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The potato blight population in Northern Ireland

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SUMMARY
Samples of late blight from 13 and 33 Northern Ireland potato crops in 2013 and 2014, respectively, were characterised for mating type, phenylamide resistance and SSR genotype. Fewer samples were obtained in 2013 than 2014 because dry weather in April and May 2013 limited the number of late blight outbreaks, whereas in 2014 the weather was conducive to infection. SSR analysis was carried out on single-lesion isolates of *Phytophthora infestans* in 2013, while in 2014 it was done on DNA samples from single lesions collected on FTA cards. In 2013, the incidence of both phenylamide resistance and the A2 mating type declined compared with 2012 (to under 5%), but in 2014, the incidence of phenylamide-resistant strains and of the 13_A2 genotype increased to over 30%. As in previous years, the population proved highly clonal: all A2 isolates belonged to the 13_A2 genotype; the predominant A1 genotype was 8_A1 in both years, but in 2014, 5_A1, 6_A1 and 12_A1 were also detected. The major A1 genotype in Northern Ireland since the mid-1990s has been 8_A1 (NI-1), whereas in Great Britain the newer 6_A1 has been the most frequent A1 genotype since 2007. The EuroBlight Late Blight Tool box was used to analyse and visualise 2013-2014 Northern Ireland *P. infestans* SSR data.

KEYWORDS
Phytophthora infestans, Northern Ireland, mating type, phenylamide resistance, SSR, population structure

INTRODUCTION

Late blight has challenged potato growers in Ireland since its arrival in 1845 and the subsequent Irish Potato Famine (1845-48). For much of the 20th century, global *Phytophthora infestans* populations were dominated by a single clonal A1 lineage, designated US-1. From the mid-1970s, migration events spread new strains of both mating types worldwide, displacing US-1 and triggering ongoing population changes. In Europe, some regions now have recombinant populations, while others have remained largely clonal.

In Northern Ireland, the A2 mating type was first identified in 1987 (Cooke *et al.,* 1995). By the mid-1990s, the Northern Ireland *P. infestans* population was highly clonal, with one major and

some minor A1 genotypes; the A2 mating type occurred at low frequency and no US-1 was detected (O'Sullivan *et al.,* 1995; Carlisle *et al*., 2001). In the period 1998-2002, the A2 mating type was not detected and there was a clonal A1 population with two major genotypes, designated NI-1 (equivalent to SSR genotype 8_A1) and NI-2 (5_A1) (Cooke *et al.,* 2006).

In the last 10 years, marked changes have occurred in *P. infestans* populations in Europe; new genotypes have been identified, most notably 'Blue 13' or 13_A2 (Cooke *et al.,* 2012a). These new types may have originated as sexual recombinants in mainland Europe and some have proved more aggressive and harder to control. In Northern Ireland, in 2005 the first A2 isolates since 1995 were detected and in 2007 the 13_A2 genotype was identified for the first time (Cooke *et al.*, 2009). Since 2008, *P. infestans* population studies in Northern Ireland have continued as part of all-Ireland projects led by Teagasc (Kildea *et al.,* 2010), currently MonPESC: **Mon**itoring **P**athogen **E**volution for **S**ustainable **C**ropping). Isolates have been characterised phenotypically (mating type, phenylamide resistance) and genotypically (SSR, RG57, mtDNA and *Pep* allozymes). A close association between markers has indicated a highly clonal population: only five genotypes were identified and within these, isolates had similar SSR patterns, shared a common mating type, RG57 fingerprint, *Pep* allozyme genotype, mtDNA haplotype and sometimes the same phenylamide resistance status (Cooke *et al.*, 2014). The major genotypes were 13_A2 (Blue 13), the only A2 detected (A2 genotypes present in Northern Ireland in the 1990s were not found) and 8_A1 (NI-1), the commonest genotype in the 1990s. The frequencies of these two genotypes fluctuated from year to year in inverse proportion to each other. Of three other A1 genotypes detected, two were present in the UK in the 1990s (5_A1/NI-2 and 12_A1); the third ('Pink 6', 6_A1), first identified in Great Britain in 2004 and in Northern Ireland in 2009 (Kildea *et al.*, 2013), has been the commonest A1 genotype in Great Britain since 2007 (Cooke *et al.,* 2012a). Results of *P. infestans* population studies up to 2012 in Northern Ireland have been reported in papers presented at previous EuroBlight Workshops and published in the Proceedings (e.g. Cooke *et al.*, 2012b; Cooke *et al.,* 2014). This paper reports results for 2013-14 and demonstrates the use of the new EuroBlight Late Blight Toolbox population tools on the Northern Ireland population.

MATERIALS & METHODS

Collection, isolation and storage of Phytophthora infestans isolates

Blighted potato leaf material was collected mainly from commercial seed crops by members of the Northern Ireland Department of Agriculture and Rural Development (DARD) Agri-food Inspection Branch. Once received, the blighted material was incubated and isolates established as previously described (Kildea *et al.,* 2010). In 2014, duplicate single lesions from each sample were also squashed onto FTA cards following the EuroBlight protocol (www.euroblight.net).

