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First results of an EU-wide genotype monitoring of *Phytophthora infestans* using FTA cards

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SUMMARY
The aim of this study was to characterize the genotypic structure of *P. infestans* isolates collected over the 2012 season in several European countries to investigate whether populations underwent major changes over the last few years and to establish a baseline for further population change studies. The success rate (genotype specified) of the methodology using FTA cards for the sampling was generally quite high. From the data it can be concluded that, in general, in each of the fields sampled one genotype is dominating the population. The frequency of some well-known genotypes was different across the countries where samples were taken, however in almost every country the genotype 13_A2 was detected. The genotype 6_A1 was most frequent in the United Kingdom, 1_A1 was mainly found in Belgium and France. The methodology used in this study was proven as a good tool to investigate the population changes of *Phytophthora infestans* in Europe.

KEYWORDS
Phytophthora infestans, genotype, monitoring

INTRODUCTION

The Oomycete *Phytophthora infestans* is the causal organism of potato late blight, the most important disease in potato, the second most important arable crop in Europe. Migration of genotypes between Mexico, North America and Europe has occurred several times throughout history, likely linked to the movement of infected tubers used as seeds. Until the 1980s, the A2 mating type was not present in Europe but now both A1 and A2 mating-types co-occur in many European regions (Spielman *et al.*, 1991) allowing for sexual recombination and the formation of oospores. Apart from tubers, oospores are another possibility for the pathogen to survive the sometimes harsh North-European winters. Apart from Mexico, in Europe oospores have only been reported as a source of primary inoculum in Scandinavia and the North-East part of the Netherlands. However, increased genetic diversity has been observed in other parts of Europe which could be a consequence of sexual reproduction. Although both mating types have been

present since the late 1990s, *P. infestans* populations in France and Switzerland, as characterized with SSR's (simple sequence repeats) have been described as more or less clonal (Knapova and Gisi 2002). Also, significant population changes have been described recently as the likely result of import of foreign *P. infestans* genotypes or more frequent sexual recombination. Over the past decade this has resulted in a higher level of aggressiveness of *P. infestans* isolates (Kiezebrink and Shaw 2006) making disease outbreaks more severe and disease control more difficult. Recently the appearance of new SSR genotypes in European populations was described with novel SSR markers (Lees *et al.*, 2006). Using this methodology in 2009 Lees *et al.* detected a new frequent genotype with a characteristic 154 bp allele in locus D13 which was named as 13_A2 or 'blue13'. Another example of a new SSR genotype is the 33_A2 or 'green 33' isolate recently found in locations in The Netherlands (Schepers 2012).
The aim of this study was to characterize the genotypic structure of *P. infestans* isolates collected over the 2012 season in several European countries to investigate whether populations underwent major changes over the last few years and to establish a baseline for further population change studies. Due to the high level of polymorphism, random distribution throughout the genome and co-dominance, microsatellites (SSR) are ideal molecular markers to determine the genetic structure of populations.

MATERIAL AND METHODS

Sampling and Genotyping P. infestans
300 FTA classic Cards (GE Healthcare\Whatman) with 4 sampling zones each were distributed within the network of Syngenta field workers together with sampling instructions. Four lesions were sampled from each infected field. Data on the origin of the sample (location, host, and cultivar) were also recorded and stored. Following the field season, cards were collected and processed. SSR genotypes were determined using the standardized 12-plex Euroblight set of *P. infestans* SSR's (Li et al 2010) and GeneMapper software. Known clonal lineages (e.g. Blue 13, Green 33 etc.) were identified. All other genotypes were assigned to the "miscellaneous group". This group therefore contained many different genotypes in contrast to the other groups containing one clonal lineage.

RESULTS AND DISCUSSION

Distribution of samples and intralocation variability
From all countries in Europe 402 samples were received. 322 samples could be successfully processed giving an overall success rate of the genotype determination of 80%. However, there were big differences across countries, in CH, DE and PL the success rate was between 40 and 75 %, in the other countries (BE, CZ, DK, FR, NL, UK) the success rates were from 92 to 100 %. The 322 successful samples originated from 83 locations. More than one sample was available for 72 locations.

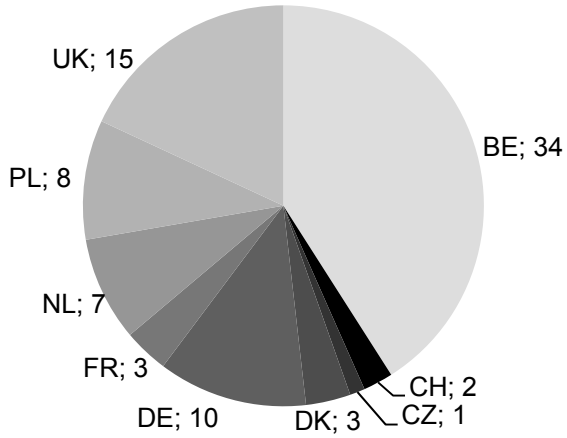

Figure 1. *Sample location country distribution*

The 72 locations with multiple successful samples were evaluated for the variability of the genotypes. For each location 2 to 16 (four FTA cards) samples were available, however the majority (89 %) was limited to one card (2 to 4 samples) and only in 8 cases more than one card was received from the same location. To allow for a simple discrimination, the results of the analysis were clustered into 3 classes representing the percentage identical genotypes within the samples from one field: 83 – 100 % / 60 – 75 % / 43 – 50 %. As can been seen in Figure 2 in about 50 % of the cases the genotype of all samples taken from the same location were nearly identical and in 80 % of the cases one genotype was dominant in more than half of the samples taken from one location.

