Monitoring virulence and mating type of *Phytophthora infestans* in the Netherlands in 2004 and 2005.

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Introduction

In the Netherlands and elsewhere in the world, *Phytophthora infestans* is a major constraint to potato cultivation. Daily grower attention is required to prevent crops from being infected and destroyed. Annually, an average of 10 – 15 fungicide applications are needed to control potato late blight in the Netherlands. The annual cost of control amount to approximately 130M€ or 20% of the farm gate turn over (KWIN 2006).

It is expected that new, highly resistant, potato varieties are going to play a major role in future potato late blight control strategies and the effort to reduce the fungicide input in potato cultivation. However, *P. infestans* has proven to be highly efficient in adapting to restraints to population development such as newly introduced host plant resistance. So far, the lifespan of resistance genes was therefore generally short.

Introduction of combinations of resistance genes will, at least theoretically, slow down adaptation of the *P. infestans* population towards virulence. Continuous monitoring of the virulence spectrum, towards old and new R-genes is however necessary. A thorough knowledge of developments concerning the virulence spectrum provides additional opportunities to exploit R- gene resistance in a more durable way.

The goal of the work described in this paper was to collect and characterize *P. infestans* isolates in the Netherlands in 2004 and 2005 to gain insight in developments in the virulence spectrum of the Dutch *P. infestans* population.

Materials & Methods

Bait fields, containing old and new resistant material, were established at three locations, Lelystad, Valthermond and Vredepeel, in the Netherlands in 2004 and 2005. Black's R-gene differential set R0 – R11 (Black *et al.,* 1953; Malcomson & Black, 1966) and genotypes or cultivars provided by breeding companies were planted in 6 plants plots per genotype on all three locations. The plots were not treated with fungicides. Infection was monitored on a weekly basis, infected plant material was collected and occurring *P. infestans* was cultured. *P. infestans* isolates were subsequently stored in liquid nitrogen and characterised. A representative number of isolates per location, solanum genotype and year were characterized for AFLP-fingerprint (primer combination E21/M16), mating-type and virulence spectrum. 52 isolates obtained in 2004 and 62 isolates obtained in 2005 were characterized.

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The mating type test was initiated by co-inoculating the isolate to be tested and a A1 or A2 tester strain on pea-agar. Isolate IPO98014 was used as the A1 tester strain whereas isolate IPO655-2A was used as the A2 tester train. Formation of oospores was checked microscopically.
The virulence spectrum of the isolates was determined using Black's R-gene differential set (R0 – R11), by spray inoculating the lower side of two detached leaflets per genotype with a sporangial suspension (1 x 10^4 sporangia per ml). Inoculated leaflets were incubated in petri dishes containing 1.5 % water agar in a climate chamber at 15°C and 16 hours light per day as described by Flier and Turkensteen (1999). Following one week of incubation, necrosis and sporulation were quantified visually with the aid of a stereo microscope. Compatibility was assumed if at least 5 % of a leaflet was necrotic and sporulation could be detected. Two replicate experiments were carried out.

Results

AFLP
AFLP results are given in Table 1. The level of polymorphism within the 6 *P. infestans* populations is shown in terms of heterozygosity and the number of polymorphic loci. Fourteen and twenty eight loci (out of 78) were polymorphic in 2004 and 2005 respectively. Seven and ten groups of identical isolates were distinguished in 2004 and 2005 respectively. Genetic distances between the 6 *P. infestans* populations are shown in Figure 1.

Table 1. Analysis of genetic variability of *P. infestans* isolates based on AFLP results. Fourteen and twenty eight loci (out of 78) were polymorphic in 2004 and 2005 respectively.

year	Location	Population	# isolates	Average heterozygosity	# polymorphic loci	% polymorphic loci
2004	Lelystad	1	13	0	0	0
	Vredepeel	2	17	0.07	12	16
	Valthermond	3	19	0	0	0
2005	Lelystad	4	23	0.02	8	10
	Vredepeel	5	24	0.04	12	15
	Valthermond	6	19	0.10	23	29
2004	All locations	1,2,3	49	0.07	14	18
2005	All locations	4,5,6	66	0.08	28	35

Mating type
Table 2 gives an overview of the results of the mating type determination of the 2004 and 2005 isolates on all three locations.

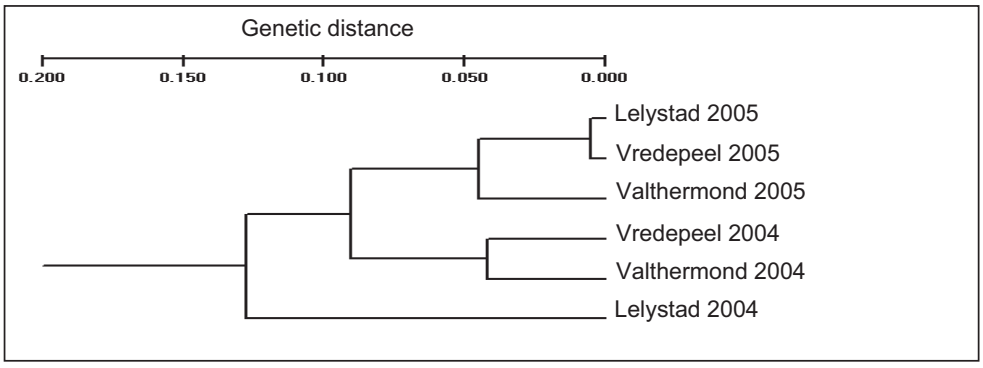

Figure 1. Dendrogram of the P. infestans population for years and locations.

Table 2. Mating type of the bait field isolates collected in 2004 and 2005 in Lelystad, Valthermond and Vredepeel.

