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Ongoing changes in the Irish potato late blight population

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SUMMARY
As part of a continuing all-Ireland late blight project, the 2010 and 2011 Irish *Phytophthora infestans* populations were characterised phenotypically and genotypically. The 2010 season was not conducive to late blight and only 51 viable single lesion isolates were obtained, all from Northern Ireland, but in 2011 more favourable weather allowed collection of nearly 200 isolates from across the island of Ireland. In 2010, over 70% of isolates characterised were A2 mating type and over 80% were metalaxyl-resistant. In contrast, in 2011, overall only 16% of isolates were A2 and only 30% were metalaxyl-resistant. SSR analysis showed that all A2 isolates were the 13_A2 Blue 13 genotype, while the A1 isolates belonged mainly to the older genotype 8_A1. The highly clonal structure of the Irish *P. infestans* population was confirmed using mitochondrial haplotyping, allozyme genotyping and RG57 fingerprinting.

KEYWORDS
Phytophthora infestans, Ireland, mating type, metalaxyl resistance

INTRODUCTION

The 'new' *Phytophthora infestans* population arrived in Ireland in the early 1980s (O'Sullivan & Dowley, 1991; Cooke *et al*., 1995) and by the mid-1990s it was dominated by two A1 clonal lineages NI-1/IE-1 and NI-2/IE-2 with the A2 mating type present at a low frequency (Carlisle *et al*., 2001; Griffin *et al*., 2002). This situation continued up to 2007 with very few A2 isolates detected after 1995 and phenylamide resistance present, but manageable with an anti-resistance strategy. However, the major changes in the *P. infestans* population structure in Great Britain which began in 2005 when the genotype 13_A2, also referred to as 'Blue 13' was first detected (Cooke *et al*., 2007) were a cause for concern. In 2007, this genotype was first detected in Northern Ireland (Cooke *et al*., 2009) and an all-Ireland project on potato late blight was initiated. Genotypic and phenotypic characterisation of *P. infestans* isolates from throughout the island of Ireland in 2008 and 2009 showed the presence of 'Blue 13' in both years and by 2009 it was the dominant genotype (Kildea *et al*., 2010) It was detected mainly in the eastern counties, with limited numbers in the north-west and none in the south-east. Here we report results of population characterisation in 2010 and 2011.

MATERIALS & METHODS

Collection, isolation and storage of Phytophthora infestans
Surveys of the Irish *P. infestans* population were carried out during the 2010 and 2011 growing seasons. Blighted potato leaf material was collected mainly from commercial crops by members of the Seed Certification Division of the Irish Department of Agriculture, Food and the Marine (DAFM; formerly the Department of Agriculture, Fisheries and Food, DAFF), the Northern Ireland Department of Agriculture and Rural Development (DARD) Quality Assurance Branch Potato Inspectors and Teagasc potato advisors. Once received the blighted material was incubated and isolates established as previously described (Kildea *et al*., 2010).

Mating type, metalaxyl sensitivity and SSR determination
Mating type was determined as described by Cooke *et al.* (2009). The sensitivity of isolates to the fungicide metalaxyl was determined using a floating leaf disk assay (Kildea *et al*., 2010).

The isolates were genotyped by SSR analysis (Kildea *et al*., 2010) using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) and in accordance with the protocol developed by EUCABLIGHT. Genotypes were determined by comparing fragment sizes with isolates previous genotyped (kindly supplied by D.E.L. Cooke, James Hutton Institute).

Selected isolates were also subjected to mitochondrial haplotyping (after Griffith & Shaw, 1998), allozyme genotyping (Carlisle *et al*., 2001) and DNA fingerprinting using the moderately repetitive probe RG57 (Goodwin *et al*., 1992) as described by Cooke *et al.* (2006).

RESULTS

2010
Very few outbreaks of late blight occurred in Ireland in 2010, probably because unusually dry weather early in the season prevented the development of primary infections and secondary spread. Ten outbreaks were sampled in Northern Ireland and yielded 51 isolates, of which 33 were successfully cultured and tested for mating type. However, the majority of these isolates (71% of those metalaxyl sensitivity tested and 58% of those mating typed) were obtained from a single site, AFBI Crossnacreevy, where blight differentials and selected cultivars were grown unsprayed for monitoring. In the Republic of Ireland, only six outbreaks were sampled and due to viability problems, no isolates were characterised.