Mating type, phenylamide sensitivity and SSR determination

Mating type was determined as described by Cooke *et al*. (2006). The sensitivity of isolates to the phenylamide fungicide metalaxyl was determined using a floating leaf disk assay (Cooke *et al.,* 2006). For selected isolates, genotypes at the polymorphic allozyme locus, *Pep-1* (peptidase), were determined using cellulose acetate electrophoresis (Carlisle *et al*., 2001). In 2013, isolates were genotyped by SSR analysis at the James Hutton Institute (JHI) using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) in

accordance with the protocol developed by EUCABLIGHT. In 2014, SSR analysis was carried out by JHI directly on the DNA collected on FTA cards.

Use of the EuroBlight Late Blight Toolbox
SSR characterisation data for the 2013 and 2014 Northern Ireland isolates were imported into the EuroBlight Late Blight Toolbox. The Toolbox was then used to visualise and analyse the data and compare the Northern Ireland population structure with populations in Great Britain and elsewhere in Europe (using Belarus and Sweden as examples). Bruvo distances were used for Principal component analysis (PCA); these are calculated genetic distances between individuals at microsatellite loci that can accommodate differences in ploidy (Bruvo *et al*., 2004).

RESULTS

Population characterisation 2013-2014
Dry weather early in the 2013 season (late May-June) prevented primary infection development and the first field outbreak of late blight was not reported until 17th July (the latest 1st report since 1981 apart from 19th July in 2010). Weather suitable for the spread of blight occurred in July and August, but there were few primary infection sources and not many outbreaks were reported. Blight samples were obtained from only 13 sites, of which 12 were commercial crops and one was an allotment; 54 isolates were established (up to five per site). In 2014, the weather was more conducive to late blight with mild night temperatures and more rainfall early in the season. The first field outbreak was identified relatively early on 9th June and subsequently blight was reported in all potato-growing areas. Blight samples were obtained from 33 sites, which comprised 27 commercial crops and six trial sites. Sixty *P. infestans* isolates were obtained from 30 sites (up to five per site), and 75 samples on FTA cards from 33 sites were SSR genotyped.

In 2013-2014, as in the whole period since 2005, the annual percentage of isolates containing phenylamide-resistant strains showed a similar trend to the annual proportion of A2 mating type isolates (Figures 1-2). This is indicative of hitch-hiking selection: growers in Northern Ireland now make little use of phenylamide fungicides and so the incidence of phenylamide-resistant strains is not related to their selection by phenylamide usage, but depends on the incidence of the invariably phenylamide-resistant 13_A2 genotype. This is further evidence for the clonality of the Northern Ireland *P. infestans* population.

In 2013, the incidence of both phenylamide resistance and the A2 mating type declined compared with 2012 (5% phenylamide resistance, 2% A2 mating type, Figures 1-2). In 2014, the incidence of phenylamide-resistant strains increased to 31%; although the incidence of A2 mating type isolates was only 7%, the incidence of the 13_A2 genotype in DNA samples collected on FTA cards was 33%. This indicated a problem which became particularly apparent in 2014 when SSR genotyping of the Northern Ireland population was done using DNA collected onto FTA cards for the first time rather than DNA from agar cultures of the pathogen. The 13_A2 genotype proved much more difficult to isolate into axenic culture than the 8_A1 genotype so that mating type determination on isolates in culture under-estimated the incidence of A2 types (whereas phenylamide resistance testing using spore suspensions generated from blight samples on leaves did not impose this bias). Of 11 sites where 13_A2 was detected, three sites were sampled onto FTA cards only (isolation not attempted), from three sites A2 mating type isolates

were obtained, but from five sites only A1 isolates were obtained although 13_A2 and A1 genotypes were detected in the DNA from FTA cards and phenylamide resistance testing showed the presence of resistant strains.

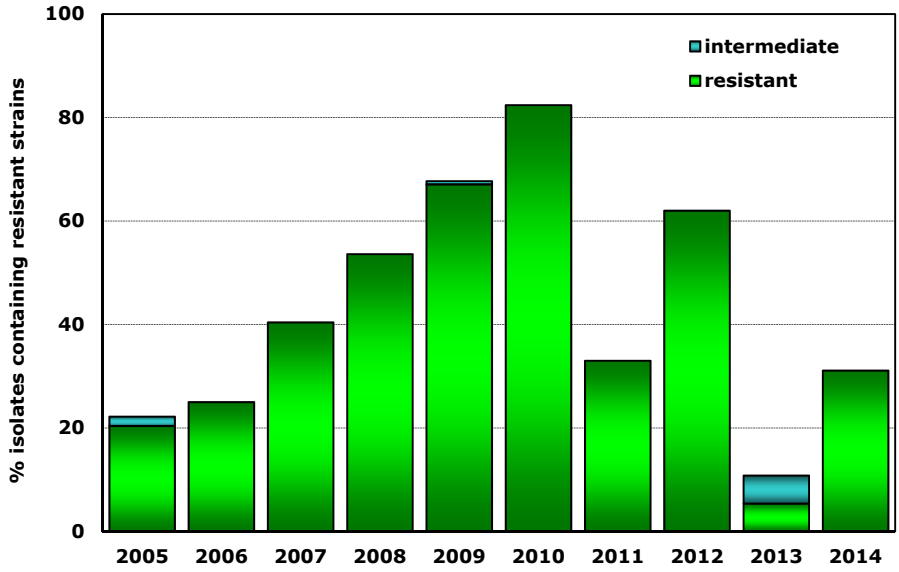

Figure 1. *The percentage of Northern Ireland Phytophthora infestans isolates containing phenylamide-resistant strains, 2005-2014*

