The status of Scottish late blight populations

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Like an ominous shadow, *Phytophthora infestans* has followed its principal hosts, potato and tomato, out of the New World and across the globe. This oomycete fungus is the cause of late blight (Fig. 1), an abiding threat to potato growers that requires vigilance and expensive fungicide applications for effective control. The patterns of migration of this pathogen reveal a fascinating history[1] and provide a classic example for the study of fungal population biology and evolution. Its mid-19th century arrival in W. Europe, and the ensuing economic, social and political impact, have been well documented.

P. infestans is a diploid, heterothallic fungus with two mating types (termed A1 and A2), which are found with equal frequency in its Mexican centre of diversity[2]. Within this freely interbreeding population, there is a wealth of genetic diversity. In Europe, however, the initial introduction from the Americas appears to have been of limited size, as the population comprised only the A1 mating type. Moreover, DNA-based genetic analysis has revealed that this population was of a single clonal lineage and represented a classic example of reduced population diversity as a result of passing through a 'genetic bottleneck'. Despite being a clone, the population was capable of adaptation, overcoming the single major R genes for resistance incorporated from wild potato species in the first half and middle of the 20th century. The mechanisms by which such somatic mutations are generated and disseminated are poorly understood in *Phytophthora*.

Figure 1 Typical foliar symptoms of potato blight: a large lesion with a necrotic central zone surrounded by profuse white 'fluffy' sporulation.

In the early 1980's, the status of European blight populations changed markedly. As a result of the dry summer of 1976, Mexican potatoes were imported into Europe bringing with them a much more diverse population of *P. infestans* that comprised A1 and A2 mating types. The 'new' population rapidly displaced the 'old' one, suggested to be as a result of its increased fitness over the existing 'old' clone which had been 'weakened' by 130 years of asexual propagation (Müller's ratchet). A major component in the chemical armoury against blight was also threatened at this time. Overuse of the curative phenylamide fungicide, metalaxyl, resulted in high selection pressure on the fungus and a build up of insensitive isolates markedly reduced the fungicide's efficacy. The arrival of the A2 mating types in the Mexican imports (not discovered until 1981 and not announced until 1984!), added a new dimension to the pathogen's biology. The occurrence of the two mating types raised the possibility that the fungus could undergo its sexual cycle in infected potato plants and form long-lived oospores. These could then be incorporated into the soil with several adverse consequences.

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Figure 2 Geographical spread of sites from which late blight samples were collected.

The presence of a sexual cycle allows a regular re-assortment of its genes. While it has been suggested that such re-assortment may be deleterious as it breaks up 'winning combinations' of genes, in general, evolutionary theory suggests that this would result in a more flexible genetic system, enabling the fungus to adapt more rapidly to man's control efforts. Should this be the case, an accelerated erosion of plant resistance, including both major gene (R-gene) and field resistance resulting from combinations of several to many loci (Quantitative Trait Loci), may occur. Erosion of the resistance of existing (and future) commercial cultivars represents a serious threat to potato production. An accelerated adaptation to existing and new fungicide active ingredients may occur also, threatening one of the most effective means of control. In addition to the, as yet unproven, genetic consequences, there are epidemiological implications of the addition of a durable soil-borne phase into the *P. infestans* life cycle. These hypotheses have yet to be tested fully in W. European systems, though earlier disease outbreaks (observed in Sweden[3]) and a need for longer crop rotations may be an inevitable consequence of a residual soil-borne source of inoculum.

It was against this background that the studies reported here were undertaken at SCRI in collaboration with the Scottish Agricultural Science Agency (SASA), to elucidate the current status of Scottish blight populations and allow predictions of the significance of a changing population to the potato industry (growers, breeders, advisors and scientists alike).

A survey was undertaken by collecting isolates during the 1995, 1996 and 1997 seasons. Samples of blighted plant material were kindly provided by potato inspectors, colleagues in other institutes, and members of the public, as well as being collected by SCRI and SASA staff. In total, 82 sites were sampled, resulting in a collection of 499 *P. infestans* isolates (Fig. 2). Reflecting the varied pattern of potato production in Scotland, a range of sites was sampled: two thirds were commercial farms and the rest were private gardens or allotments. To assess diversity both within and between sites, as many as 15 isolates were collected from each site (the average number of isolates per site was six). The isolates were examined phenotypically for mating type, sensitivity to metalaxyl fungicide and, to a limited extent, virulence against major potato resistance (R) genes. The isolates were also DNA-fingerprinted using the amplified fragment length polymorphism (AFLP) method.

