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Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype

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Phytophthora infestans was isolated from potato leaves and tubers collected from different parts of Finland in 1990–96 and Norway in 1993–96. Isolates were assessed for mating type, resistance to metalaxyl and virulence phenotype. In Finland 15% of 200 isolates tested and in Norway 25% of 642 tested were A2 mating type. Differences in the A1/A2 ratio were evident among regions and A2 was not found in northern areas. In Finland the frequency of metalaxyl-resistant isolates, among 1834 tested, decreased from 59% in 1990–95 to 2% in 1996. In Norway 59% of 491 isolates tested were resistant to metalaxyl in 1996. Among 269 Finnish and 105 Norwegian isolates, all known virulence genes were found in both countries and race 1.3.4.7.10.11 was the most common (resistance gene *R9* was not included in the differential set). Oospores were observed in potato leaves from three locations in the southern part of Norway. The implications of the 'new' populations in the Nordic countries are discussed.

Keywords: mating type, metalaxyl resistance, *Phytophthora infestans*, potato late blight, virulence phenotype

Introduction

Late blight caused by *Phytophthora infestans* is globally the most important potato disease.

The pathogen is heterothallic, with two mating types designated A1 and A2. Until the 1980s, the A2 mating type was restricted to central Mexico and only the A1 mating type was distributed worldwide (Fry *et al*., 1993). Hohl & Iselin (1984) reported that isolates with A2 mating type were present in Switzerland from 1981. Thereafter, reports of 'new' populations of *P. infestans* consisting of both mating types have come from most parts of the world. In these populations, sexual reproduction may be expected to occur, which would have important consequences for potato late blight control (Spielman *et al*., 1991; Fry *et al*., 1993). Indications of sexual reproduction of the pathogen have been reported from the Netherlands (Drenth *et al*., 1994), Poland (Sujkowski *et al*., 1994) and Sweden (Andersson *et al*., 1998).

Insensitivity to phenylamide fungicides is common in *P. infestans* populations in several countries (Gisi & Cohen, 1996). Race diversity has increased after the migration of 'new' populations (Schöber & Turkensteen, 1992). Why the 'old' genotypes are displaced by 'new' genotypes is not understood, but differences in aggressiveness between genotypes are probably involved (Day & Shattock, 1997).

Mating type A2 was reported in Sweden in 1987 (Kadir & Umaerus, 1987). In 1988, metalaxyl resistance was found among seven isolates tested from one county in Norway (Magnus & Hjønnevåg, 1989). There are also reports of reduced sensitivity to metalaxyl in Sweden (Olofsson, 1989).

The main objective of this study was to characterize the population of *P. infestans* in Finland and Norway in order to decide the best control strategies for potato late blight in this part of Europe. A survey of mating types in different regions is needed as a basis for predicting the extent of sexual reproduction. The present study also aimed to show if oospores are formed in potato fields in the Nordic countries. Monitoring metalaxyl sensitivity is essential to permit optimal use of this fungicide. To reduce problems with R-genes in potato breeding programmes, complex races are needed for testing late blight resistance. Acquiring knowledge of the virulence pattern of the population is important when selecting these isolates.

From a global perspective, the Nordic populations of *P. infestans* are interesting because, in this region, potatoes are grown far north. Many of the potato fields are geographically isolated and therefore it is of

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interest to follow the possible migration of the new blight population into these areas.

Materials and methods

The population surveys of *P. infestans* in Finland and Norway started independently in the early 1990s and therefore details in methods are somewhat different.

Collection of samples

Potato leaves and tubers naturally infected by *P. infestans* were collected from Finland during 1990–96 and from Norway during 1993–96 (Table 1, Fig. 1). The majority of the Norwegian samples originated from farm fields and untreated experimental plots. The samples were taken from 21 cultivars, but mainly from the cvs. Beate, Pimpernel and Saturna. Leaf samples were collected mainly during August and September. Tuber samples were collected during the storage season. Some of the tuber samples came from the same fields as the leaf samples. In 1993 and 1994, usually, one leaf isolate was obtained from each of 65 fields in Norway (Table 1). In 1995 and 1996, up to 24 isolates were collected from each of 50 fields (Table 1). In some fields, the leaf samples were taken from different plants along a transect of 100–200 m. From field experiments the samples were taken at random within $\approx 500\,m^2$. Sometimes several samples were taken from a single plant.

The Finnish samples were obtained mainly from untreated field plots of cv. Bintje, which were established at five experimental stations of the Finnish Agricultural Centre and Potato Research Institute to monitor the onset of blight epidemics at different locations. Additional samples were provided by potato growers. The field samples were collected when the first blight symptoms appeared in potato crops, between the end of July and the first week of September. Three consecutive samples were taken from the plots, unless frost killed the crop; at the onset of the epidemic and at 10 and 20 days thereafter. Each sample consisted of 20–30 leaflets, each containing a single lesion (diameter < 1 cm). The sampling was carried out according to international guidelines for monitoring fungicide resistance (Sozzi *et al*., 1992; Williams & Gisi, 1992).

