The use of SSR markers to examine Scottish Phytophthora infestans *populations*

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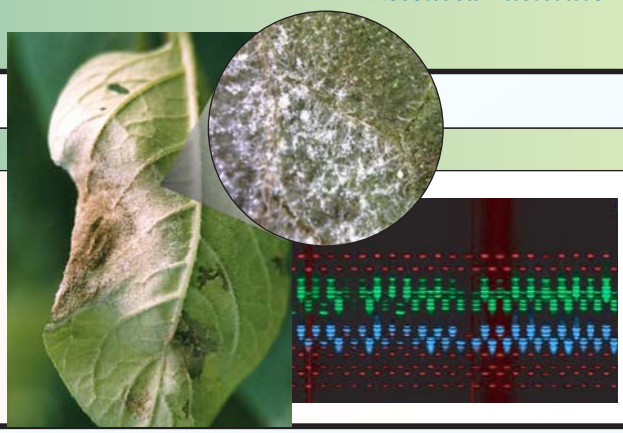


Introduction

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 measuring 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) and 2003 (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.

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. 4) with five markers was performed.

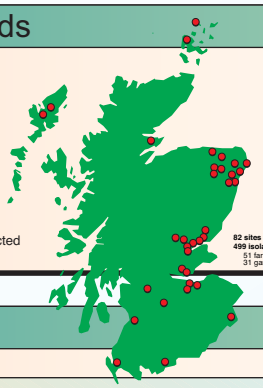


Fig. 1 Blight samples collected between 1995-7

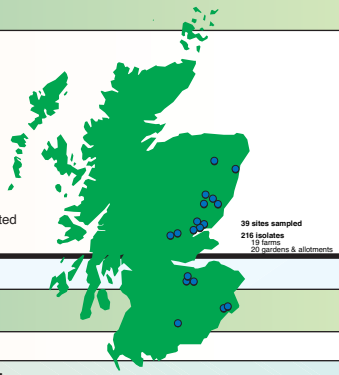


Fig. 2 Blight samples collected in 2003

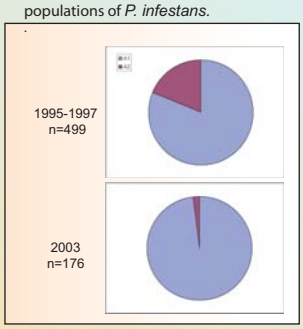
Results

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 80 sites the A2 frequency was lower still (data not shown)

Such reductions have also been seen in other countries but the cause is not known. Detailed analysis of SSR data will shed light on whether the A2

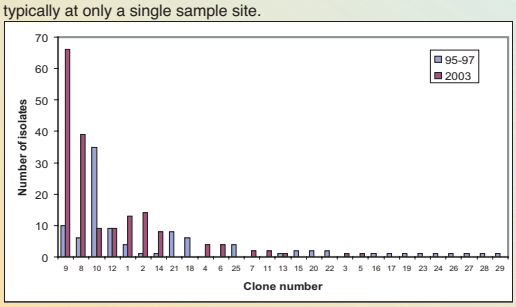
Fig. 3. Mating type frequency for the two populations of *P. infestans*.



SSR genotype diversity and frequency

Data from Fig. 4 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. 5). Twenty nine different genotypes were observed and the most commonly found types were seen in both 1995-7 and 2003 populations. Many combinations were found at a very low frequency, typically at only a single sample site.

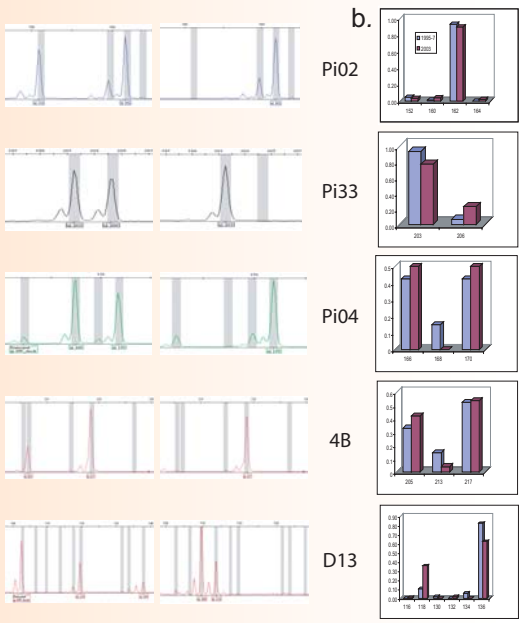
Fig. 5 The frequency of each genotype (as derived by SSR analysis) and their frequency over the two time periods.



SSR allele diversity and frequency

A sample of 176 isolates from 2003 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 the 1995-7 *P. infestans* isolate collection. The number of alleles for each marker ranged from two to six and Fig. 4a shows examples of the different alleles observed. The overall frequency of each allele for the five markers is plotted in Fig. 4b and shows that the alleles and their overall frequencies for the 1995-7 and 2003 populations were broadly

Fig. 4. SSR data for the 1995-7 and 2003 populations of *P. infestans*.
a. Electropherograms of typical allele sizes are shown for each of the fivemarkers.
b. The overall frequency of the different alleles from samples of the two populations.



Conclusions

This study indicates that the frequency of the A2 mating type is declining in Scotland. 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. The population does not appear to have changed markedly in the five years between surveys but further analysis of 2004 and BPC FaB samples is needed to complete the picture.

Acknowledgements

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