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The potato blight population in Northern Ireland in 2012: ongoing changes and fungicide performance

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SUMMARY
Nearly 100 isolates of *Phytophthora infestans* obtained from all potato-growing regions of Northern Ireland in 2012 were characterised for metalaxyl resistance, mating type and *Pep* allozyme genotype. A small sub-sample was also analysed for SSR genotype. The marked decline in the incidence of the A2 mating type between 2010 and 2011 (from over 70% to 10%) was partially reversed: 37% of isolates proved to be A2. All A2 isolates were metalaxyl-resistant and the vast majority were from the south and east of Northern Ireland (Cos. Down and Antrim). Overall, 62% of isolates were metalaxyl-resistant. All A2 isolates were *Pep 96/96* and SSR analysis of three showed that they were Blue 13 (13_A2). All but two A1 isolates were *Pep 100/100*; these two, which were *Pep 96/96*, were shown to belong to the Pink 6 (6_A1) genotype by SSR (the first finding in Ireland since 2009), whereas the other A1 isolates analysed by SSR were all 8_A1. The Northern Ireland *P. infestans* population remains highly clonal. In small-scale field trials in Belfast in 2010, 2011 and 2012, a standard programme of two applications of mandipropamid + fluazinam followed by eight applications of fluazinam gave good control of foliar and tuber blight and there was no evidence for a decline in performance of fluazinam, which is the fungicide most widely used for potato blight control in Northern Ireland.

KEYWORDS
Phytophthora infestans, Northern Ireland, mating type, metalaxyl resistance, allozyme genotype

INTRODUCTION

Since the 'new' *Phytophthora infestans* population arrived in Northern Ireland in the early 1980s (Cooke *et al*, 1995), the population has remained clonal. Up to 2007, it was dominated by a few A1 clonal lineages with the A2 mating type at low frequency (Carlisle *et al.*, 2001; Cooke *et al.,* 2006). From 2007 onwards, the population has undergone major upheavals following the appearance in Northern Ireland (Cooke *et al.*, 2009) of genotype 13_A2, also referred to as 'Blue 13' (Cooke *et al.*, 2012a). Genotypic and phenotypic characterisation of *P. infestans* isolates from the island of Ireland was initiated as part of an all-Ireland project on potato late blight and

this showed the presence of 'Blue 13' throughout Ireland in 2008 and by 2009 it was the dominant genotype (Kildea *et al.*, 2010). The 2010 season was not conducive to late blight and only 51 viable isolates were obtained, all from Northern Ireland; over 70% were A2 mating type and over 80% were metalaxyl-resistant, but most were from a single site (Cooke *et al.*, 2012b). In 2011, blight was widespread and nearly 200 isolates were obtained from across the island of Ireland, of which 100 were from Northern Ireland. In both Northern Ireland and the Republic of Ireland there was a dramatic reduction in the incidence of the A2 mating type; only 10% and 22% of isolates from Northern Ireland and the Republic of Ireland, respectively, proved to be A2. In Northern Ireland, the A2 isolates were from only five sites, all were metalaxyl-resistant and SSR analysis showed that all were the 13_A2 Blue 13 genotype, while the A1 isolates belonged to older genotypes, mainly 8_A1; the situation was similar in the Republic of Ireland. In contrast, in Great Britain, although the incidence of Blue 13 also declined abruptly to only 10% of isolates (Cooke, D.E.L., personal communication), the population was dominated by the newer genotype 6_A1 ('Pink 6'), which was found in Northern Ireland at low frequency only in 2009. The all-Ireland late blight project concluded in 2012 and did not include population characterisation for that year, so to obtain information on whether the decline of Blue 13 would continue a short-term investigation was initiated. Here we report results of this and of fungicide performance of standard programmes in 2010-2012.

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).