Isolation methods

In Norway isolations were mainly made on plates with rye B agar (Caten & Jinks, 1968), but with 2% glucose instead of sucrose, amended with $400\,mg\,L^{-1}$ pimaricin and $200\,mg\,L^{-1}$ ampicillin, and incubated at 18 °C in darkness. In Finland the isolation of *P. infestans* was carried out according to the methods described by Tantius *et al*. (1986) and Shattock *et al*. (1990). The rye agar was prepared from rye porridge flakes (Nalle, Melia Oy). Fifty grams of rye flakes was boiled for 3 min in 2 L of distilled water. The porridge was then filtered through cheesecloth and the volume of the filtrate was adjusted to 1 L by adding distilled water. Forty grams of sucrose and 30 g of agar were added and the filtrate was autoclaved at 120 °C for 20 min (rye F agar). The isolation medium was amended with $200\,mg\,L^{-1}$ vancomycin, $100\,mg\,L^{-1}$ rifamycin, $500\,mg\,L^{-1}$ nystatin and $100\,mg\,L^{-1}$ neomycin sulphate after autoclaving (rye FA agar).

Pure isolates were normally obtained after 1 or 2 transfers of hyphal tips on media containing antibiotics. Isolates were maintained on rye B or F agar without antibiotics at 18 °C and were transferred every 4–6 weeks until further characterisation or storage. Isolates were stored on rye B agar slants under mineral oil at 18 °C, or as 5 mm rye B agar plugs in dimethyl sulfoxide (DMSO) at − 130 °C (WE Fry, Cornell University, personal communication).

In Norway 688 isolates were obtained. In Finland 1463 leaf samples were collected, but only 250 of them were isolated on agar (Table 1). Isolates from single lesions have previously been shown to be single genotypes (Koh *et al*., 1994; Sujkowski *et al*., 1994). In this paper, the term 'isolate' is used to designate either pure cultures of the pathogen obtained from single lesions and maintained on agar, or sporangial suspensions collected from a single lesion without establishing a culture on agar.

Mating type determination

In Norway 642 isolates and in Finland 200 isolates were examined for mating type. *P. infestans* isolates CBS 430.90 (A1) and CBS 429.90 (A2) from Centraalbureau voor Schimmelcultures, Baarn, Netherlands were used to determine the mating type in Norway. In Finland isolates 90209 (A1) and 88055 (A2) from the Netherlands, obtained from Cyanamid Forschung GmbH, Germany, were used. Mycelial plugs (5 mm diameter) of the isolates were paired with the tester isolates (60–65 mm apart) on separate 90 mm plates with rye B agar in Norway and rye F agar in Finland. After 10–18 days of incubation at 18–20 °C in darkness, or at about 22 °C in the laboratory in daylight, the plates were examined for oospores at the hyphal interfaces between the isolates. If a plate yielded oospores, the test isolate was rated as the opposite mating type of the known isolate. Test isolates producing oospores with both A1 and A2 were scored as A1 A2. These could be self-fertile isolates or a mixture of mating types, but this was not determined.

Response to metalaxyl

The metalaxyl sensitivity of 491 Norwegian isolates from 1996 was determined on rye B agar plates (diameter 90 mm) with or without $10\,mg\,L^{-1}$ of technical-grade metalaxyl. The fungicide was dissolved in 0·1% DMSO. The control medium contained 0·1% DMSO without metalaxyl. Mycelial plugs (5 mm diameter) were cut from the margin of actively growing colonies of *P. infestans* and placed with the mycelium in

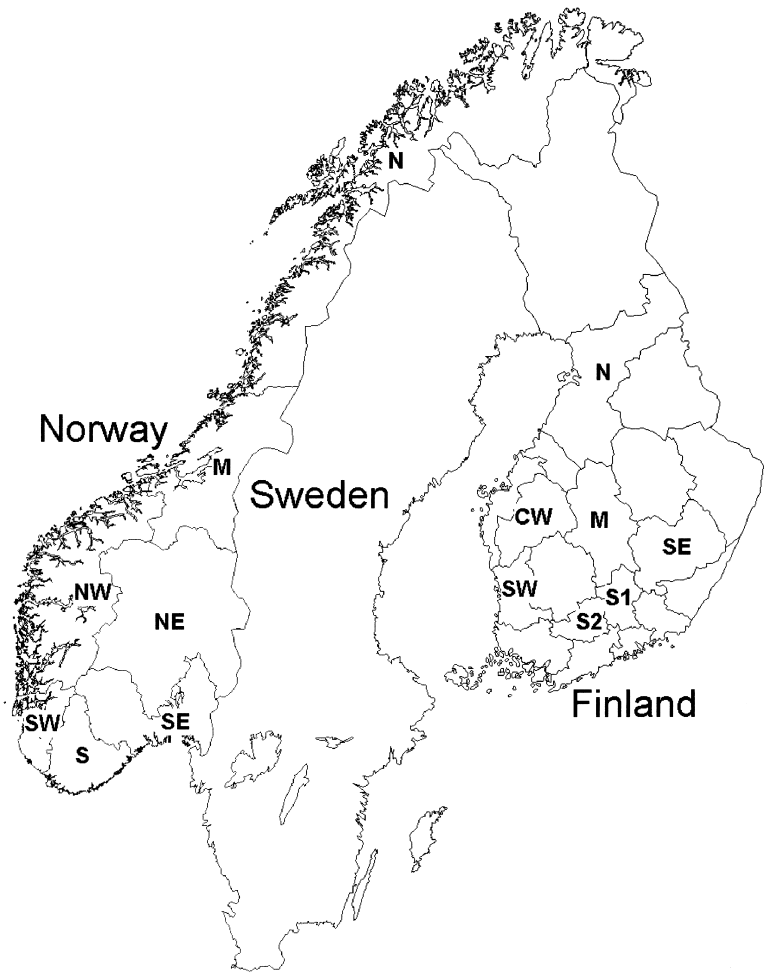

Figure 1 Regions in Norway and Finland where *Phytophthora infestans* samples were collected during the survey (see also Table 1).

contact with the test medium in the middle of the plates. The plates were incubated for up to 21 days at 18 °C in darkness. Colony diameters were measured in two perpendicular directions on all plates when the control had a diameter of at least 30 mm. The measurements were averaged and corrected for the size of the agar plug. There were two replicate plates for each isolate tested. Sensitive, intermediate and resistant isolates were defined as exhibiting < 10%, 10–60% and > 60% growth, respectively, on metalaxyl-amended agar, relative to growth on the control (Shattock, 1988).

