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# Project Report

# Variation in *Phytophthora infestans*: characterisation of A1 mating types and additional A2 mating types from 2005

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# 1. Summary

The aim of this research has been to complete a characterisation of GB isolates of *Phytophthora infestans* from the Fight Against Blight (FAB) campaign 2005, begun in 2005, and to include miscellaneous isolates also collected in 2005. Isolates have been characterised using molecular fingerprinting and sensitivity to metalaxyl so that direct comparisons can be made with isolates collected in the 1990s and analysed in the same way. The study in 2005 detected a much increased frequency of A2 isolates. Many variants, including some which were fully resistant to metalaxyl, were detected among the FAB A2 isolates. This contrasted with the A2s from the 1990s which were not variable and were never fully resistant to metalaxyl.

The results of the present study of the A1 isolates from the FAB collection showed the presence of many different fingerprints and different sensitivities to metalaxyl. One of the fingerprints (type I) was predominant, being found at 16 of the 41 sites. The next most frequent variant was found at 5 sites. Other variants were found at three, two or only one site. As was the case for the A2 isolates from 2005, some A1 isolates were sensitive to metalaxyl, more were intermediate and most were resistant.

Analysis of the 24 miscellaneous isolates of both A1 and A2 mating type showed similar results to those of the FAB isolates; three of the A1 isolates were of type I fingerprint. Fifteen other fingerprints were detected at three, two or one site. Both A1 and A2 isolates showed a full range of variation for metalaxyl sensitivity.

Combining all FAB and miscellaneous isolates has shown that both A1 and A2 mating type isolates are highly variable for molecular fingerprint and for sensitivity to metalaxyl. This is in contrast to the populations analysed in the 1990s where the A1 mating type was highly variable but the rare A2 mating type was non-variable and never fully resistant to metalaxyl. The predominant A1 type I was the only variant detected in the 1990s when it was present at a lower frequency. The predominant variant ten years ago which was detected at half of the sites sampled was not detected among 2005 isolates.

# 2. Experimental Section

## 2.1 Introduction

The unexpectedly high frequency of A2 mating types detected in samples obtained in the Fight Against Blight (FAB) campaign in 2005, prompted a study of the molecular genotype and the metalaxyl sensitivity of the A2 isolates. On the last occasion when GB isolates of blight were characterised (1995-8), the frequency of the A2 mating type was not more than a few percent, the molecular fingerprint of A2s as determined by the RFLP probe RG57 was largely of a single genotype and almost all isolates were sensitive to metalaxyl (Day *et al.,* 2004*).* When the A2 mating types from 2005 were similarly characterised, they belonged to one of seven molecular genotypes, none of which had been detected in the old collections. Moreover, approximately half of the A2s were resistant to metalaxyl (BPC report, Shaw *et al.,* 2006).

The present study aimed to characterise the A1 isolates from the 2005 FAB collection in the same way and also to characterise both A1 and A2 isolates collected by the authors from a variety of sites, mainly in England and Wales. This was done to extend the study of new A2 isolates and to find out if A1 genotypes had also changed from those determined in 1995-8.

## 2.2 Material and methods

A1 isolates from the FAB collection and A1 and A2 isolates from the authors' collection (now referred to as miscellaneous isolates) were each stored in October, 2005, at room temperature within plastic cryotubes each containing sterile water and several sterile rye grains. Where several miscellaneous isolates had been made from some sites and both mating types had been detected, one isolate of each mating type was selected for characterisation. Cultures were revived on rye A agar and grown on in pea-water broth to generate approximately 150mg of dried mycelium which was used for DNA extraction. Mitochondrial haplotypes were determined using the PCR method (Griffith and Shaw, 1998) but using an annealing temperature of 62°C. The determination of RG57 genotype was carried out using the protocol of Pipe and Shaw (1997) as modified by Nagy *et al.,* (2006).

Sensitivity of the isolates to metalaxyl was determined *in vitro* using a modification of the method of Shattock (1988). Three replicate inocula, each of 4mm diameter, were placed towards the edge of plates of pea-water agar containing 0 or 50µg /ml metalaxyl for each isolate. There were three replicates for each isolate. The hyphal extension over 7 days' incubation at 20°C was measured and the mean growth on 50µg/ml was expressed as a percentage of that made on 0µg/ml metalaxyl. Isolates showing growth relative to the control of 0 - 20% were classed as sensitive, S, of 20 - 40% were classed as intermediate, I, and of 40 - >100% as resistant, R.

## 2.3 Results

*Molecular characterisation*
The fingerprints revealed by RFLP probe RG57 are shown in Table 1. Presence or absence of each of the 25 bands scored is shown for each isolate where 1,3,5 etc indicates presence of bands 1,3 and 5 but absence of bands 2 and 4. Thirty-three different fingerprints were scored. One fingerprint, referred to here as type I was identified in isolates from 19 sites. All of these isolates were of A1 mating type; 16 were FAB isolates and three were miscellaneous isolates. The next most frequent fingerprint was type II, represented by five isolates, all from FAB sites. Types III, IV and V were each represented by three isolates and all but two isolates were from miscellaneous sites. Types VI (FAB and misc.), VII (misc.), VIII (misc.), and IX (FAB) were represented by two isolates each. The remaining 16 FAB isolates and eight miscellaneous isolates all had different fingerprints.