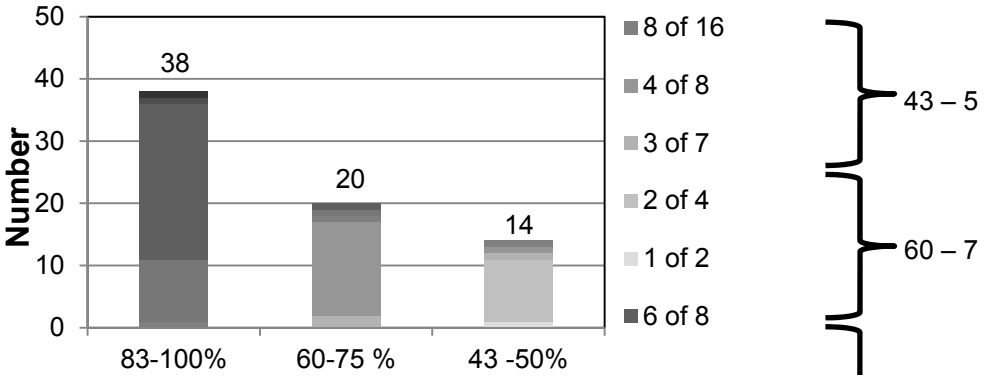

Figure 2. *Percentage identical genotypes within the samples from one location based on samples from the available 72 multi-sample locations*

DISTRIBUTION OF GENOTYPES

Taking all samples across Europe together, the most dominant genotype identified was 13_A2 ("Blue13", Table 1). Also a lot of genotypes not belonging to the four defined genotypes were found ("Others"). The genotype 6_A1 with about 10 % of the total samples was with the exception of 2 locations in Belgium exclusively found in the UK; here it was the dominant genotype. 1_A1 with about 9 % of the total samples was mainly found in Belgium and France (one location with 8 samples). Also interesting to notice was the distribution in Poland with the vast majority of the samples being classified as "Others".

Table 1. *Genotypes of samples from different countries*

Country	Number of samples with genotype					Total number per country	Relative per country (%)
	13_A2	6_A1	1_A1	33_A2	"Others"		
Belgium	72	2	19	7	16	116	36.0
United Kingdom	25	29	1	-	12	67	20.8
Germany	21	-	-	-	21	42	13.1
Poland	3	-	-	1	25	29	9.0
Netherlands	8	-	2	1	16	27	8.4
France	9	-	8	-	3	20	6.2
Denmark	-	-	-	-	11	11	3.4
Switzerland	6	-	-	-	-	6	1.9
Czech Republic	1	-	-	-	3	4	1.2
Total (#)	145	31	30	9	107	322	
Relative (%)	45.0	9.6	9.3	2.8	33.2		100

However, as shown before we have about 50 % of locations where multiple genotypes were detected. Therefore, the distribution of the genotypes in the different locations as shown in Table 2 is also quite interesting. The geographical distribution of the most frequent genotypes 13_A2, 6_A1 and 1_A1 are shown in Figures 3 and 4, however, some locations are too close together to be distinguished.

Table 2. *Genotypes of samples from different locations*

Country	Number of locations	Number of locations with genotype				
		13_A2	6_A1	1_A1	33_A2	"Others"
Belgium	34	21	2	9	3	12
United Kingdom	15	9	9	1	-	6
Germany	10	8	-	-	-	8
Poland	8	3	-	-	1	7
Netherlands	7	4	-	3	2	6
France	3	2	-	1	-	2
Denmark	3	-	-	-	-	3
Switzerland	2	2	-	-	-	-
Czech Republic	1	1	-	-	-	1
Total	83	50	11	14	6	45