In Finland, the response to metalaxyl of 1009 *P. infestans* isolates from 1990–92 was studied according to Ciba-Geigy guidelines for metalaxyl resistance monitoring (Fitzgerald, personal communication). For isolates from 1993–96, the international guidelines for resistance monitoring (Williams & Gisi, 1992) and the floating leaf-disk method (Sozzi *et al*., 1992) were used. In 1993–95, 683 isolates and in 1996, 142 isolates were tested. Leaf disks (15 mm diameter) were cut with a cork borer from leaves of cv. Bintje grown in the greenhouse for 4–5 weeks. Six leaf disks were floated in Petri plates (50 mm diameter) each containing 7 mL distilled water or metalaxyl (Ridomil 25 WP, CGA 48988) in 0·1, 1·0, 10·0 and 100·0 mg L^{-1} concentrations. Sporangia from pure cultures or from leaf samples with single lesions were inoculated on leaves of cv. Bintje to increase the inoculum. Then sporangia were collected into distilled water with a paintbrush and the spore concentration was adjusted to 100 000 sporangia mL^{-1}. Twenty microlitres of sporangial suspension was placed in the centre of each leaf disk. Test plates were incubated in the greenhouse under continuous light (natural daylight 6–18 h and artificial light (Airam HgMT-D 400 W) 18–6 h depending on the time of the year) at 15°C for 5–6 days. The relative humidity in the greenhouse was maintained at 100% for the first two days of incubation and at 80% thereafter. After incubation, the leaf disks were observed using a stereo microscope to estimate growth and sporulation. The area of each leaf disk producing sporulating mycelium was estimated. The isolates were rated as resistant if they sporulated on leaf disks in 100 mg L^{-1} metalaxyl. Those sporulating in metalaxyl concentrations of 1 or 10 mg L^{-1} were rated intermediate, and those sporulating only in water or 0·1 mg L^{-1} metalaxyl were rated sensitive.

Table 1 Origin of *Phytophthora infestans* isolates and numbers of tests for characters in isolates from Norway (1993–96) and Finland (1990–96)

Country/Region[a]	Year	Fields (*n*)	Isolates (*n*)	Tested for		
				Mating type	Metalaxyl sensitivity	Virulence
Norway						
Northern (N)	1993–94	2	3	3		
Middle (M)	1993–94	11	12	12		
	1995	4	11	11		
	1996	3	48	42	48	10
North-west (NW)	1993–94	11	14	14		
	1995	3	3	3		
	1996	7	48	47	48	8
South-west (SW)	1993–94	10	11	11		
	1995	14	46	46		
	1996	9	85	71	85	10
South (S)	1993–94	2	2	2		
	1995	2	11	11		
	1996	2	55	53	55	11
South-east (SE)	1993–94	20	22	22		
	1995	13	30	30		
	1996	21	158	137	158	33
North-east (NE)	1993–94	7	8	8		
	1995	14	22	22		
	1996	8	97	95	97	33
'Unknown'	1993–94	2	2	2		
Finland						
Northern (N)	1990–91	10	124	1	124	1
	1992–93	6	148	3	148	1
	1994–95	1	21	0	21	19
	1996	no blight				
Central West (CW)	1990–91	15	92	0	92	0
	1992–93	3	50	0	50	2
	1994–95	1	4	4	4	4
	1996	2	35	4	34	0
Middle (M)	1990–91	no blight				
	1992–93	2	108	0	108	4
	1994–95	2	42	27	42	34
	1996	no blight				
South-west (SW)	1990–91	6	50	0	50	1
	1992–93	3	139	1	139	1
	1994–95	4	67	24	67	57
	1996	1	13	0	13	0
South 1 (S1)	1990–91	8	64	0	64	1
	1992–93	8	192	7	192	7
	1994–95	4	60	24	60	36
	1996	1	52	9	52	13
South 2 (S2)	1990–91	8	172	0	172	0
	1992–93	4	186	30	186	7
	1994–95	3	86	62	86	79
	1996	1	42	3	42	0
South-east (SE)	1990–91	7	27	0	27	0
	1992–93	1	60	1	60	2
	1994–95	no blight				
	1996	no blight				

[a]Counties in regions in Norway – N: Finnmark, Troms, Nordland; M: Nord-Trøndelag, Sør-Trøndelag; NW: Møre og Romsdal, Sogn og Fjordane, Hordaland; SW: Rogaland; S: Vest-Agder, Aust-Agder; SE: Telemark, Vestfold, Akershus, Østfold; NE: Buskerud, Hedmark, Oppland; in Finland – N: Pohjois-Pohjanmaa, Keski-Pohjanmaa, CW: Etelä-Pohjanmaa, Österbotten, M: Keski-Suomi, SW: Satakunta, Varsinais-Suomi, S1: Päijät-Häme, S2: Kanta-Häme, SE: Etelä-Savo. See also Fig. 1.

