Using SSR markers to investigate Scottish *Phytophthora infestans* populations and the epidemiology of potato late blight

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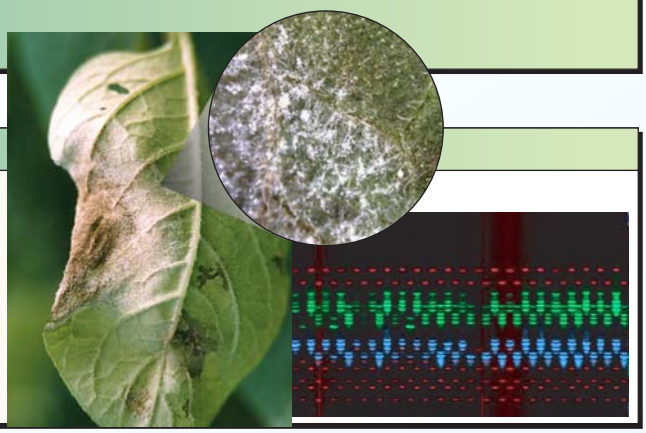


Introduction

The A2 mating type has now been identified in many parts of Europe and an increase in *P. infestans* genetic diversity is apparent. Large imbalances in mating type frequency have been observed with the A2 mating type comprising between 50 and <1 percent of the population. Such anomalies are poorly understood but will clearly affect the prevalence of the long-lived oospores and influence the genetic diversity and adaptability of populations.

P. infestans populations in Scotland were monitored from 1995 to1997 and in 2003 and 2004 during surveys of late blight outbreaks in both farms and gardens. Mating type was tested and powerful new DNA-based markers called SSRs were developed and used for examining genetic diversity. Changes in population structure were monitored over this period and will be examined in the context of existing disease management.

Materials and methods

Isolates were collected in 1995, 1996 and 1997 (Fig.1) , 2003 and 2004 (Fig. 2). In addition to the samples collected by survey staff, sampling instructions, envelopes and a cropping questionnaire were sent to seed potato inspectors and amateur gardeners.

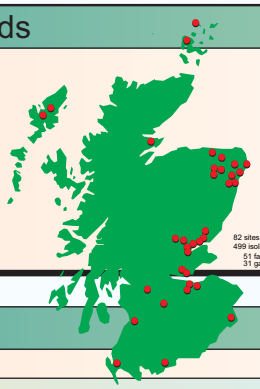


Fig. 1 Blight samples collected between 1995-7

An ideal sample consisted of 3 diseased leaves from each of 5 plants within a focus. Mating type and metalaxyl sensitivity tests were carried out on agar plates and SSR analysis (Fig. 3) was performed.

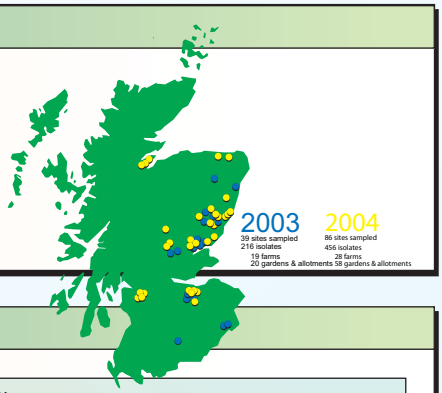


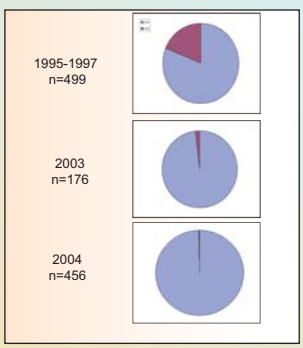
Fig. 2 Blight samples collected in 2003 and 2004

Results

Mating type

As reported previously, the frequency of the A2 type in 1995-97 was 19%. However in 2003 the A2 type was only recovered from one of 39 sites and the frequency was only 2% (Fig. 3). In 2004 amongst over 450 isolates from 86 sites only a single A2 isolate was found.