Mating type	Farm sites	Farm isolates	G/A sites	G/A isolates
A1 only	72.7	83.3	56.9	57.8
A2 only	18.9	8.0	7.8	5.1
A1 & A2	8.4	8.7	35.3	37.1

Table 1 Mean percentage of sites and isolates from each of the mating type categories (A1, A2 or a mixture of both mating types) collected between 1995 and 1997. Results are categorised according to site type, farm or garden/allotment (G/A).

The majority (80%) of isolates collected were A1, 19% were A2, and 1% produced oospores without the presence of the opposite mating type (so-called self-fertile types). The type of site from which they came and whether or not that site comprised a single or a mixture of both mating types, can be seen in Table 1. The A1 mating type alone was found on 73% of farm sites, compared with 57% of garden and allotment sites. Since a variable number of isolates was collected at each site, the percentage of isolates belonging to either mating type differs from the percentage of sites where one or both were recovered. Populations of mixed mating types were four times more common in gardens and allotment populations than in farms. Studies in the Netherlands also have shown a similar pattern which is thought to result from amateur growers keeping home-grown seed, co-cultivating tomato and potato, having generally higher disease incidences, and using compost containing peelings from imported potatoes. No clear changes in mating type frequency,

occurrence or distribution were observed over the 3 years, suggesting the populations are relatively stable.

Response to a range of metalaxyl concentrations was scored on the basis of each isolate's ability to grow on amended agar media relative to growth on unamended media. Isolates were classified as resistant, sensitive or intermediate. Of 444 isolates examined, approximately equal percentages were resistant (42%) and sensitive (44%) with 14% showing an intermediate response. In this case, however, a trend was apparent over the course of the study. The number of sensitive isolates was similar each year but the number of intermediates increased as the number of fully resistant isolates decreased. It is interesting that 70% of the intermediate isolates occurred in mixed mating type populations compared with only 23% from single mating type populations. As some reports show, metalaxyl resistance may be governed by a single, semi-dominant gene and heterozygotes have the intermediate phenotype. Thus, the occurrence of high numbers of intermediate types on mixed mating type sites is indicative of sexual recombination. The majority of such mixed populations were from gardens and allotments in which there is a risk of fully resistant A2 isolates developing. Significantly, only four of 82 A2 isolates tested were resistant to metalaxyl.

	Fungicide program		Samples treated with fungicide	
	Untreated	Treated	Phenylamide ingredient	
			-	+
Response to metalaxyl	% Isolates (n=166)	% Isolates (n=133)	% Isolates (n=58)	% Isolates (n=66)
Resistant	27	67	58	76
Sensitive	60	28	29	24
Intermediate	13	5	12	0

Table 2 Percentage of isolates classified as resistant, sensitive, or intermediate in response to metalaxyl. Sites were sub-divided into those untreated and treated with fungicides; the latter being further sub-divided into those treated with or without phenylamides.

An analysis of 299 isolates from 46 samples, for which fungicide application data were available, shows the impact that phenylamides have on *P. infestans* population structure (Table 2). Only 27% of isolates from untreated sites were resistant compared with 67% from fungicide-treated sites, and where phenylamide was a component of the fungicide, this figure rose to 76%. Considering that 96% of A2 isolates were either sensitive or intermediate in their response to metalaxyl, this suggests phenylamides played a significant role in limiting the A2 population size.

The virulence characteristics of representative isolates of a range of 1995 and 1997 samples were assessed in 'whole plant' tests. Considerable variation was noted in virulence phenotypes but no significant differences were observed between the 1995 and 1997 samples. Since collecting virulence data is time-consuming and recent publications[4] have reported a lack of correlation of virulence with epidemiological or molecular characters, it was not pursued further in this study.

In collaboration with colleagues at The University of Wales, Bangor, 31 isolates were examined using restriction fragment length polymorphism analysis (RFLPs) with the moderately repetitive RG57 probe, and eight RG57 fingerprints were identified, hinting at the breadth of genotypic diversity in Scottish populations. In agreement with the phenotypic data, isolates from mixed A1 and A2 sites yielded a mixture of RG57 fingerprints, again suggestive of sexual recombination. Fourteen isolates from nine single mating type farm samples had five different fingerprints but no variation was observed within a sample from a single farm. Thus, single clones probably initiate epidemics on farms but the overall Scottish population consists of many RG57 clones.

