Genetic analysis of Scottish *Phytophthora infestans* populations with emphasis on within-outbreak diversity in 2004

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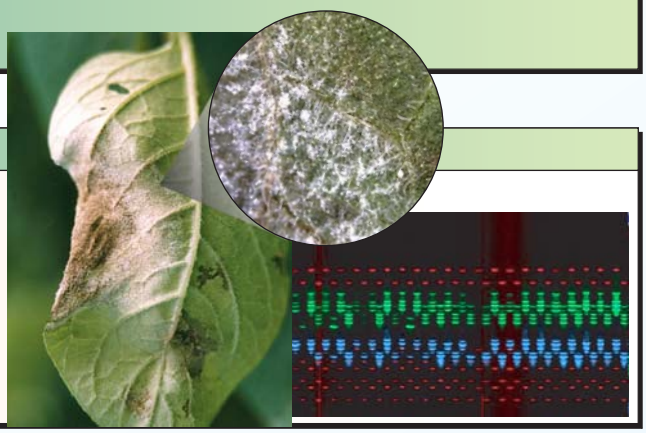


Introduction

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.

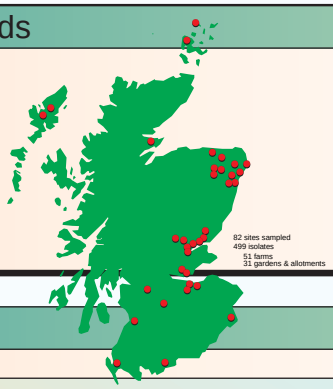


Fig. 1 Blight samples collected between 1995-7

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 was performed. In most cases multiple isolates were obtained from each outbreak and the population structure was examined (Fig. 3).

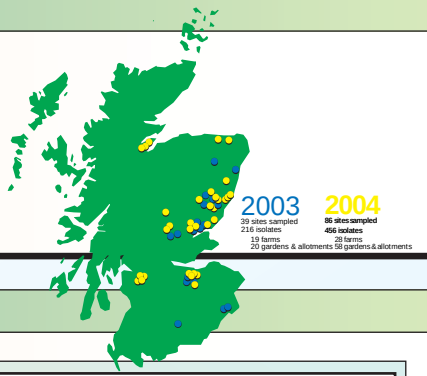


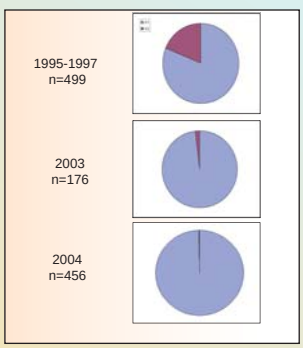
Fig. 2 Blight samples collected in 2003 and 2004

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%. In 2004 amongst over 450 isolates from 86 sites only a single A2 isolate was found.

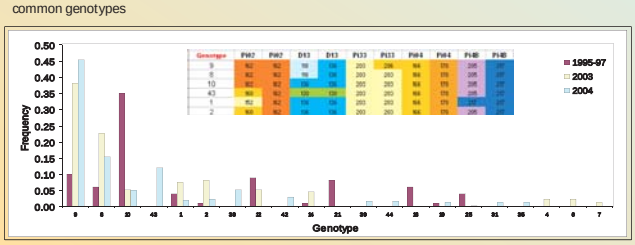
This is in contrast to England and Wales were recent evidence suggests an increase in the A2 mating type to over 30% (British Potato Council Fight Against Blight. David Cooke and David Shaw (unpublished data). Detailed analysis of SSR data will shed light on whether the A2 genotypes remain present in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



SSR genotype diversity and frequency

SSR data from over three sampling periods was examined 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). 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. 5 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



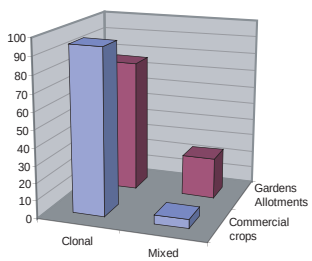


Fig. 3. The proportion of *P. infestans* outbreaks from commercial (n=20) or GA (n=51) crops that comprised isolates of a single (clonal) or mixture of SSR genotypes.

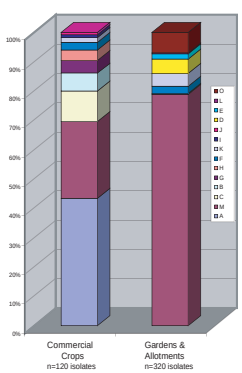


Fig. 4. The frequency of each of the 14 clonal types amongst 440 isolates of *P. infestans* from blight outbreaks in commercial or GA crops sampled in Scotland in 2004.

Within outbreak SSR diversity (2004 data)

Isolates were tested with twelve polymorphic SSR markers and the genetic fingerprint data for the multiple isolates sampled from 84 individual outbreaks examined. The majority of outbreaks in both commercial and garden and allotment (GA) crops were clonal (Fig 3). A higher proportion of GA outbreaks comprised a mixture of genotypes which likely reflects the diversity of crops grown in city allotments.

A total of 14 clonal types were discriminated amongst the 440 isolates. The distribution of these clones on commercial and GA crop is shown in Fig. 4. With the exception of the most common clone (M), that comprised 78% of GA and 26% of farm isolates, there was relatively little overlap between the two pathogen populations. For example, clone A, found in more than 50% of commercial outbreaks, was not found in GA sites.

The overall lack of pathogen diversity within each outbreak suggests clonal rather than sexually derived oospore inoculum was responsible for the Scottish blight outbreaks sampled in 2004. The differences between commercial and GA pathogen clones suggests low levels of inoculum movement between the sampled crops.

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. The diversity in each outbreak supports the absence of oospore-borne inoculum 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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