Mating type, metalaxyl sensitivity and SSR determination

Mating type was determined as described by Cooke *et al.* (2006). The sensitivity of isolates to the fungicide metalaxyl was determined using a floating leaf disk assay (Cooke *et al.,* 2006). For selected isolates, genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase) and *Pep-1* (peptidase), were determined using cellulose acetate electrophoresis (Carlisle *et al.*, 2001). A sub-sample of isolates was 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.

Field evaluation of fungicide performance against foliar blight 2010-2012

Tubers cv. Up-to-Date were planted in April or May of each year (Table 1) at AFBI Headquarters, Newforge, Belfast, Northern Ireland in fully randomised blocks with five replicate plots per treatment as described by Cooke & Little (2010). Each plot (2.8 x 3.0 m^2) contained four rows of ten tubers. Pairs of rows of unsprayed plants adjacent to each treated plot served as an infection source and were inoculated in July of each year (Table 1). In these rows, two leaves on every fourth plant were inoculated with recent Northern Ireland isolates of *P. infestans.* In 2010 and 2011, 50% of leaves were inoculated with A2 metalaxyl-resistant isolates, 25% with A1 resistant isolates and 50% with A1 sensitive isolates, while in 2012 (following the decline in the incidence of the A2 mating type), 25% were inoculated with A2 resistant isolates, 25% with A1 resistant

isolates and 50% with A1 sensitive isolates. When required, plots were misted after inoculation, usually for 2-3 h daily at dawn and dusk to encourage spread of blight.

Table 1. *Field trials for the control of potato blight, 2010-2012: dates of field operations*

Year	Planting date	Fungicide application dates		Inoculation	Desiccation	Harvest
		First	Last			
2010	28 April	18 June	19 August	7 July	25 August, 1 September	21 September
2011	4 May	29 June	31 August	6 July	15, 20 September	31 October
2012	21 May	26 June	30 August	2 July	6, 13 September	9 October

Fungicide formulations were applied at manufacturers' recommended rates in *c*. 300 litres water/ha using a Cooper Pegler CP15 knapsack sprayer. The first applications were made before inoculation in the third or fourth week of June of each year (Table 1) and ten treatments were applied at 7-day intervals (as far as possible) until the end of August. In each year, the standard programme comprised two sprays of mandipropamid (150 g /ha as 'Revus', Syngenta) tank-mixed with half-rate fluazinam (100 g/ha as 'Shirlan', Syngenta) followed by eight sprays of full-rate fluazinam (200 g/ha). Other programmes included in the trial are not reported here (for results of 2012 trial mandipropamid + fluazinam/mancozeb programme, see poster Cooke & Nugent, in this volume).

Foliage blight was assessed on each drill of each sprayed plot twice weekly from the time that blight was first seen in them until haulm destruction, using the ADAS key (Anonymous, 1976) with added 0.01% and 10% categories. Plots were desiccated with diquat dibromide ('Reglone', Syngenta) in late August or early September and tubers harvested in September or October (Table 1). The yield from each plot was graded and recorded; the number and weight of blighted, soft-rotted tubers was recorded and they were then discarded. The number and weight of firm blighted tubers >35 mm was assessed (and diseased tubers discarded) in November-December in each year. The remaining healthy tubers were stored and re-assessed in late January-February, after which the final marketable yield was determined. In each trial, the Area Under the Disease Progress Curve (AUDPC) was calculated from the untransformed percentage foliage blight for each plot.

RESULTS

Population characterisation 2012

The weather in summer 2012 was very cool and wet between May and September. Until the third week in June, minimum temperatures were generally well below 10°C and so conditions were too cold for development of blight. The first outbreak was reported on 22 June as it became warmer. Blight was encouraged by the high proportion of rainy days as well as by very high rainfall on certain days (22 June, 44.4 mm; 27 June, 53.6 mm; 24 September, 52.0 mm) and outbreaks were reported in all potato-growing areas of the Province. A total of 34 crops were sampled (Co. Antrim, 11; Cos. Down & Armagh, 15; Co. Londonderry & Tyrone, 8) and 99 *P. infestans* isolates obtained (up to five isolates per site).

