sexual reproduction generates new genotypes and that occasional fit recombinants (e.g., genotype 28) become widespread as a result of rapid asexual reproduction. However, testing this hypothesis will be difficult because of the many genotypes that can survive asexually between seasons.

Because RG57 DNA fingerprinting has also been used to assess genotypic diversity in *P. infestans* populations in central and northern Mexico (13), we can compare those populations with our isolates from The Netherlands. The only difference in the fingerprinting analyses was the scoring of fragment 14a, which was not scored in the fingerprints of the Mexican isolates because it often comigrates with fragment 14. However, fragment 14a is found in Mexican isolates as well. All the fragments identified in the isolates collected in The Netherlands are also present in Mexican isolates, and isolates collected in central Mexico appear to have a few additional polymorphic RG57 fragments (11, 12, 15, 22, 23, 24a, and 25a) at low frequencies (13). This indicates that the present Netherland isolates belong to a subpopulation that originated from the *P. infestans* population in Mexico.

LITERATURE CITED

1. Bowman, K. O., Hutcheson, K., Odum, E. P., and Shenton, L. R. 1971. Comments on the distribution of indices of diversity. Pages 315–359 in: Statistical Ecology. Vol. 3, Many Species, Populations, Ecosystems, and Systems Analysis. G. P. Patil, E. C. Pielou, and W. E. Waters, eds. The Pennsylvania State University Press, University Park. 462 pp.
2. Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S., and Shattock, R. C. 1990. Restriction fragment length polymorphism of mitochondrial DNA of *Phytophthora infestans*. Mycol. Res. 94:1123-1128.
3. Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S., and Shattock, R. C. 1991. DNA polymorphism in *Phytophthora infestans:* The UK experience. Pages 272-294 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. British Mycological Society, Cambridge University Press, Cambridge.
4. Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
5. Deahl, K. L., Goth, R. W., Young, R., Sinden, S. L., and Gallegly, M. E. 1991. Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. Am. Potato J. 68:717-726.
6. Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-10.
7. Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C., and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81:1330-1336.
8. Giddings, N. J., and Berg, A. 1919. A comparison of the late blights of tomato and potato: A Preliminary Report. Phytopathology 9:209-210.
9. Gittins, R. 1985. Canonical Analysis: A Review with Applications in Ecology. Biomathematics Series, 12. Springer-Verlag, Berlin. Page 351.
10. Goodwin, S. B. 1991. DNA polymorphism in *Phytophthora infestans:* The Cornell experience. Pages 256-271 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. British Mycological Society, Cambridge University Press, Cambridge.
11. Goodwin, S. B., Allard, R. W., Hardy, S. A., and Webster, R. K. 1992. Hierarchical structure of pathogenic variation among *Rhynchosporium secalis* populations in Idaho and Oregon. Can. J. Bot. 70:810-817.
12. Goodwin, S. B., Drenth, A., and Fry, W. E. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr. Genet. 22:107-115.
13. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
14. Hohl, H. R., and Iselin, K. 1984. Strains of *Phytophthora infestans* with A2 mating type behavior. Trans. Br. Mycol. Soc. 83:529-530.
15. Hooker, W. J., ed. 1981. Compendium of Potato Diseases. The American Phytopathological Society, St. Paul, MN.
16. Klimzack, L. J., and Prell, H. H. 1984. Isolation and characterization of mitochondrial DNA of the oomycetous fungus *Phytophthora infestans*. Curr. Genet. 8:323-326.
17. Lewontin, R. C. 1972. The apportionment of human diversity. Evol. Biol. 6:381-398.
18. Malcolmson, J. F. 1985. *Phytophthora infestans* A2 compatibility type recorded in Great Britain. Trans. Br. Mycol. Soc. 85:531.
19. Mosa, A. A., Kato, M., Sato, N., Kobayashi, K., and Ogoshi, A. 1989. Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Japan. Ann. Phytopathol. Soc. Jpn. 5:615-626.
20. Shaw, D. S., Fyfe, A. M., Hibberd, P. G., and Abdel-Sattar, M. A. 1985. Occurrence of the rare A2 mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with A1 mating types. Plant Pathol. 34:552-556.
21. Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W. K., Tooley, P. W., and Fry, W. E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans?* Plant Pathol. 40:422-430.
22. Tantius, P. H., Fyfe, A. M., Shaw, D. S., and Shattock, R. C. 1986. Occurrence of the A2 mating type and self fertile isolates of *Phytophthora infestans* in England and Wales. Plant Pathol. 35:578-581.
23. Turkensteen, L. J. 1973. Partial resistance of tomatoes against *Phytophthora infestans,* the late blight fungus. Agric. Res. Rep. 810. Instituut voor Plantenziektenkundig Onderzoek, Wageningen-Nederland, Meded. 633.
24. Yeh, F. C., Cheliak, W. M., Dancik, B. P., Illingworth, K., Trust, D. C., and Pryhitka, B. A. 1985. Population differentiation in logdepole pine, *Pinus contorta* spp. *latifolia:* A discriminant analysis of allozyme variation. Can. J. Genet. Cytol. 27:210-218.
25. Zhang, Q., Webster, R. K., and Allard, R. W. 1987. Geographical distribution and associations between resistance to four races of *Rhynchosporium secalis*. Phytopathology 77:352-357.