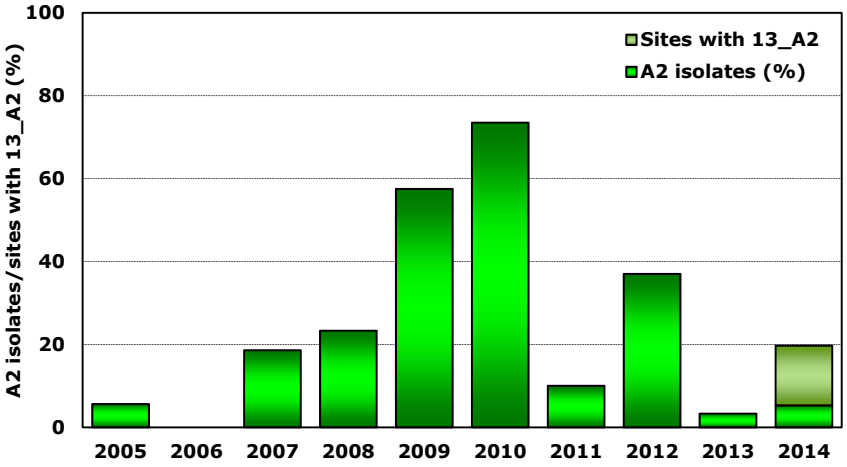

Figure 2. *The percentage of Northern Ireland Phytophthora infestans isolates of the A2 mating type, 2005-2014*

SSR genotype characterisation of 2013 and 2014 isolates revealed that all A2 mating type isolates belonged to the 13_A2 type and no other A2 genotypes were detected (Fig. 3). In 2013, of 53 isolates characterised by SSR, the only 13_A2 isolate was obtained from a tuber grown in an allotment in Belfast; this represented 2% of the detected genotypes. In 2014, as noted above, 13_A2 was identified at 11 sites (eight commercial crops and three trial sites) and constituted 33% of the detected genotypes (25 of 75 samples genotyped).

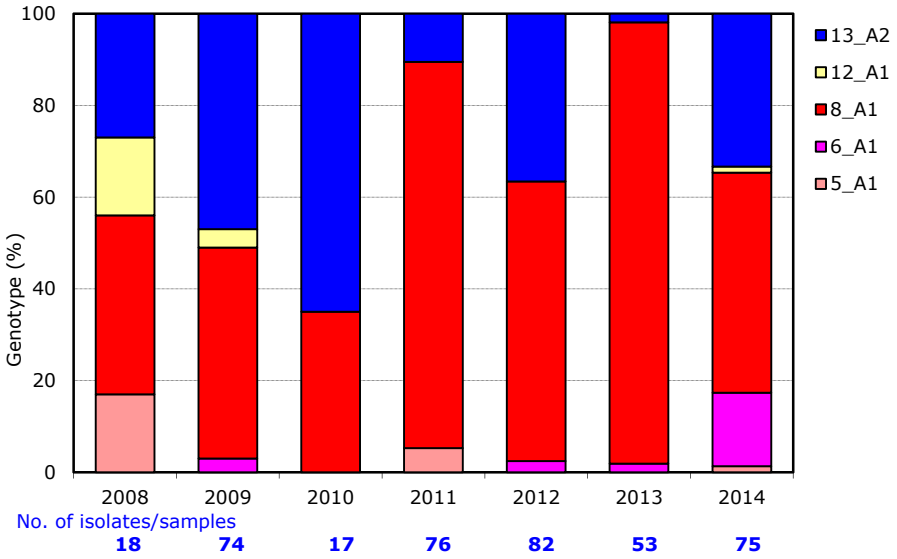

Figure 3. *Northern Ireland Phytophthora infestans genotypes 2008-2014*

Four A1 genotypes were identified by SSR in 2013-2014 (Fig. 3). In 2013, all but one A1 isolate belonged to the 8_A1 genotype (51 isolates, 96% of detected genotypes). One 2013 A1 isolate was not successfully characterised by SSR, but was phenylamide-sensitive with the *Pep* allozyme genotype *96/96*, typical of 6_A1 (Cooke *et al.,* 2014) and was tentatively assigned to that genotype (2%). In 2014, A1 types were identified in 50 of the 75 FTA samples analysed and these belonged to four genotypes, 8_A1 again predominated (48% of all samples genotyped), 6_A1 was present in 16% of samples (its highest detected occurrence in Northern Ireland), and 5_A1 and 12_A1 were each represented by a single sample.

Comparison of the *P. infestans* genotype occurrence in Northern Ireland with that in Great Britain between 2008 and 2014 (Cooke, D.E.L., personal communication) showed a clear difference between the two geographical regions throughout the period. While in both regions the only A2 genotype detected was 13_A2, in Great Britain the commonest A1 genotype detected in every year was 6_A1, while in Northern Ireland it was 8_A1. In years when 13_A2 was less frequent its place was taken by 6_A1 in Great Britain, but 8_A1 in Northern Ireland (Fig. 3).