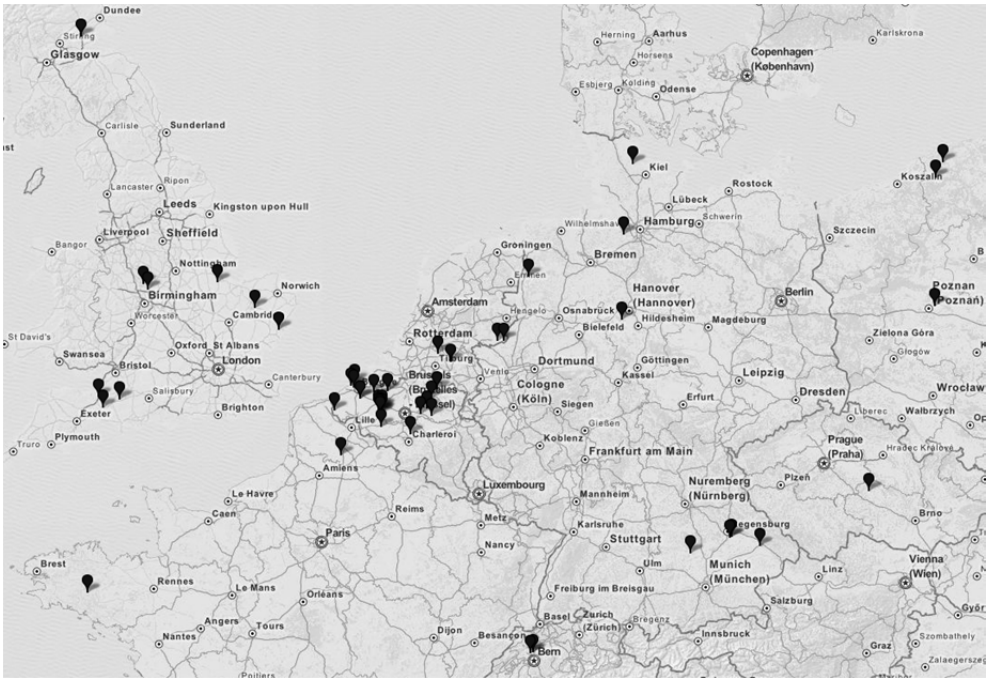

Figure 3. *Locations where 13_A2 ("Blue13") was present*

Figure 4. *Locations where 6_A1 ("Pink 6", light) and 1_A1 ("GS008", dark) were present*

CONCLUSIONS

The success rate (genotype specified) of the methodology was generally quite high with the exception of FTA cards coming from Germany and Poland which had a much higher failure rate (34 and 60 %) than the cards from the other countries (average 5 %). The reason for this is still under evaluation.

80 % of the samples from the same location had a dominant (> 50 %) genotype; in 50 % of the locations only one genotype has been found. Assuming that there is no bias due to "close by" sampling (samplers have been asked to sample from distant parts on one field) it can be concluded that in general in each field sampled one genotype is dominating the population.

The frequency of some genotypes was different across the countries where samples were taken, however in almost every country the genotype 13_A2 was detected. The genotype 6_A1 was frequent in the United Kingdom and rarely also in Belgium where 1_A1 was dominating the population. This genotype was also important in France but only minor in The Netherland's and The United Kingdom. A few isolates of the "new" genotype 33_A2 were detected in Belgium and also in The Netherland's and Poland. However, other studies have shown that it is also present in the United Kingdom (D. Cooke, pers. communication). In the majority of samples from Poland, The Netherland's and Germany different genotypes than the ones mentioned so far were found ("Others").

The methodology used in this study has proven as a good tool to investigate the population changes of *Phytophthora infestans* in Europe.

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REFERENCES

Gisi U, Walder F, Resheat-Eini Z, Edel D, Sierotzki H., 2011. Changes of Genotype, Sensitivity and Aggressiveness in *Phytophthora infestans* Isolates Collected in European Countries in 1997, 2006 and 2007. Journal of Phytopathology 159(4):223–232.

Kiezebrink DT, Shaw DS., 2006. Assessing resistance to late blight disease in new Sarpo clones. In: Westerdijk CE, Schepers HTAM. (eds) "Proceedings Ninth Workshop European Network for Development of an Integrated Control Strategy of Potato Late Blight". PPO-Special Report, Tallinn, Estonia, Vol. 11, pp 29–34.

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

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams NA, Cooke DEL., 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. Plant Pathol 55:311–319.

Lees AK, Cooke DEL, Stewart JA, Sullivan L, Williams NA, Carnegie S., 2009. *Phytophthora infestans* population changes: implications. In: Schepers HTAM. (ed) "Proceedings 11th Workshop European Network for Development of an Integrated Control Strategy of Potato Late Blight". Hamar, Norway, PPO-Special Report, Vol. 13, pp 55–60.

Li Y., Govers F., Mendes O., Testa A., Jacobsen E., *et al.*, 2010. A new set of highly informative SSR markers for *Phytophthora infestans* population analysis assembled into an efficient multiplex. Mol. Ecol. Resour. 10: 1098–1105.

Schepers, H.T.A.M., 2012. Nog veel vragen over gevoeligheid fluazinam voor Green 33 : Interview met Huub Schepers. Aardappelwereld 66(4):28 - 29.

Spielman LJ, Drenth A, Davidse LC, Sujkowski LS, Gu W, Tooley PW, Fry WE., 1991. A second world wide migration and population displacement of *Phytophthora infestans*. Plant Pathol 40:422–430.