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Changes within the Irish potato late blight population

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SUMMARY
As part of an all-Ireland late blight initiative the 2008 and 2009 Irish *Phytophthora infestans* populations have been characterised both phenotypically and genotypically. Over both seasons a collection of 659 single lesion *P. infestans* isolates was established. To characterise these isolates they were subject to mating type tests, phenylamide sensitivity assessments and SSR analysis. In both seasons the presence of the A2 mating type 'Blue 13' was confirmed, with increasing numbers of the strain detected in 2009. Their detection was predominantly in the eastern counties, with limited numbers detected in the north-west and none detected in the south-east. The presence and rapid spread of 'Blue-13' has confirmed that the Irish late blight is currently undergoing a dramatic change.

KEYWORDS
Phytophthora infestans, Ireland, A2 mating type, metalaxyl resistance

INTRODUCTION

Following the introduction of the A2 mating type into Ireland in early the 1980s (O'Sullivan & Dowley, 1991; Cooke *et al*, 1995) and the establishment of the 'new' *P. infestans* population that had become prevalent in Europe during the previous years (Tooley *et al.,* 1993) the Irish population became dominated by clonal lineages of a limited number of *P. infestans* genotypes (Carlisle *et al.,* 2001; Griffin *et al.,* 2002). During the same period the frequency of phenylamide resistance has fluctuated depending on the usage of the fungicide. During the period 2000-2007 a decrease in resistance was detected in the population within the Republic of Ireland despite the continued usage of the fungicide (LJ Dowley, *personal communication*).

In Great Britain a similar *P. infestans* population structure was observed up until 2005 when the genotype 13_A2, also referred to as 'Blue 13' was first detected (Day *et al.,* 2004; Cooke *et al.,* 2007). Since then 13_A2 has rapidly spread throughout Great Britain, where it now dominates the *P. infestans* population (Lees *et al.,* 2009). In 2007 it as first detected in Northern Ireland (Cook *et al.,* 2009). With increased aggressiveness and virulence reported on commonly cultivated commercial cultivars (Lees *et al.,* 2009; White & Shaw, 2009) and the lack of an apparent fitness penalty previously associated with phenylamide resistance, determining its presence and/or

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prevalence throughout both the Republic of Ireland and Northern Ireland is essential to ensure the best disease control strategies can be implemented.

MATERIALS & METHODS

Collection, isolation and storage of Phytophthora infestans

Surveys of the Irish *P. infestans* population were carried out during the 2008 and 2009 growing seasons. Blighted potato leaf material was collected from mainly commercial crops by members of the seed certification within the Irish Department of Agriculture Fisheries and Food (DAFF), the Northern Irish Department of Agriculture and Rural Development (DARD) Potato Inspection Service and Teagasc potato advisors. Once received the blighted material was incubated in a moist environment for approximately 24 hrs to promote sporulation. To establish single lesion isolates sporulating mycelium was transferred to antibiotic pea agar (riampicin 50 mg/l) or antibiotic rye agar (rifampicin 25 mg/l and natamycin 25 mg/l). Once pure cultures were established they were maintained on rye agar either as plates or slants.

Mating type, metalaxyl sensitivity and SSR determination

The mating type of each isolate was determined on unamended carrot agar with known reference isolates of the A1 or A2 mating types and as described by Cooke *et al.* (2009). The sensitivity of the isolates to the fungicide metalaxyl was determined using a floating disk assay as described by Cooke (1986). Isolates were deemed sensitive if showing sporulation only on the untreated disks, intermediate if showing sporulation on the untreated and 2 mg/l metalaxyl amended disks only, and resistant if sporulating on all three treatments (0, 2 and 100 mg/ metalaxyl).