Use of the EuroBlight Late Blight Toolbox

The EuroBlight Late Blight Toolbox genotype frequency maps for 2013 and 2014 clearly show the differences in genotype frequencies between Northern Ireland, Great Britain (England, Scotland and Wales) and mainland European countries, in both years (Fig. 4). Northern Ireland was the only location where the A1 genotype 8_A1 predominated.

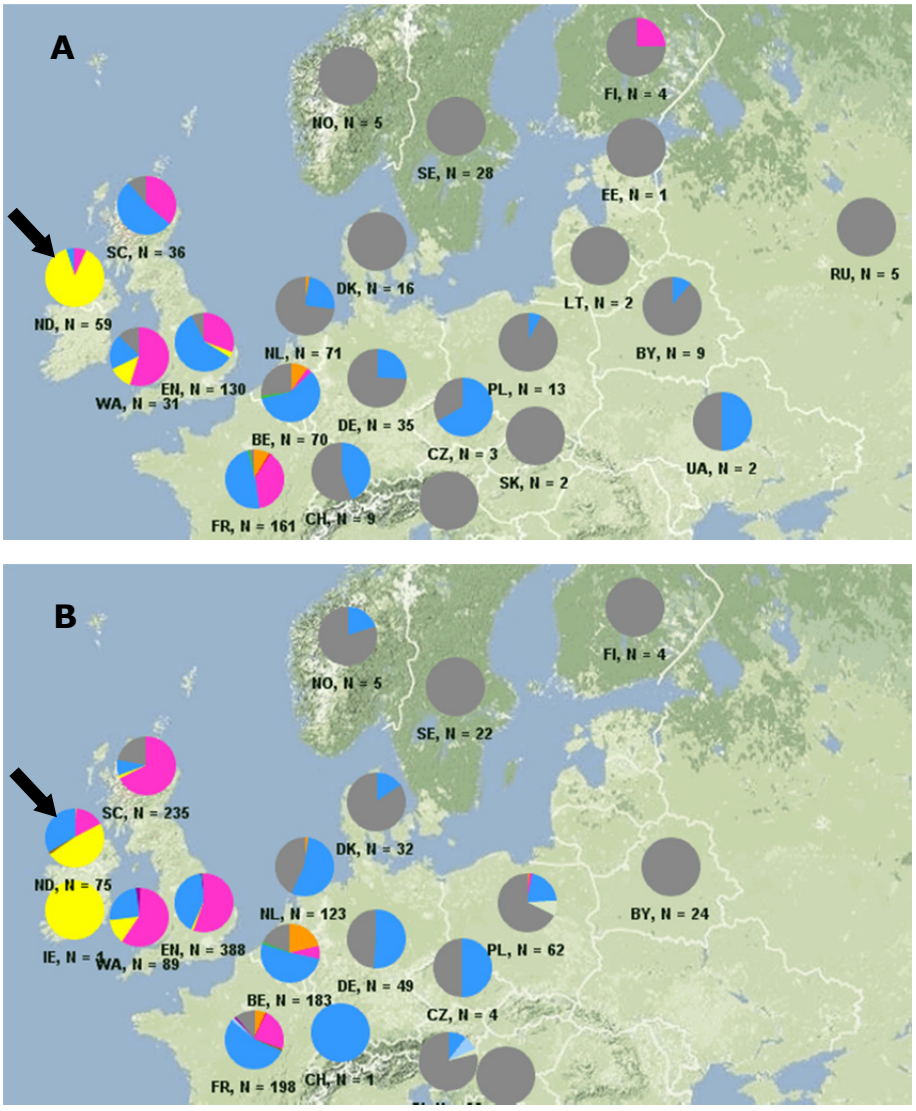

Figure 4. *Euroblight Potato Late Blight Toolbox genotype frequency maps for 2013 (A) and 2014 (B) showing Phytophthora infestans genotype frequencies in Northern Ireland (ND, arrowed) compared with those elsewhere in Northern Europe*

The number of analysed samples per multilocus genotype in Northern Ireland, England, Scotland and Wales was compared with Belarus and Sweden on the basis of data submitted to the EuroBlight Late Blight Toolbox (Table 1). This showed that in Belarus and Sweden the number of samples per multilocus genotype was 1.0 in both years (i.e. every analysed sample represented a unique genotype), whereas in Northern Ireland the corresponding figure was 3.9 and 3.6 in 2013 and 2014, respectively, and in Great Britain it was between 2.8 and 6.6. This result is in agreement with the *P. infestans* populations in Belarus and Sweden being recombinant in contrast to populations in the UK, which exhibited considerable clonality.