Virulence tests

The number of virulence genes of an isolate was determined from the number of compatible interactions on a set of single *R*-gene differential potato clones, according to Tooley *et al.* (1986). A subset of isolates representing all the important potato-growing areas in the two countries was used. In Norway 75 isolates were tested. The differential clones used in Norway were obtained from Svalöf Weibull AB, Potato Department, Sweden. In Finland, 269 Finnish and 30 Norwegian isolates were tested. The differentials *R0*, *R1*, *R2*, *R4*, *R5*, *R7*, *R8*, *R10* and *R11* were obtained from Dr J. G. Harrison at the Scottish Crop Research Institute, Dundee, Scotland. The SCRI British set consisted of 19 clones, but only clones including single *R*-gene were used in tests. The clones including *R3* and *R6* were obtained from Hanne Grethe Kirk, LKF-Forædlingsstationen, Vandel, Denmark.

Fully or near-fully expanded leaflets were picked from plants at early flowering stage. In Norway, for each isolate four detached leaflets of each differential were placed with abaxial side up on moist filter paper in 150-mm-diameter Petri plates. Two 50 μL drops of a sporangial suspension ($2{\cdot}5 \times 10^4$ sporangia mL^{-1}) were placed on each leaflet. In Finland, the virulence test was done using the same procedure as for testing metalaxyl resistance. Six leaf disks (15 mm diameter) of each potato clone were floated in distilled water in 50-mm plastic Petri plates and inoculated with a 20 μL drop of a sporangial suspension containing 10^5 sporangia mL^{-1}. After seven days incubation at 15°C under low light (16 h cold white fluorescent light and 8 h dark), the leaflets or leaf disks were examined with a stereo microscope for sporulation of *P. infestans*. If sporulation was observed the interaction was rated compatible, and if no sporulation was observed, the interaction was rated incompatible. In some incompatible reactions a hypersensitive response was observed. Only those tests in which there were large, profusely sporulating lesions on the susceptible potato clone *r* (the clone without any *R*-genes) were used. Eighteen of the 105 Norwegian isolates were not tested on the potato clone *R10*.

Race diversity for the Norwegian and Finnish populations was calculated using the normalised Shannon diversity index (Goodwin *et al.*, 1992). The virulence complexity (average number of virulence genes per isolate) was also calculated.

Oospore formation *in vivo*

During the collection of *P. infestans*-infected plant material in Norway in 1996, leaflets with two lesions were sampled from some locations. Isolations from the lesions and mating type determination were carried out according to the procedures described. Each leaflet was put on a moist filter paper in a 90-mm Petri plate and placed at 15–18 °C in darkness to let the lesions merge. Afterwards the Petri plates containing the leaves were put at − 20 °C until further examination. The leaves were thawed at 20–24 °C and examined for the presence of oospores.

Statistics

The statistical analysis of frequencies of *P. infestans* isolates in different categories was performed using SAS (SAS Institute Inc.). The data were analysed with the SAS GENMOD procedure. The procedure allows the user to define the type of distribution of the data (SAS Institute Inc., 1993). The data were assumed to have a binomial distribution. The procedure performs logit transformation of the data before statistical calculations. The results of the analyses are expressed as probability values (p) related to chi-square values.

Results

Mating types

In Finland, the A2 mating type was detected for the first time in 1992, but only in 3% of the isolates. During 1993–96 the frequency was in the range 18–22% each year. In Norway, A2 was found during the first year of the survey and its frequency varied between 22 and 26% throughout the survey. In both countries there was a considerable variation ($P < 0{\cdot}001$) in the proportion of A1 and A2 among regions (Figs 1 and 2). In the northern (N) and middle parts (M) of Norway, and the northern (N) and south-eastern (S1, SE) parts of Finland only A1 was found. In southern Norway (NW, SW, S, SE, NE), the frequency of A2 ranged from 10 to 59%. But in one isolated growing area, Lærdal (NW), only A1 was found among the 45 isolates tested from five sites. In Finnish regions where both mating types were present (CW, M, SW, S2) the proportion of A2 varied from 8 to 33%. In Norway in 1995 and 1996, when more than one isolate was collected from each field, 34% and 39% of the fields, respectively, had both mating types present. In Finland in 1992–96, both mating types were present in 9 of 14 fields (64%) when more than one isolate was collected. In both countries A1 A2 isolates were very rare (< 1%). In Norway the frequency of A2 was higher ($P < 0{\cdot}001$) among isolates from leaves than from tubers (Table 2).