AFLPs yielded total genomic DNA fingerprints consisting of around 70 bands per isolate. Using a single primer combination, almost 300 isolates were fingerprinted and the presence/absence data for 15 easily scored polymorphic bands (markers) per isolate were recorded. Considerable molecular diversity was observed, with over 163 (56%) of the isolates having unique marker combinations. A cluster analysis of the fingerprint data from each individual isolate did not resolve any clear grouping of isolates by site, mating type, metalaxyl sensitivity or season. This is consistent with a 'metapopulation model' in which short-lived, local populations are being replaced continually by new genotypes that either migrate from neighbouring populations or are generated through sexual recombination. Averaging the AFLP marker diversity within each site using the computer package POPGENE did, however, reveal a trend. The population was clustered into three groups; in the first group, seven of the ten sites were of A2 only or A1/A2 mixed type, compared with only eight out of 44 sites in the other two major groups. Comparisons of the annual average number of isolates per fingerprint and average number of fingerprints per site do not indicate any trend to increasing

fingerprint complexity *e.g.* as a result of extensive sexual recombination. AFLP fingerprints showed a greater resolution than RFLPs with the RG57 probe.

Conclusions The extensive database generated in this study highlights the complexity and dynamic nature of blight populations in Scotland. The samples from throughout the country were of both mating types, and also included self-fertile isolates. They showed different levels of resistance to the phenylamide fungicides, and a range of virulences was observed.

In agreement with a similar survey in England and Wales, the A2 mating type is present but at a much lower frequency than the A1 type. While the implication of such an imbalance is, for now at least, good news for growers (reduced risk of sexual recombination and thus oospore formation), the reasons are unclear. Unpublished reports of A2 isolates in Scotland as early as 1984 would seem to dispel the theory that it is a recent import and has thus had insufficient time to spread. It is possible that the A2 strains found here are less well adapted to Northern European conditions than the A1 strains. However, 25 and 15% of isolates from commercial potato fields in Norway and Finland[5] respectively were found to be A2's. Since such strains are very likely from the same European population, poor adaptation is also an unlikely reason for the differences in frequency. The results of this survey suggest that the sensitivity of the A2 strains to metalaxyl is the principal factor limiting their numbers. Their prevalence in gardens and allotments is probably a consequence of a combination of poor crop hygiene (discussed above) and the fact that gardeners do not have access to metalaxyl.

Our molecular data suggest that the A2s are of a different genetic background to the majority A1 population. However, the same data and the phenotype and site data (discussed above) also suggest that crossing does occur, albeit infrequently. The eventual consequences of such interbreeding will be a blurring of genotype boundaries. It is also likely that more A2 metalaxyl insensitive isolates will emerge and thus the impact of metalaxyl sensitivity, as a limiting factor on A2 spread, will be reduced. Between 1995 and 1997, the frequency of A2's remained unchanged but ongoing monitoring of mating type and metalaxyl resistance is necessary to check for such population changes.

The risks of oospore formation are clearly greater in gardens and allotments, although mixed populations were also found on commercial farms. Growers, both amateur and professional, should therefore remain assiduous in their monitoring and control strategies and be on the lookout for early infections that may indicate soil-borne inoculum (i.e. oospores). Amateur growers must, as always, avoid using poor quality potato seed and avoid contaminating garden compost with either their own infected material and more importantly that of imported potato material.

The low and stable frequency of isolates fully resistant to metalaxyl suggests Scottish growers are using the phenylamide group of fungicides prudently. However, the rise in the number of isolates of intermediate resistance should be monitored to ensure the longevity of this active ingredient.

The information generated in this project on the current status of Scottish blight populations will help plant health inspectors assess the risk of early epidemic development through oospores. It will also help breeders concerned with the effects of shifts in pathogenicity on the durability of resistant cultivars, as well as guiding policy on fungicide resistance build-up.

The future As well as detailed studies on the host pathogen interaction[6], ongoing work in the Unit of Mycology, Bacteriology and Nematology is targeted at PCR-based detection of oospores in soil, the development of new co-dominant DNA markers (SSR and SNP-based) that can be applied to study the population biology of *P. infestans* in more detail, as well as detailed epidemiological studies on the behaviour of particular isolates in the field.

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