Table 1. *Comparison of the number of isolates/DNA samples (N) per multilocus genotype (MLG) in Belarus (BY), Sweden (SE), Northern Ireland (ND), England (EN), Scotland (SC) and Wales (WA) in 2013 and 2014*

Population	N	MLG	N/MLG
2013			
BY	9	9	1.0
SE	28	26	1.1
ND	59	15	3.9
EN	130	30	4.3
SC	36	6	6.0
WA	31	11	2.8
2014			
BY	24	24	1.0
SE	22	22	1.0
ND	75	21	3.6
EN	377	57	6.6
SC	235	55	4.3
WA	89	15	5.9

PCA of Bruvo distances from the 2013 and 2014 data from Northern Ireland, England, Scotland, Wales, Belarus and Sweden showed that the Northern Ireland data clustered together and this clustering was largely associated with genotype 8_A1 in 2013 (Fig. 5) and with 6_A1, 8_A1 and 13_A2 in 2014 (Fig. 6).

Multiple Spanning Network (MSN) trees for the same countries for 2013 and 2014 similarly indicated clustering of the Northern Ireland data with the circles representing *P. infestans* genotypes in Northern Ireland being large (i.e. representing clonal genotypes) compared with the small dots indicative of single isolate genotypes in Belarus and Sweden (Fig. 7-8).

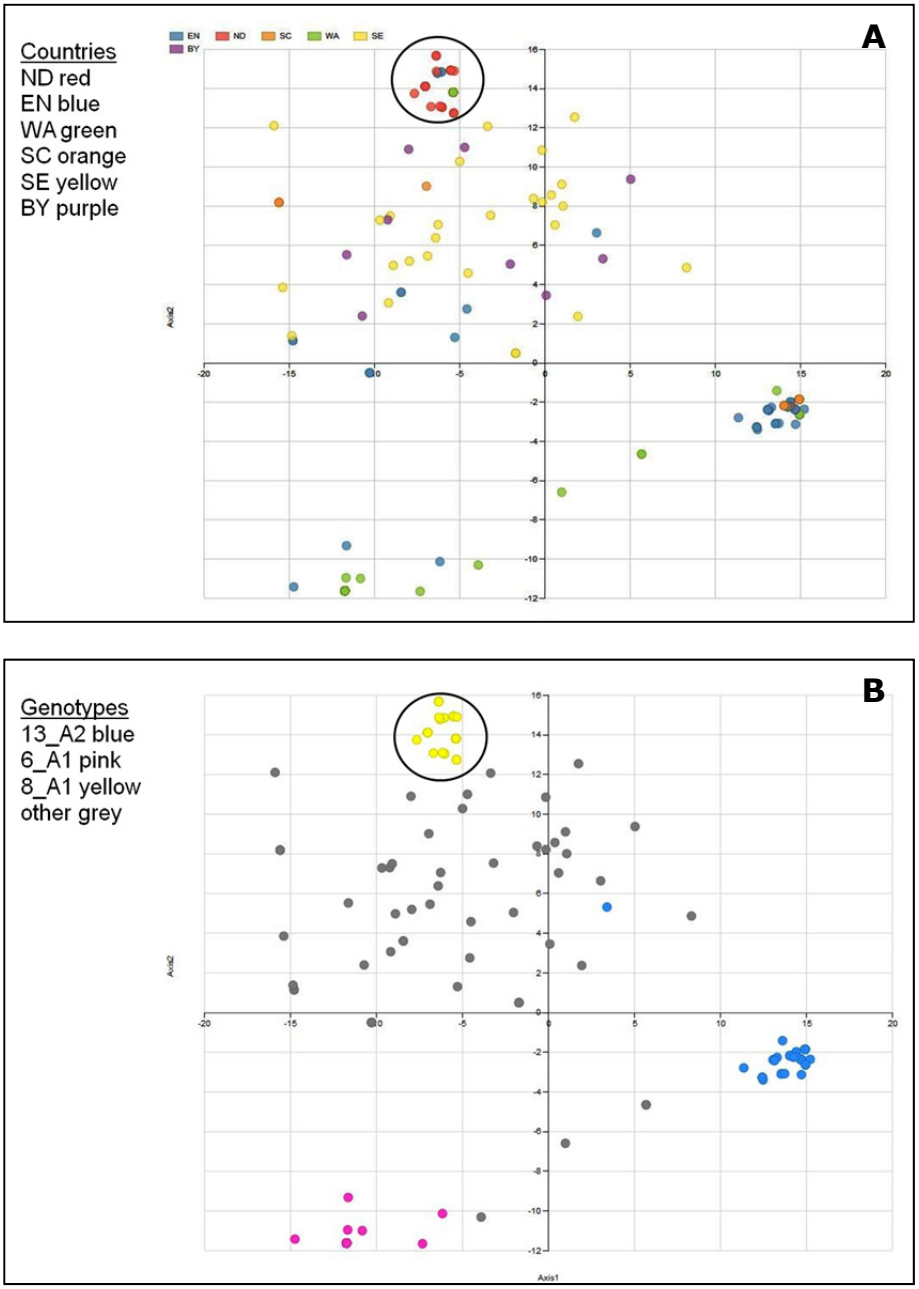

Figure 5. *Euroblight Potato Late Blight Toolbox Principal Component Analyses of Phytophthora infestans SSR data with Bruvo distances as input. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2013; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