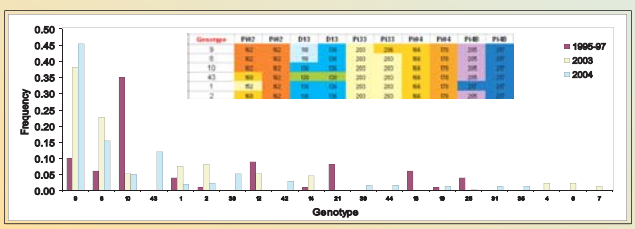
This is in contrast to England and Wales were recent evidence suggests an increase in the A2 mating type to over 30% (British Potato Council Fight Against Blight. David Cooke and David Shaw (unpublished data). Detailed analysis of SSR data will shed light on whether the A2 genotypes remain present in the population.



SSR genotype diversity and frequency

Data from Fig. 3 was processed further to consider the combinations of different alleles at all five SSR loci. Each unique combination represents a different P. infestans genotype and the number of genotypes and their frequency over each time period are presented (Fig. 4). Forty seven different genotypes were observed amongst 708 isolates. Five of the six most prevalent genotypes were sampled over all three periods but 37 genotypes were unique to a single sample period. Many combinations were found at a very low frequency, typically at only a single sample site.

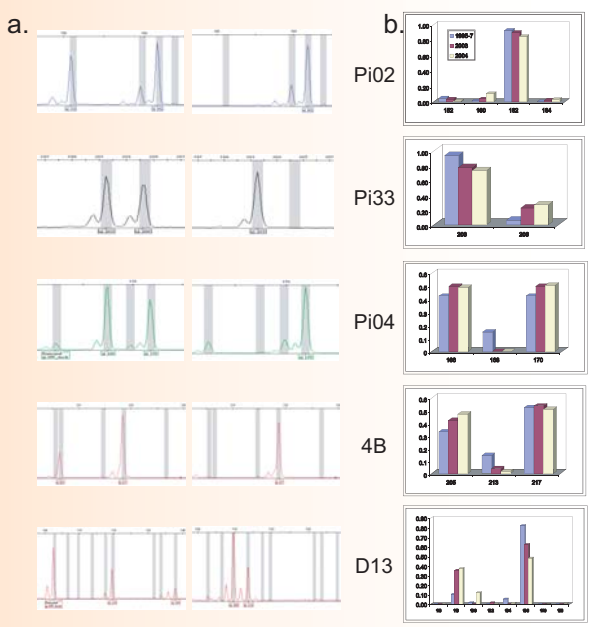
Fig. 4 The frequency of the most prevalent 20 of 47 SSR genotypes (as derived by SSR analysis) over the three survey periods 1995-7 (n=100), 2003 (n=173) and 2004 (n=435). The inset details the five most common genotypes



SSR allele diversity and frequency

Isolates from 2003 and 2004 were tested with twelve polymorphic SSR markers and the data for five of these markers was compared with that from a sample of 100 isolates from 1995-7. Although new alleles have been observed in other parts of the UK (SCRI, unpub. data from BPC Blight campaign) none were observed in Scotland. The overall frequency of each allele for the five markers is plotted in Fig. 3b and the trend observed in four of the five loci is indicative of a gradual shift in population structure.

Fig. 3. SSR data for the 1995-7, 2003 and 2004 populations of *P. infestans*.
a. Electropherograms of typical allele sizes are shown for each of the five markers.
b. The overall frequency of the different alleles from samples of the three populations.



Conclusions

This study indicates that the frequency of the A2 mating type has declined between 1995-2004. This suggests that the risk of oospores as a source of primary inoculum is also decreasing. The reasons for the decline are, however, not clear and the SSR data indicates that the population remains very diverse. A gradual change in allele frequencies suggests a trend but further analysis in the context of Eucablight and BPC FaB from GB samples is needed to complete the picture.

Acknowledgements

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