Overall, 62% of isolates were metalaxyl-resistant (Figure 1) with the highest proportion of resistant isolates found in Cos. Down & Armagh (74%) and Co. Antrim (69%) compared with only 27% in Cos. Londonderry & Tyrone (Figure 2). This compares with the 2011 figure of 33% metalaxyl-resistant isolates overall.

Eighty-four isolates from 31 crops were tested for mating type and 37% proved to be A2 (Figure 3), compared with only 10% in 2011. All of the A2 isolates were metalaxyl-resistant and the majority were obtained from the south-east (Figure 2), while 30% of A1 isolates were metalaxyl-resistant. Only one A2 isolate was from Cos. Londonderry & Tyrone (4.5%, n=22), whereas 36% from Co. Antrim (n=33) and 62% from Down & Armagh (n=29) were A2.

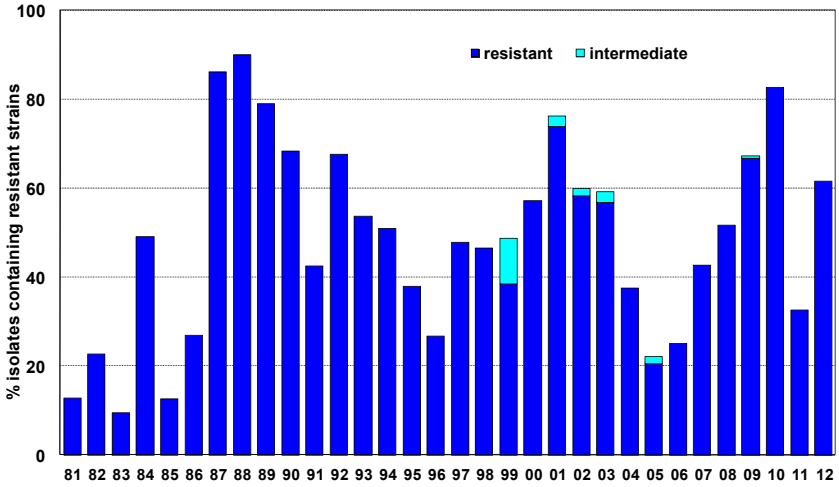

Figure 1. *The percentage of Northern Ireland Phytophthora infestans isolates containing metalaxyl-resitant strains, 1981-2012*

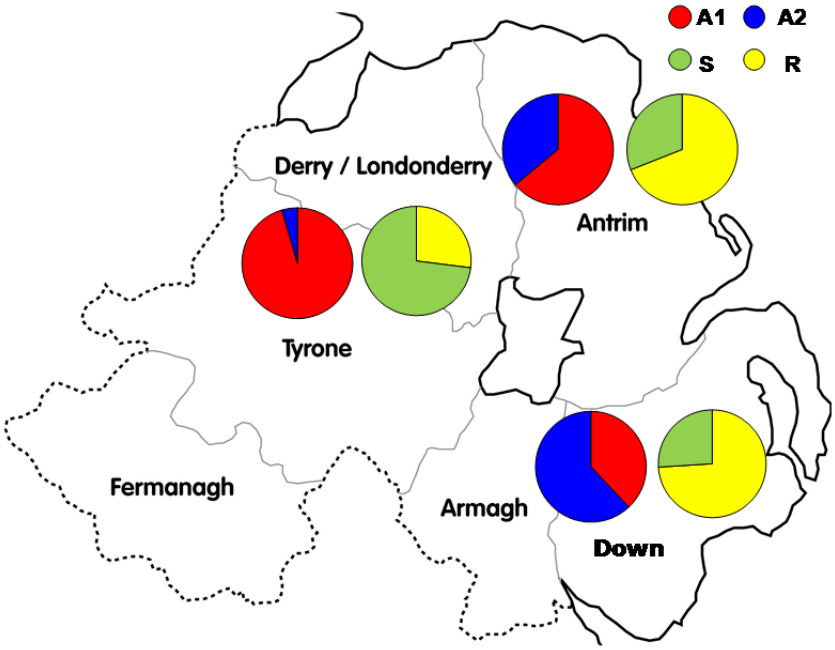

Figure 2. *Mating type and metalaxyl sensitivity of Nothern Ireland Phytophthora infestans isolates, 2012*