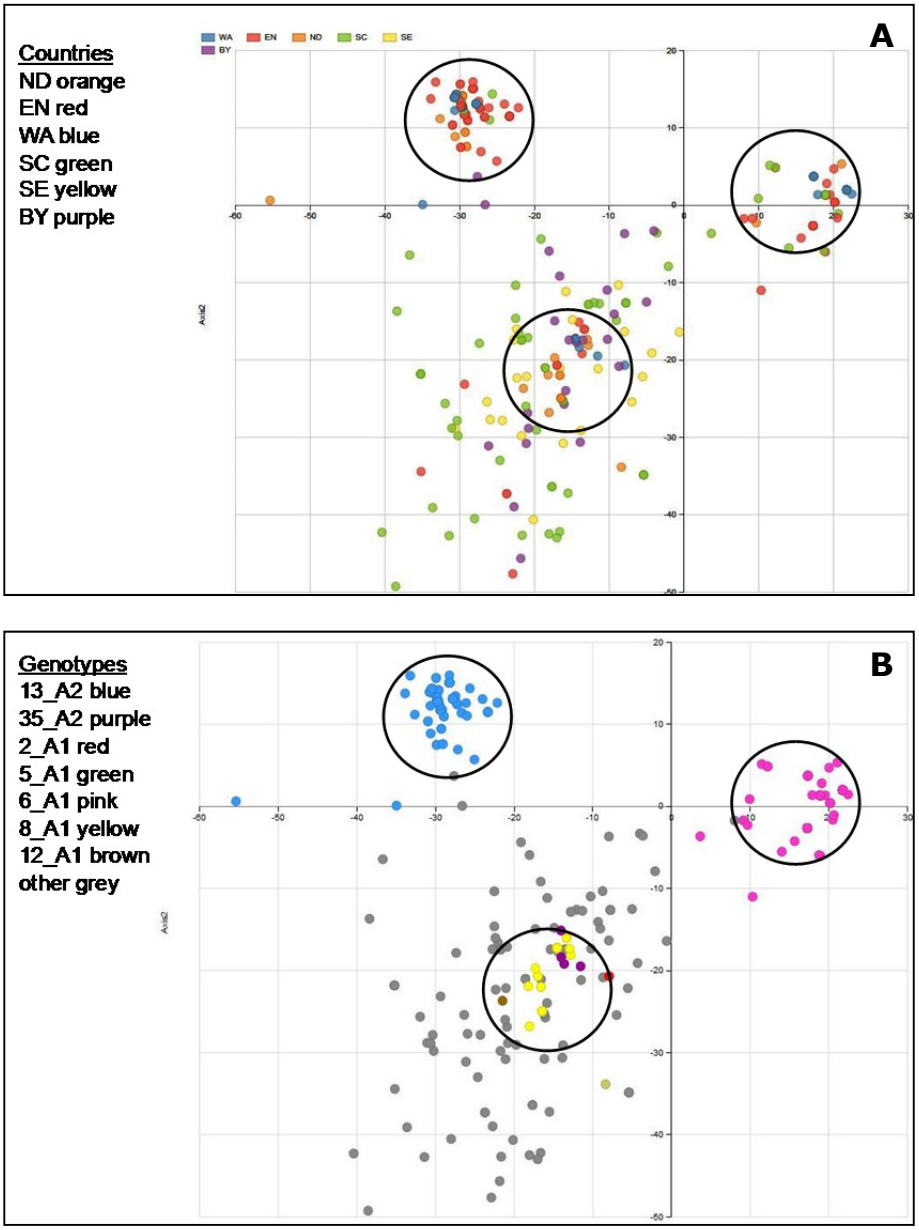

Figure 6. *Euroblight Potato Late Blight Toolbox Principal Component Analyses of Phytophthora infestans SSR data with Bruvo distances as input. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2014; data shown by Country (A) and by Genotype (B), 6_A1 (right), 8_A1(left below) and 13_A2(left above) clusters circled*