In Northern Ireland, the frequency of the A2 mating type was 73% in 2010 (compared with 56% in 2009) and as in previous years it was more frequent in Co. Down (south-east) than in the north-west (Fig. 1). However, the sampling was biased because 80% of A2 isolates were from AFBI Crossnacreevy in Co. Down. Metalaxyl-resistant isolates dominated the population (82%), particularly in Co. Down (Fig. 1). All A2 isolates tested were metalaxyl-resistant, whereas only one A1 isolate was resistant.

2011
The weather in 2011 was more conducive to late blight. In Northern Ireland, 27 outbreaks were sampled and 100 isolates were obtained (the majority from outbreaks in the north-west) and in the Republic of Ireland 13 outbreaks were sampled and 91 isolates obtained.

In both Northern Ireland and the Republic of Ireland there was a dramatic change in mating type frequencies; of isolates tested for mating type, only 10% and 22% of isolates from Northern Ireland and the Republic of Ireland, respectively, proved to be A2 (Fig.2). The A2 isolates were from only five sites in Northern Ireland and three sites in the Republic of Ireland.

Eighty-six isolates from Northern Ireland were tested for metalaxyl sensitivity: only 33% proved to be resistant and no isolates with intermediate sensitivity were detected. The proportion of metalaxyl-resistant isolates was higher in Co. Down (56%) than in the north-western counties (Antrim, Londonderry, Tyrone; 18%; Fig. 2). Seventy-two isolates from the Republic of Ireland were tested for metalaxyl sensitivity: only 26% proved to be resistant, but 19% were intermediate (Fig. 2). All of the A2 isolates tested from Northern Ireland tested for metalaxyl sensitivity proved to be resistant, but of the 19 A2 isolates from the Republic of Ireland tested, five were resistant, 13 were intermediate and one was sensitive. Of the A1 isolates tested, eight out of 62 from Northern Ireland were resistant and 14 out of 53 from the Republic of Ireland were resistant and one was intermediate.

SSR analysis of a collection of isolates from Northern Ireland (2010 and 2011) and the Republic of Ireland (2011) detected only three genotypes, 5_A1, 8_A1 and 13_A2 (Blue 13). Genotype 6_A1 (Pink 6), which had been identified in 2009 (Kildea *et al.*, 2010), was not detected in 2010-2011. The majority of A1 isolates in both Northern Ireland and the Republic of Ireland belonged to the 8_A1 genotype. All A2 isolates analysed were 13_A2.

Mitochondrial haplotyping, allozyme genotyping and RG57 fingerprinting all confirmed the limited number of multilocus genotypes detected by SSR. The 5_A1 isolates were mtDNA Ia; *Gpi, Pep 100/100, 100/100* with a common RG57 fingerprint. All 8_A1 isolates were mtDNA IIa, *Gpi, Pep 100/100, 100/100* and all shared a common RG57 fingerprint. Similarly all 13_A2 SSR isolates were mtDNA Ia; *Gpi, Pep 100/100, 96/96* and these also shared common RG57 fingerprints (a variant lacking one band was detected in a few isolates).