Metalaxyl resistance

In Finland metalaxyl-resistant isolates were predominant during 1990–95. Statistically significant differences ($P < 0{\cdot}001$) between regions in the frequency of sensitivity to metalaxyl were observed (Table 3). In 1996, only 2% of the isolates were resistant and they originated from the south-west coast (SW), where metalaxyl had been widely used for late blight control. In 1996 in Norway, 59% of all isolates were resistant to metalaxyl. The proportion of metalaxyl-resistant isolates from different regions in Norway and Finland ranged from 0 to 100% (Table 4). Differences in the frequency of

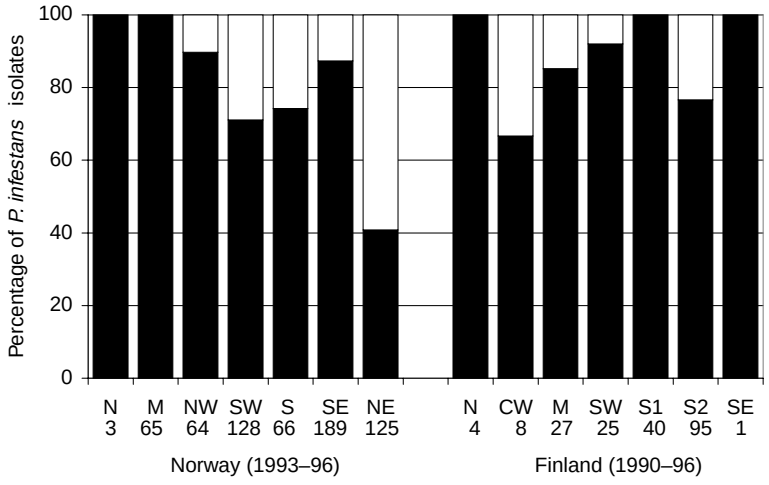

Figure 2 Frequency (percentage) of A1 (■) and A2 (□) mating type isolates of *Phytophthora infestans* in different regions of Norway (1993–96) and Finland (1990–96) (see Table 1 and Fig. 1 for details of regions). Sample sizes for each region are indicated below region codes.

Table 2 Percentage of the A2 mating type among *Phytophthora infestans* isolates from leaf, tubers and stems

	Norway (93–96)		Finland (90–96)	
Origin of isolates (tissue)	% A2	Isolates tested (*n*)	% A2	Isolates tested (*n*)
Leaf	30·3	446	17·1	152
Tuber	13·8	196	10·7	28
Stem			16·7	12

Table 3 Metalaxyl sensitivity[a] among isolates of *Phytophthora infestans* from different regions in Finland during 1990–95

Region[b]	MS (%)	MI (%)	MR (%)	Isolates tested (*n*)
N	29·7	12·3	58·0	293
CW	9·6	9·6	80·8	146
M	29·3	20·0	50·7	150
SW	19·5	30·5	50·0	256
S1	24·1	9·5	66·5	316
S2	31·8	15·1	53·2	444
SE	12·6	23·0	64·4	87

[a]MS, metalaxyl sensitive; MI, intermediate metalaxyl sensitive; MR, metalaxyl resistant.
[b]Refers to region codes in Table 1.

metalaxyl sensitivity were evident between isolates from the two countries ($P < 0{\cdot}001$), and between regions within countries ($P < 0{\cdot}001$).

Norwegian tuber isolates were more often resistant than isolates from leaves ($P < 0{\cdot}001$). This difference was not observed among Finnish isolates (Table 5). The frequency of resistant leaf isolates throughout the growing season did not show any clear pattern (data not shown). The proportion of intermediate isolates was in general (with some exceptions) highest in areas with the highest frequency of the A2 mating type (Tables 3 and 4 and Figs 1 and 2). Metalaxyl resistance was most frequent among A1 isolates ($P < 0{\cdot}001$) both in Norway and in Finland (Table 6).

Virulence tests

With the exception of virulence for resistance gene *R9*, which was not tested, all known virulence genes were found in isolates from Finland and Norway. Most of the isolates (89%) were able to overcome four or more *R*-genes. The virulence complexity (average number of *R*-genes overcome) was 5·30 in Finland and 5·78 in Norway. Two Norwegian isolates were virulent on all R-gene differential potato clones that were tested. The most common race was 1.3.4.7.10.11 in both countries (Table 7). From Norway, 105 isolates were tested and 38 different races were detected. In Finland, 66 different races were found among 269 isolates tested (Table 7). The normalised Shannon diversity index was 0·44 in Norway and 0·35 in Finland. The frequency of virulence genes was similar in Norway and Finland, except for virulence genes 2 and 8 (Fig. 3).

No obvious regional pattern emerged from the data on races in Norway and Finland. There was no correlation between race and mating type or race and metalaxyl resistance (data not shown).

Oospore formation in vivo

A total of 27 leaflets with two separate lesions were collected from six fields where both mating types were present. In four of the 27 leaflets both mating types were present and oospores, according to the descriptions of oospores of *P. infestans* (CMI description no. 838), were observed. These four leaves originated

Table 4 Metalaxyl sensitivity[a] among isolates of *Phytophthora infestans* from different counties in Norway and Finland during 1996

Country/county (region)[b]	MS (%)	MI (%)	MR (%)	Isolates tested (*n*)
Norway				
Nord-Trøndelag (M)	0·0	0·0	100·0	48
Sogn og Fjordane (NW)	0·0	2·2	97·8	46
Hordaland (NW)	0·0	100·0	0·0	2
Rogaland (SW)	18·8	64·7	16·5	85
Aust-Agder (S)	7·3	29·1	63·6	55
Telemark (SE)	0·0	100·0	0·0	1
Vestfold (SE)	5·4	27·0	67·6	37
Østfold (SE)	0·0	2·7	97·3	73
Akershus (SE)	0·0	2·1	97·9	47
Buskerud (NE)	22·2	77·8	0·0	18
Oppland (NE)	31·3	68·7	0·0	32
Hedmark (NE)	74·5	19·1	6·4	47
Finland				
Dagsmark (CW)	100·0	0·0	0·0	3
Kokemäki (SW)	7·7	69·2	23·1	13
Lammi (S1)	90·4	9·6	0·0	52
Tammela (S2)	100·0	0·0	0·0	42
Ylistaro (CW)	96·9	3·1	0·0	32

[a]MS, metalaxyl sensitive; MI, intermediate metalaxyl sensitive; MR, metalaxyl resistant.
[b]Refers to region codes in Table 1.