The isolates were genotyped by SSR analysis using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) and in accordance to the protocol developed by EUCABLIGHT. DNA from each isolate was extracted from freeze dried mycelia of 14 day old cultures grown in either unamended liquid pea broth or on unamended pea agar. Post-PCR processing analysis was performed on an Applied Biosystems 3130*xl* genetic analyzer and the subsequent DNA fragments were sized automatically using the Applied Biosystems Genemapper® software, version 4.0. Genotypes were determined by comparing fragment sizes with isolates previous genotyped (kindly supplied by David Cooke, SCRI).

RESULTS

In 2008 234 single lesion *P. infestans* isolates were established from 55 samples collected from throughout Ireland (203 isolates from the Republic of Ireland; 31 from Northern Ireland). Weather conditions in 2009 were extremely favourable for the spread of *P. infestans* and 425 single lesion isolates (266 from Republic of Ireland; 159 Northern Ireland) were established from 93 samples.

In 2008 and 2009 the A2 mating type was found in almost an identical frequency in both the Republic of Ireland (25% in 2008 and 50% in 2009) and Northern Ireland (23% in 2008 and 56% in 2009). In both seasons the A2 isolates were found predominantly in the Eastern counties (Fig 1.). In both seasons metalaxyl resistant isolates dominated the populations in both the Republic of Ireland (62% in 2008; 52% in 2009) and Northern Ireland (54% in 2008; 68% in 2009). All A2 isolates tested were metalaxyl resistant. These isolates did not appear to suffer a fitness penalty as they were detected in crops in early June 2009.

SSR analysis of a collection of the A1 mating type isolates confirmed the presence within the Irish *P. infestans* population of the genotypes 8_A1, 5_A1, 12_A1 and 6_A1 (commonly referred to as 'Pink 6'). All A2 mating type isolates genotyped to date were confirmed as 13_A2 (commonly referred to as 'Blue 13').

DISCUSSION

The results presented indicate the Irish *P. infestans* population is currently undergoing a dramatic change. Since its first detection in Northern Ireland in 2007, the 'Blue-13' genotype has spread at a rapid pace similar to that observed in Great Britain (Lees *et al.,* 2009). The apparent east-west divide present with the country in relation to the presence of 'Blue-13' is surprising. Why such a divide exists, and why it has been maintained over the two years of sampling justify further investigation.

Possible problems associated with the presence of 'Blue-13' in the Irish *P. infestans* can be viewed in both the immediate and medium-term time scales. The fact that these isolates do not appear to suffer fitness penalties previously associated with phenylamide resistance reduces the potential of these fungicides in fungicide control regimes. In the medium-term the presence of A1 and A2 mating type isolates within the population provides the potential for the sexual recombination of *P. infestans* and the associated risks.

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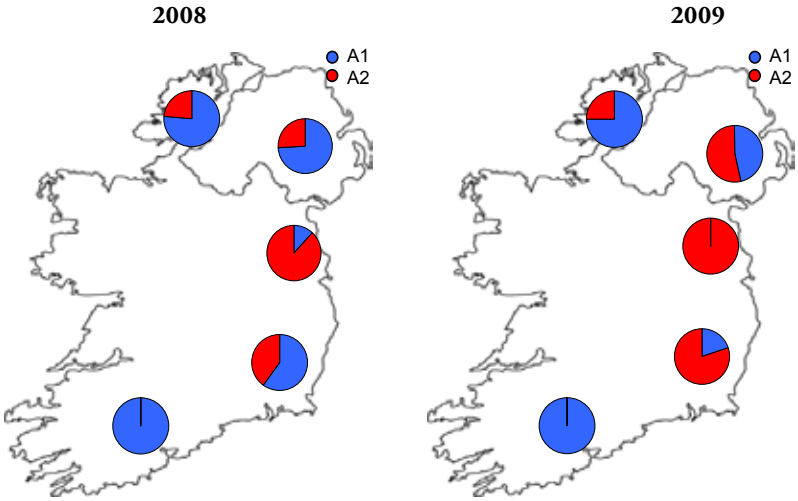

Fig 1. *Frequency of the A2 mating type within the main potato growing regions of Ireland and Northern Ireland in 2008 and 2009.*