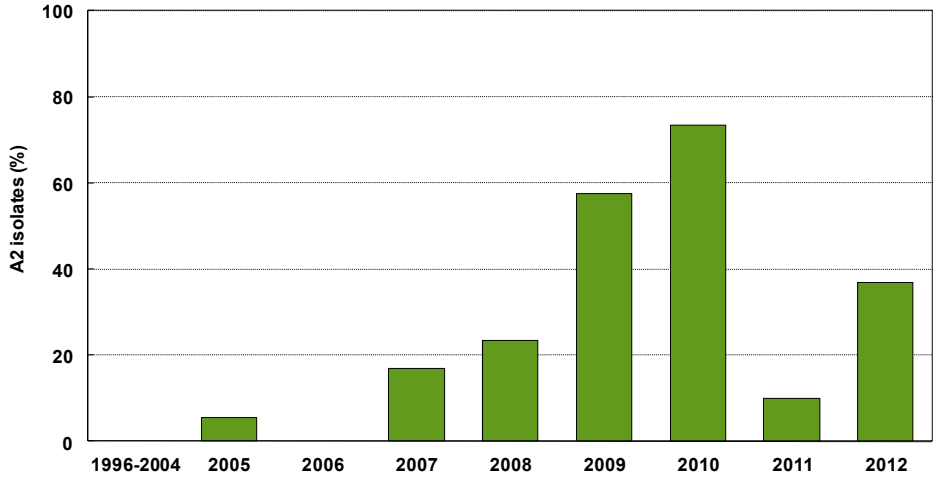

Figure 3. *The percentage of Northern Ireland Phytophthora infestans isolates of A2 mating type, 19966-2012*

All isolates characterised for *Gpi* proved to be *100/100*. Of 82 isolates characterised for *Pep,* all of 30 A2 isolates were *96/96,* while of 52 A1 isolates, 50 were *Pep 100/100* and two were *96/96.* Nine of the isolates were SSR genotyped at JHI and this showed that the three A2 isolates (from three different sites) were all Blue 13 (two 13_A2_1 and one 13_A2_37), while of six A1 isolates, four (from three sites) were 8_A1 (two 8_A1, two 8a_A1) and two (from a single site) were 6_A1. The two 6_A1 isolates were the two A1 isolates with *Pep 96/96.*

Field evaluation of fungicide performance against foliar blight 2010-2012

In each year, the standard programme (two applications of mandipropamid + half-rate fluazinam followed by eight fluazinam applications) achieved good foliar blight control (Table 2), while the adjacent, inoculated infector drills had 95-100% foliar infection by the final assessment. Foliage blight was greatest in 2012 probably as a result of some tuber blight in the seed and conditions very conducive to infection, but was still under 5% at the final assessment, whereas plots where the first two applications of another programme were made at very reduced rate were completely killed by blight (data not shown). Tuber blight was also well controlled, although in 2012 extensive soft rotting made accurate assessment impossible. Yields were extremely low in 2012 reflecting the very poor growing conditions, with low temperatures and little sunshine as well as a high incidence of blackleg (*c.* 30% of plants affected), but were similar in all programmes.