Table 5 Metalaxyl sensitivity[a] among isolates of *Phytophthora infestans* from leaves and tubers of potatoes in Norway (1996) and Finland (1990–93)

Origin of isolates (tissue)	MS (%)	MI (%)	MR (%)	Isolates tested (*n*)
Norway (leaf)	18·9	29·8	51·3	376
Norway (tuber)	0·0	18·3	81·7	115
Finland (leaf)	24·9	16·7	58·4	774
Finland (tuber)	32·3	10·3	57·4	223

[a]MS, metalaxyl sensitive; MI, intermediate metalaxyl sensitive; MR, metalaxyl resistant.

Table 6 Metalaxyl sensitivity[a] among isolates of *Phytophthora infestans* of A1 and A2 mating type in Norway (1996) and Finland (1990–96)

Mating type	MS (%)	MI (%)	MR (%)	Isolates tested (*n*)
A1 –Norway	9·8	18·4	71·8	326
A2 –Norway	29·6	56·5	13·9	115
A1 – Finland	61·9	14·2	23·9	155
A2 – Finland	75·9	17·2	6·9	29

[a]MS, metalaxyl sensitive; MI, intermediate metalaxyl sensitive; MR, metalaxyl resistant.

from three fields located in the southern part of Norway. Oospores were found in leaves of cv. Peik from Hedmark county (23 isolates tested; 17 A1, 4 A2, 2 A1 A2), cv. Beate from Buskerud county (18 isolates tested; 2 A1, 16 A2) and in cv. Oleva from Aust Agder county (30 isolates tested; 24 A1, 6 A2). All three cultivars showed intermediate resistance to *P. infestans*.

Discussion

It is not known how long the 'new' population of *P. infestans* has been in the Nordic countries. In 1985, 1 out of 12 isolates from Southern Sweden was mating type A2 (Kadir & Umaerus, 1987). The A2 mating type was first detected in Finland during 1992 and in 1993 in Norway. It was detected in Denmark during 1996 (Bødker *et al*., 1998). Throughout the period 1993–96, the frequency of A2 was stable and averaged 20% in Finland and 25% in Norway (data not shown). The A2 frequency has shown different patterns in other European countries. In England and Wales the A2 mating type was detected during 1981 (Tantius *et al*., 1986). The percentage of A2 varied between 7 and 15% of the isolates in surveys from 1985 to 1988 (Shattock *et al*., 1990). Later surveys from 1993 to 1995 showed A2 frequencies of 1–3% (Day & Shattock, 1997). In the Netherlands the frequency of A2 was 25% in 1985 and 11% in 1990 (Drenth *et al*., 1993). In Germany the proportion of A2 has increased from 6% in 1985 to 41% in 1987 and then decreased to 22% in 1990 and 7% in 1994 (Schöber-Butin *et al*., 1995). In Poland A2 was first detected in 1988. The frequency reached 44% by 1989 and then decreased to 12% by 1991 (Sujkowski *et al*., 1994). These reports show that in most European countries the A2 frequency has declined, although it might be difficult to compare frequencies in different

Table 7 Number of isolates of different races among isolates of *Phytophthora infestans* from Finland (1990–96) and Norway (1996)

Race	Finland	Norway	Race	Finland	Norway
1	1		1.4.7.8.11		1
7	1		1.4.7.10.11	5	2
1.3	2		1.5.6.10.11	1	
1.4	2	1	2.3.4.7.11	1	
1.11	1		3.4.7.8.11		1
3.11		1	3.4.7.10.11	2	1
4.11	1	1	1.2.3.4.10.11		1
1.2.4		1	1.2.4.5.7.11	1	
1.3.4	1		1.2.4.7.10.11	3	
1.3.7	3		1.3.4.5.6.11	2	
1.3.10	1		1.3.4.5.7.11		1
1.3.11	3		1.3.4.5.10.11	1	
1.4.7	2	3	1.3.4.6.7.11	1	1
1.4.10	2		1.3.4.7.8.10		1
1.4.11	4	1	1.3.4.7.8.11		1[1][a]
1.7.11	1		1.3.4.7.10.11	96	19
1.10.11	1		1.3.4.8.10.11	1	
2.3.10		1	1.4.5.6.10.11	1	
2.4.10	1		1.4.5.7.8.11	1	
4.10.11	1	2	1.4.5.7.10.11	1	1
1.2.4.7	1		1.4.6.7.10.11	1	
1.2.4.10	1		2.3.4.7.10.11	1	
1.3.4.7	4	4[3][a]	3.4.7.8.10.11	1	1
1.3.4.11	12		1.2.3.4.5.7.11	2	
1.3.7.11	5		1.2.3.4.6.10.11		1
1.4.7.10	3		1.2.3.4.7.10.11	3	8
1.4.7.11	4	2[1][a]	1.3.4.6.7.10.11	6	
1.4.10.11	7		1.3.4.7.8.10.11	10	4
3.4.7.10		2	1.2.3.4.5.6.10.11	1	
3.4.7.11	1	1	1.2.3.4.5.7.10.11	2	1
3.7.10.11	1		1.2.3.4.6.7.10.11	1	1
4.7.10.11	2		1.2.3.4.6.8.10.11		1
1.2.4.10.11		1	1.2.3.4.7.8.10.11	1	7
1.2.7.10.11	1		1.3.4.5.6.7.10.11	2	
1.3.4.5.7	1		1.3.4.6.7.8.10.11	1	
1.3.4.7.10	3	1	1.2.3.4.5.6.7.10.11	1	
1.3.4.7.11	33	18[13][a]	1.2.3.4.5.6.8.10.11	1	
1.3.4.10.11	6	3	1.2.3.4.6.7.8.10.11		5
1.3.7.10.11	2		1.2.3.5.6.7.8.10.11		1
1.4.5.6.11	1		1.3.4.5.6.7.8.10.11	1	
1.4.6.10.11	1		1.2.3.4.5.6.7.8.10.11		2
Total number of isolates				269	105
Total number of races				66	38