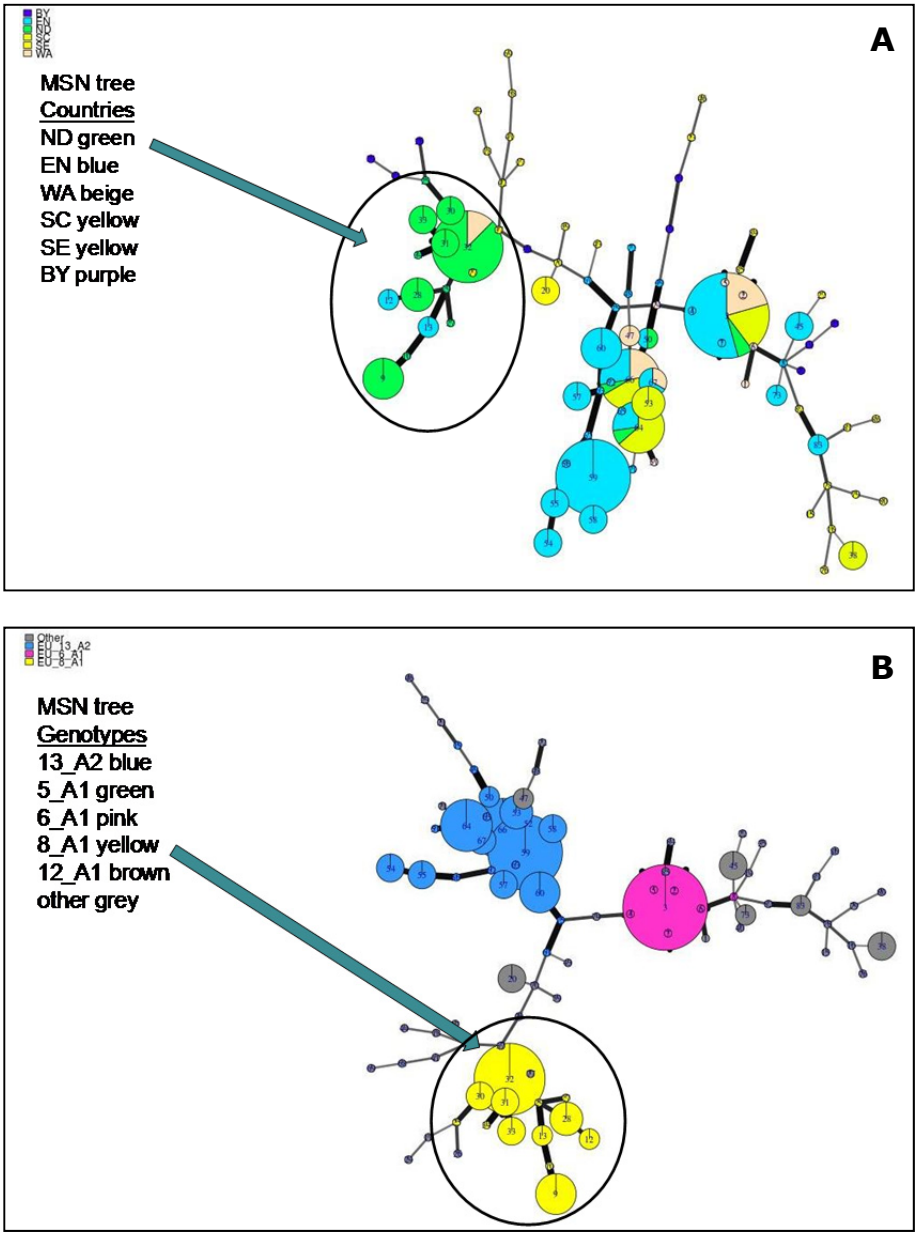

Figure 7. *Euroblight Potato Late Blight Toolbox Multiple Spanning Network Trees of Phytophthora infestans SSR data. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2013; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