DISCUSSION

The Irish *P. infestans* population is continuing to undergo dramatic changes. All characterisation techniques employed in this study support the view that the population remains highly clonal and there is no current evidence of recombination, although the potential for this exists (Nyongesa *et al.*, 2012). Only three multi-locus genotypes were detected in 2010-2011 and two of these (8_A1, 5_A1) are genotypes present in the Irish population since at least the mid-1990s. The most notable feature was the marked decline in the incidence of the Blue 13 (13_A2) genotype which had dominated the population in 2009 and (in Northern Ireland) 2010, but in 2011 was only detected in 10% of Northern Ireland and 22% of Republic of Ireland isolates from a very limited number of sites. A decline has also occurred in Great Britain (Cooke, D.EL., personal communication). The other 'new' genotype Pink 6 (6_A1), which had been found at a few sites in 2009, was not detected at all in 2010-2011. This finding is in marked contrast to the situation in Great Britain, where, as the frequency of Blue 13 declined in 2010 and 2011, there was a concomitant increase in Pink 6 (Cooke, D.E.L., personal communication). In the island of Ireland, the decline in Blue 13 was associated with an increase in the genotype 8_A1, which as NI-1 and IE-1 was the commonest type in the mid-1990s. There was thus a loss of both 'new' genotypes from the population in 2011, which may possibly be related to the very dry weather and low incidence of blight in 2010. The decline in Blue 13 also resulted in the population becoming predominantly metalaxyl sensitive, so that use of phenylamide-containing formulations might once again be an option for control of late blight in Ireland.

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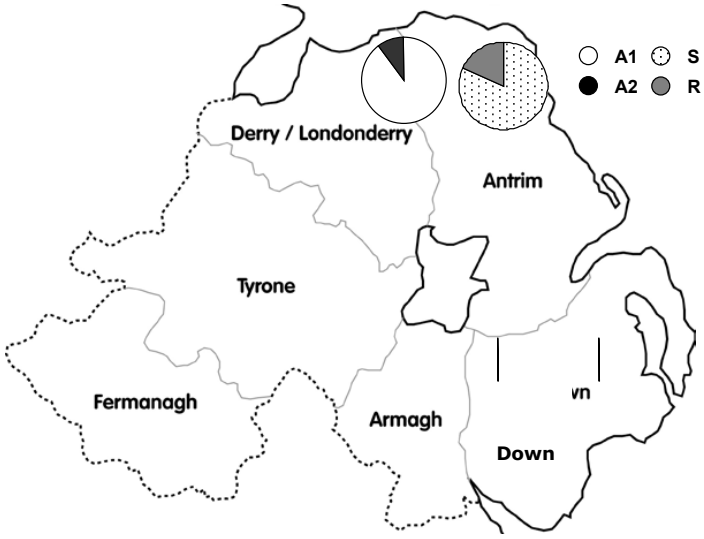

Fig 1. *Mating type and metalaxyl sensitivity of isolates in Northern Ireland, 2010.*

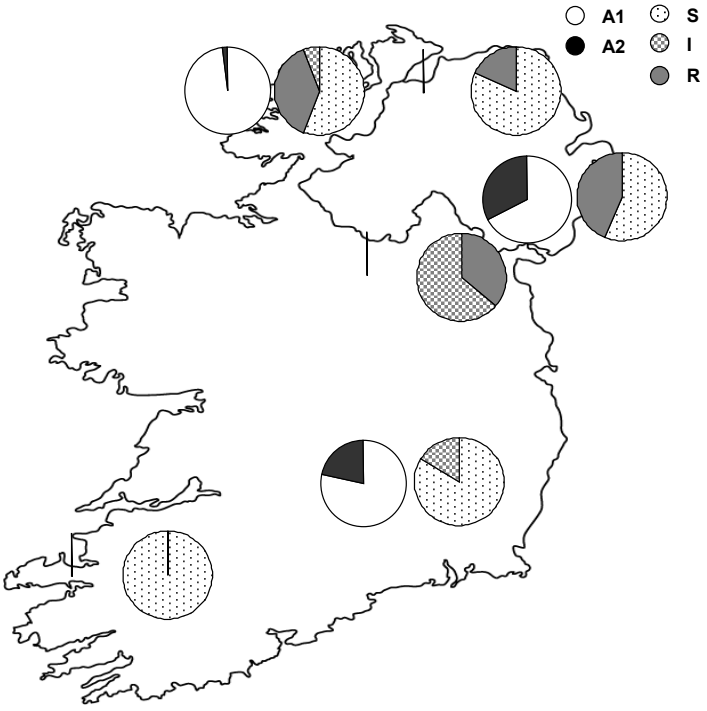

Fig 2. *Mating type and metalaxyl sensitivity of Phytophthora infestans isolates in the Republic of Ireland and Northern Ireland, 2011.*