[a]Figures in square brackets indicate number of isolates not tested on potato clone R10.

countries because the figures given are often based on a limited number of isolates and monitoring sites.

Differences in the A1/A2 ratio between regions were evident in Norway and Finland. Mating type A2 was not detected in the middle and northern parts of Norway, or the northern and south-eastern regions of Finland. From northern Norway only 3 isolates were tested, but 65 were examined from 18 fields in the middle of Norway. Most of these fields were, however, located in one growing area. The genotypes of A1 in these regions are of the 'new' population (Brurberg *et al.*, 1999). Why only A1 was detected in some areas is not clear. One reason could be that the A1 genotypes are more fit in these regions because of differences in response to climatic parameters. Mizubuti & Fry (1998) reported that clonal lineages of *P. infestans* differed in response to temperature.

In Norway the importation of seed potatoes during the last 20 years has mainly been in small quantities for experimental use. However, 280 tons of cv. Saturna in 1987, and 10 tons of cv. Provita in 1989, were imported from Sweden (K. O. Larsen, Norwegian Agricultural Inspection Service, personal communication). Considerable quantities of ware potatoes are imported each year from other European countries. In Finland seed potatoes are imported mainly from the Netherlands and Germany. Seed potatoes are mainly produced in areas on the north-west coast of Finland, and distributed to other

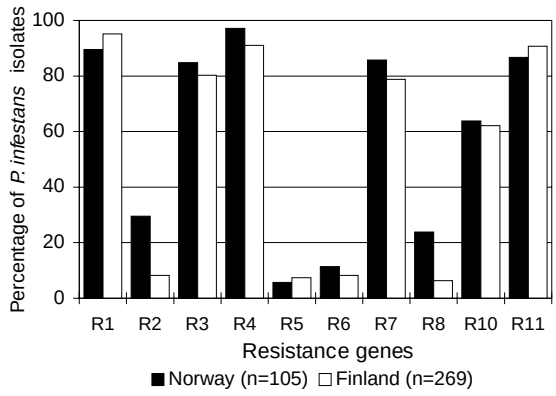

Figure 3 Frequency (percentage) of *Phytophthora infestans* isolates from Finland (1990–96) and Norway (1993–96) overcoming major-gene resistance.

potato-growing areas. Remarkably, A2 was not detected in Lammi, a research centre in the southern middle part of Finland, where much imported seed and many new cultivars have been grown.

Data from Norway showed a higher frequency of A2 among leaf isolates than among tuber isolates. No differences were found in A2 frequency from samples collected at different periods during the growing season (data not shown). If infected tubers are the main source of inoculum surviving the winter, a lower frequency of A2 isolates would have been expected early in the season. This indicates that A2 strains have better survival ability in tubers than A1 strains.

In Finland, metalaxyl-resistant strains dominated during 1990–95, while only a few resistant isolates were found during 1996. In Norway, only isolates collected during 1996 were tested for sensitivity to metalaxyl and the majority (59%) were resistant. Most of the isolates were sampled late in the season and some samples came from fields where metalaxyl had been used curatively. Both of these factors favour the occurrence of resistant isolates (Gisi & Cohen, 1996). In both countries, only prepacked mixtures of metalaxyl and mancozeb have been used. They are recommended for use only twice each season, and not to be used in seed potato production. The proportion of metalaxyl-resistant isolates in Finland during the first period of the survey and in Norway during 1996 is, however, in the same range as in other European countries (Gisi & Cohen, 1996).

There was a higher frequency of metalaxyl resistance among A1 isolates than A2 isolates. The frequency of A2 was low in some areas of Norway where metalaxyl has been used as an eradicative fungicide. This observation is, however, not evidence that the use of metalaxyl is the cause of low levels of A2 in these regions. Gisi & Cohen (1996) analysed data from different European countries and did not find any correlation between metalaxyl resistance and the proportion of either mating type, but they stated that the A2 genotypes are primarily metalaxyl-sensitive. Genetic studies indicate that there is no linkage between the locus for metalaxyl resistance and mating type locus (Lee *et al*., 1999).

In some regions of Finland and Norway, the frequency of intermediate metalaxyl-sensitive isolates was high. Most of these areas also had relatively high proportions of the A2 mating type. High levels of intermediate-resistant isolates are reported from regions with mixed populations of A1 and A2 in Mexico (Deahl *et al*., 1995), but also from populations where A1 is predominant, such as England and Wales (Day & Shattock, 1997).