	2004			2005		
Mating type	Lelystad	Vredepeel	Valthermond	Lelystad	Vredepeel	Valthermond
A1	28	1	0	1	8	21
A2	0	37	37	35	45	16
% A2	0	97	100	97	85	43

On all locations and in both years, one of both mating types was dominant except in Valthermond in 2005 where both mating types occurred in similar numbers (Table 3).

Table 3. *P. infestans* mating types per *Solanum* genotype occurring in bait fields in two years and three locations.

Genotype	Vredepeel		Lelystad		Valthermond	
	2004	2005	2004	2005	2004	2005
R 0	A2	A2	A1	A2	-	A1
R 1	A2	A2	A1	A2	A2	A1
R 2	A2	A2	A1	A2	A2	A2
R 3	A2	A2	A1	A2	A2	A1
R 4	A2	A2	A1	A2	A2	A1
R 5	A2	A2	*	A2	A2	A2
R 6	A2	A2	A1	A2	A2	A1 & A2
R 7	A2	A2	A1	A2	A2	A1
R 8	A2	A1	*	*	*	A1
R 9	A2	A1	A1	*	*	*
R 10	A2	A2	A1	A1	A2	A1
R 11	A1 & A2	A2	A1	A2	A2	A1
AM 66-42	A2	A2	A1	A2	A2	A1 & A2
Axona	-	A2	-	A2	-	A2
Biogold	A2	A2	A1	A2	A2	*
CMK-MCD1	A2	A2	*	A2	A2	A1
HZPC-02	A2	*	*	*	A2	*
HZPC-04	A2	A2	*	*	A2	*
HZPC-05	A2	A2	A1	A2	A2	A1 & A2
KA-0001	-	A2	-	A2	-	A2
KA-0002	-	A2	-	A2	-	A2
KA-95-0140	A2	A2	*	*	A2	*
Sarpo Mira	-	A2	-	*	-	*
Spirit	-	A2	-	A2	-	A1
VR -92-501	-	A2	-	A2	-	A2

–: Genotype not included in this experiment.
**: P. infestans not found in this genotype.*

Virulence

All *P. infestans* isolates displayed highly complex virulence spectra with an average of 8 to 10 virulence factors for Black's differentials R1 – R11 per isolate (Table 4). Figure 2 shows the frequency of individual virulence factors in Dutch *P. infestans* isolates in 2004 and 2005 at AFLP group level.

Table 4. Average number of virulence factors for R1 - R11 per isolate per year observed on the different locations. The maximum number of virulence factors is 11.

Location	2004	2005
Vredepeel	9.0	9.9
Lelystad	8.7	8.9
Valthermond	8.7	7.9

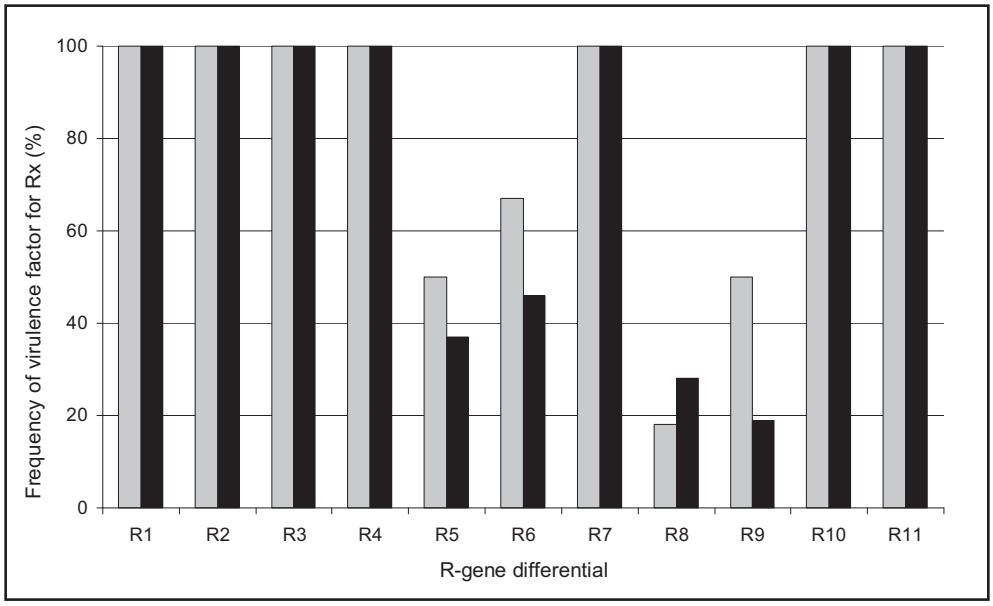

Figure 2. Frequency of virulence factors for Black's R1 – R11 in Dutch P. infestans isolates from 2004 (▒) and 2005 (■)at AFLP group level.

Discussion & Conclusions

The aim of the work described in this paper was to collect and characterize *P. infestans* isolates in the Netherlands in 2004 and 2005 to gain insight in development of the virulence spectrum of the Dutch *P. infestans* population. For this purpose bait fields containing Black's differentials and resistant breeding material were established in 2004 and 2005 at Valthermond, Lelystad and Vredepeel. Naturally occurring *P. infestans* was removed, cultured and characterized for AFLP pattern, mating type and virulence spectrum.

P. infestans was found on - and cultured from all genotypes included in the experiments indicating that, at least low level, virulence is present for all R-genes included in these experiments. With respect to virulence to Blacks R-gene differentials R1 – R11, highly complex virulence spectra were common. On average, *P. infestans* isolates contained 8 – 10 virulence factors including at least virulence for R1, R2, R3, R4, R7, R10 & R11. Considering the development of the virulence spectrum in the Dutch *P. infestans* population, virulence factors seem to accumulate without a pronounced effect on pathogenic fitness.

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