Table 2. *Performance of standard mandipropamid + fluazinam/fluazinam programme in field trials for the control of potato blight, 2010-2012*

Year	Final foliar blight assessment		AUDPC[a]	Tuber blight (% by number)	Total yield >35 mm (kg/plot)	Marketable yield (kg/plot)
	Date	Foliar blight (%)				
2010	24 August	1.24	7.7	3.63	65.0	58.6
2011	14 September	1.80	27.2	7.49	56.7	48.6
2012	4 September	4.00	87.4	3.73	30.1	26.7

[a] *Area Under the Disease Progress Curve for foliage blight development*

DISCUSSION

Mating type determination combined with *Pep* allozyme genotyping provided a useful predictor of genotype in the Northern Ireland *P. infestans* population. All A2 isolates genotyped since 2008 have proved to be Blue 13 and all of these examined to date for *Pep* allozyme genotype were *96/96*. It is therefore reasonable to assume that all A2 *Pep 96/96* isolates identified in the current study were Blue 13. In contrast, the vast majority of A1 isolates in the current population in Northern Ireland were *Pep 100/100,* but the two which were *Pep 96/96,* proved to belong to the Pink 6 genotype, the first finding of this since 2009*.* In a study of Pink 6, allozyme genotyping of 18 isolates from Great Britain, Northern Ireland and the Republic of Ireland showed that all had *Pep 96/96* (Kildea *et al.,* 2013). Therefore the low frequency of *Pep 96/96* in A1 isolates of *P. infestans* from Northern Ireland in 2012 clearly indicated that the Pink 6 genotype was rare. As in previous years, it is likely that the vast majority of A1 isolates belonged to older genotypes such as 8_A1 as was indicated by the limited SSR genotyping.

However, it should not be assumed that A1 isolates of *P. infestans* from other populations with *Pep 96/96* belong to the Pink 6 genotype, since this allozyme pattern can be associated with quite different multi-locus genotypes e.g. in the Hungarian *P. infestans* population (Nagy *et al.,* 2006).

The current study clearly indicates that Northern Ireland *P. infestans* population is continuing to change: the Blue 13 genotype, which declined so markedly in 2011, underwent a resurgence in 2012 (to 37% of isolates), although it did not return to dominating the population as it did in 2009 and 2010. The increase in Blue 13 was associated with an increase in the proportion of metalaxyl-resistant isolates within the population (since Blue 13 is invariably metalaxyl-resistant) and, as the A1 isolates found belonged to genotypes that may be either metalaxyl-resistant or -sensitive (unlike Pink 6, which is always metalaxyl-sensitive), it is probably inadvisable to encourage the use of phenylamide-containing formulations for blight control in Northern Ireland. The limited characterisation of the 2012 Northern Ireland isolates indicated that the population remained highly clonal, but distinct from that in Great Britain where Pink 6 dominated (Cooke, D.E.L., personal communication).

Field trials between 2010 and 2012 indicated continuing good performance of programmes based on mandipropamid and fluazinam. Growers are encouraged to make use of a wide range of active ingredients with different modes of action and not to rely heavily on a single fungicide (*viz.* fluazinam), but for the purposes of trials, it is useful to have a simple standard programme for comparative purposes. The performance of the standard indicated consistent good control by fluazinam and no evidence of the poor performance associated with the occurrence of the Green 33 *P. infestans* genotype as occurred in The Netherlands in 2011 (Schepers *et al.,* 2013). Examination of genotyping results from the previous all-Ireland study failed to find any isolates with the Green 33 profile in the Irish population between 2008 and 2011 (S. Kildea, personal communication) and Green 33 is rare in Great Britain (Cooke, D.E.L., personal communication), so at present this and similar genotypes are not affecting fluazinam performance in the UK and Ireland; anecdotal evidence is of good performance in Northern Ireland. Any occurrence of *P. infestans* genotypes with reduced sensitivity to fluazinam could seriously impact on late blight control in Northern Ireland as fluazinam is the most popular fungicide for blight control, used by 63-75% of seed growers in the years 2010-12 (Cooke, L. R., unpublished) and is the most widely used fungicide (in terms of spray hectares) on seed, early and maincrop potatoes (Withers *et al.,* 2013). It is therefore important to continue to monitor changes in the *P. infestans* population in Northern Ireland so that control programmes can take into account the characteristics of current genotypes.

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