In Norway the metalaxyl sensitivity test was carried out *in vitro* using metalaxyl-amended agar. This test might overestimate resistance in a field situation. However, there is good correlation between the *in vitro* and *in vivo* tests on leaf discs (Goodwin *et al*., 1996). Later, Goodwin *et al*. (1998) stated that the floating leaf disc method eliminated some of the variance in the amended-agar assay and they recommended the former test for all further studies.

As long as metalaxyl is used according to the recommendations in Finland and Norway it is unlikely that metalaxyl-resistant isolates will completely displace sensitive isolates. Several reports suggest that resistance is unstable in the population and is selected anew each year, increasing steadily during the season and declining over winter (reviewed by Gisi & Cohen, 1996). In Finland the proportion of metalaxyl-resistant isolates declined after the majority of farmers stopped using metalaxyl products during 1994–96. Day & Shattock (1997) demonstrated that metalaxyl-resistant isolates in England and Wales were less pathogenic on detached leaves than sensitive isolates. However, resistant isolates from ware potatoes imported from Mediterranean countries were not less pathogenic. The data presented here indicates that metalaxyl should be used with care in the future, and further studies are needed to find the best strategy for optimum efficiency of this fungicide in the Nordic countries.

The most common race, 1.3.4.7.10.11, in Norway and Finland, was also prevalent in France (Andrivon, 1994; Lebreton *et al*., 1998) and Switzerland (Gisi *et al*., 1995), and was among isolates collected during 1980 and 1981 in Denmark (Gürtler, 1984). In Poland during 1985–91 the most common race was 1.2.3.4.7.10.11 (Sujkowski *et al*., 1996), and in Germany it was 1.3.4.7.8.10.11 (Schöber-Butin *et al*., 1995). Race diversity (number of races among isolates tested) in Norway is somewhat higher than in Finland, and is in the same range as in other European populations (Schöber & Turkensteen, 1992; Sujkowski *et al*., 1996; Lebreton *et al*., 1998). Differences in race diversity between the two countries might be due to different sampling methods. In Norway, a few isolates were tested from a large number of fields, while in Finland, a large number of isolates were obtained from a few fields. The virulence complexity (mean number of virulence genes per isolate) in Finland (5·30) and Norway (5·78) is

somewhat higher than is found in the Netherlands (4·68) (Schöber & Turkensteen, 1992), France (4·83) (Lebreton *et al*., 1998), western Germany (4·98) (Rullich & Schöber, 1988) and eastern Germany (5·07) (Götz, 1986). In Poland, during 1985–91, the virulence complexity was higher (6·37) (Sujkowski *et al*., 1996). Race diversity calculated by the normalised Shannon diversity index showed lower values in Finland (0·35) and Norway (0·44) than from analyses of the 'new' population in the Netherlands (Drenth *et al*., 1994) and Poland (Sujkowski *et al*., 1996), but were in roughly the same range as in France (Lebreton *et al*., 1998).

Twenty Norwegian *P. infestans* isolates from 1997 were tested in Finland using the leaf disc method and the 'Finnish' potato differential set, and in Norway using both the Norwegian and the Finnish methods, with the 'Norwegian' potato differential set. The results in Finland and Norway were the same except for those on potato clone R2. More isolates were scored as virulent on R2 in Norway with both methods than in Finland (data not shown). The difference in the frequency of virulence gene 2 in Norway and Finland could be caused by the use of different *R*2 potato clones. None of the 20 isolates in the parallel test were virulent on R8, so this test did not provide any explanation for the small difference observed in the frequency of virulence gene 8 in the two countries.

Most Norwegian and Finnish potato cultivars do not contain major *P. infestans* resistance genes (Kankila *et al*., 1995; T Bjor, Agricultural University of Norway, personal communication). R1 is, however, present in at least three important cvs. R-gene selection certainly has not been of importance in generating the observed pattern of race variation in the Norwegian and Finnish *P. infestans* populations. One or more of the genes *R1*, *R3*, *R4* and *R10* are present in several European potato cvs. (Umaerus *et al*., 1983), which may explain the prevalence of race 1.3.4.10 (Schöber & Turkensteen, 1992). New populations introduced from Mexico into Europe probably carried virulence genes 5, 6, 7, 8 and 11 (Drenth *et al*., 1994). Mutation in the population also may account for the complexity of virulence (Goodwin, 1997). The virulence data provided here clearly shows that breeding programmes in the Nordic countries need complex races in their late blight resistance tests to select for apparently race-nonspecific resistance.

Oospores were found in leaves from three locations in two regions in Norway. In these fields the frequency of A2 ranged from 9 to 88%. The cultivars in which oospores were formed had medium levels of apparently race-nonspecific leaf resistance to *P. infestans*. This is common among cultivars used in Norway (T Bjor, Agricultural University of Norway, personal communication). There are reports indicating that more oospores are produced in cultivars showing intermediate levels of resistance to late blight than in more susceptible cultivars (Drenth *et al*., 1995; Hanson & Shattock, 1998).

The high and stable frequency of isolates of A2 mating type, the high number of fields with both mating types and the finding of oospores, suggest that sexual reproduction is involved in the development of *P. infestans* populations in Finland and Norway. DNA fingerprints with probe RG57 showed high levels of genetic diversity in these populations, consistent with a sexual means of reproduction (Brurberg *et al*., 1999). More work is needed to elucidate the role of oospores in the epidemiology of *P. infestans* in the Nordic countries.

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