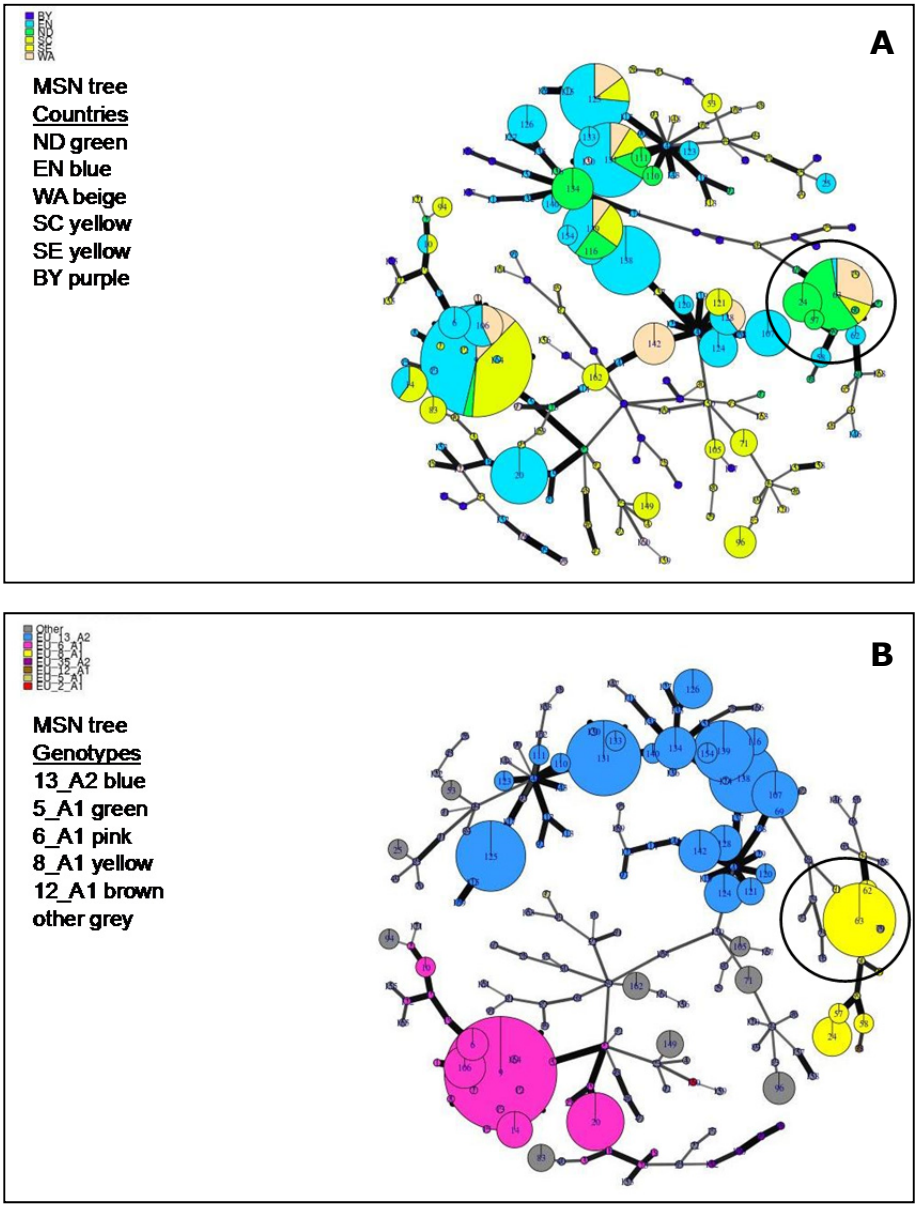

Figure 8. *Euroblight Potato Late Blight Toolbox Multiple Spanning Network Trees of Phytophthora infestans SSR data. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2014; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

DISCUSSION

Studies of the Northern Ireland *P. infestans* population, begun in 1981 when phenylamide-resistant strains were identified (Cooke, 1981), were subsequently extended to investigate mating type (Cooke *et al.,* 1995). Since the mid-1990s, a range of approaches has been used to characterise the population and these have developed as new techniques became available. Results have shown that for the past 20 years, the population has remained highly clonal, but subject to ongoing change from year to year (e.g. Cooke *et al.,* 2014). The Northern Ireland population, as might be expected, contains a subset of the genotypes/clonal lineages present in other European *P. infestans* populations, but throughout the period studied these have occurred at frequencies different from those in the neighbouring population in Great Britain so that results from Great Britain cannot be extrapolated to Northern Ireland. In contrast, comparison of *P. infestans* population studies in Northern Ireland with those in the Republic of Ireland indicates that the pathogen behaves as a single population across the island of Ireland, albeit with some regional variation (e.g. Kildea *et al.*, 2010). As elsewhere in northern Europe, the appearance of the 13_A2 genotype, first identified in 2004-2005 in isolates from The Netherlands and Germany (Cooke *et al.*, 2012a), resulted in major upheavals in the *P. infestans* population in Ireland north and south. However, remarkably, whereas elsewhere in Europe the A1 genotypes of *P. infestans* have also changed, in the island of Ireland the predominant A1 genotype has been 8_A1 (equivalent to the multilocus RG57 genotypes IE-1 and NI-1; Griffin *et al.*, 2002; Cooke *et al.*, 2006) since the mid-1990s. Genotype 6_A1, which has increased in importance in Great Britain, particularly since 2011, although present in Northern Ireland, has remained relatively infrequent.

Understanding the pathogen population is a key component of control strategies, but a number of important questions remain. Among these are:

- Why do genotype frequencies in Northern Ireland and the Republic of Ireland differ from those in Great Britain?
- Why do genotype frequencies in clonal populations such as those in Northern Ireland fluctuate markedly from year to year?
- Why do some European countries now have recombinant *P. infestans* populations while others remain highly clonal?

Clonal populations are particularly subject to the influences of factors such as fungicide usage, cultivar and environment. The fungicides used for late blight control in Great Britain and Northern Ireland are similar and in both areas most cultivars grown are moderately or highly susceptible to blight, so these factors are unlikely to explain population differences. Environmental conditions may have a role; the Irish climate is cooler and wetter with mild winters, which may favour some genotypes such as 8_A1 while militating against others such as 6_A1, which is thought to be encouraged by warmer weather particularly in the spring. In a polycyclic disease such as late blight, repeated asexual reproduction can rapidly magnify the impact of even small selection pressures and also of stochastic effects. While factors such as latent period and sporulation capacity have major influences on genotype selection during the foliar epidemic phase of the disease, the impact of bottlenecks imposed by tuber infection, overwinter survival and success of primary outbreaks also needs to be taken into account and these may well have an important role in annual population changes.

Studies of *P. infestans* populations are valuable for formulation of integrated control strategies and for decision support systems, but without a greater understanding of the drivers of population changes, they remain largely reactive. Thus while they can describe the present or

immediately past populations, they have limited ability to predict the future. One area where future risks can to some extent be predicted is that of fungicide resistance. Monitoring for changes in the pathogen genome which could be associated with fungicide resistance e.g. to the carboxylic acid amide (CAA) fungicides (e.g. benthiavalicarb, dimethomorph, mandipropamid), is already being implemented across Ireland as part of the MonPESC project to provide an early warning of the development of fungicide resistance allowing loss of disease control and crop yield to be avoided (Kildea *et al.*, 2014). Rapid characterisation of the pathogen genotypes infecting specific crops can be achieved by SSR genotyping of DNA collected on FTA cards and this is now being used in the USA with a turn-around time of 24-48 h (Fry, 2015). Such an approach could be adopted in the UK and Ireland where currently population characterisation is done on an annual basis, but would require integration of extension and research programmes and additional funding.

In the longer term, while predicting some future changes in *P. infestans* populations may be possible, forecasting the emergence of new genotypes will be challenging. It remains unclear why some European and North American populations are clonal, despite the presence of both mating types, while other European populations have become recombinant. Ploidy differences may limit the success of sexual reproduction, but why in some in some geographic areas and not others?

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