Mitochondrial haplotypes Ia and IIa were detected. 37 (19 FAB + 18 misc.) isolates were Ia and 28 (22 FAB + 6 misc.) were IIa.

*Sensitivity to metalaxyl*
Fig. 1 shows that the sensitivity to metalaxyl amongst the isolates had a continuous distribution from full sensitivity to resistance where growth on metalaxyl exceeded that on the control plates. Sensitivities for individual isolates is shown in Table 1. When divided into classes, Sensitive, Intermediate and Resistant, there were 14, 20 and 31 isolates respectively. The distribution of S, I and R classes were similar for A1 FAB isolates (Fig. 2) and for miscellaneous isolates (Fig. 3).

*Multi-marker variation*
All isolates of type I fingerprint were of A1 mating type and IIa haplotype; sensitivity to metalaxyl included sensitive, intermediate and resistant phenotypes. Isolates of type II were all of A1 mating type and Ia haplotype; metalaxyl sensitive and resistant phenotypes were present. Isolates belonging to a single fingerprint type were always of the same haplotype and the same mating type with the exception of type III where the two isolates for one site (M23) were of different mating type.

*Geographic distribution of variants*
Fig. 4 shows the sites from which the FAB A1 isolates were obtained and Fig. 5 shows the sites from which the miscellaneous isolates were obtained. Fingerprints of types I and II are identified. There appears to be a tendency for type I isolates to be coastal.

| ID | Post Code | Mt | RG57 fingerprint | Type | Metalaxyl* | ** | Mating type |
|---|---|---|---|---|---|---|---|
| 14392 | L39 | Ia | 1,2,3,5,6,7,10,13,14,16,20,21,22,24,25 | | 65 | R | A1 |
| 14608 | PE22 | Ia | 1,2,3,5,6,7,8,10,13,14,16,20,21,24,25 | | 30 | I | A1 |
| 15472 | LL57 | Ia | 1,2,3,5,7,10,13,14,16,20,21,22,24,25 | V | 25 | I | A1 |
| 15499 | LL57 | Ia | 1,2,3,5,7,10,13,14,16,20,21,22,24,25 | V | 52 | R | A1 |
| M21 | | Ia | 1,2,3,5,7,10,13,14,16,20,21,22,24,25 | V | 16 | S | A1 |
| 12988 | YO17 | Ia | 1,2,3,5,7,10,13,14,16,20,21,24,25 | VI | 65 | R | A1 |
| M14 | | Ia | 1,2,3,5,7,10,13,14,16,20,21,24,25 | VI | 22 | I | A1 |
| M34 | | Ia | 1,2,3,5,7,10,13,14,16,20,22,24,25 | | 32 | I | A1 |
| M31 | | IIa | 1,2,3,5,7,9,10,13,14,17,20,21,24,25 | VIII | 48 | R | A2 |
| M8-2 | | IIa | 1,2,3,5,7,9,10,13,14,17,20,21,24,25 | VIII | 37 | I | A2 |
| M15-2 | | Ia | 1,2,5,10,13,14,16,17,20,21,24,25 | | 32 | I | A2 |
| 12556 | CT7 | Ia | 1,2,5,10,13,14,16,17,20,22,24,25 | | 46 | R | A1 |
| M15-1 | | Ia | 1,2,5,10,13,14,16,20,22,24,25 | | 35 | I | A1 |
| 12799 | IV31 | Ia | 1,2,5,13,14,17,20,22,24,25 | | 8 | S | A1 |
| 14149 | AB51 | Ia | 1,2,5,8,10,13,14,20,22,24,25 | | 44 | R | A1 |
| 14905 | CA6 | IIa | 1,2,5,9,10,13,14,16,20,21,22,24,25 | | 56 | R | A1 |
| 13636 | NR28 | Ia | 1,2,8,10,13,14,16,17,20,21,22,23,24,25 | | 66 | R | A1 |
| M11-2 | | Ia | 1,2,8,10,13,14,16,17,20,21,22,24,25 | | 92 | R | A2 |
| 13150 | CW6 | Ia | 1,3,5,6,7,8,10,13,14,16,20,21,24,25 | | 200 | R | A1 |
| 15769 | TF10 | Ia | 1,3,5,6,7,8,9,10,13,14,16,17,20,21,22,24,25 | | 72 | R | A1 |
| 13285 | TA14 | Ia | 1,3,5,6,7,8,9,10,13,14,16,20,21,24,25 | II | 8 | S | A1 |
| 13609 | GU29 | Ia | 1,3,5,6,7,8,9,10,13,14,16,20,21,24,25 | II | 83 | R | A1 |
| 14743 | LN8 | Ia | 1,3,5,6,7,8,9,10,13,14,16,20,21,24,25 | II | 65 | R | A1 |
| 14851 | PE7 | Ia | 1,3,5,6,7,8,9,10,13,14,16,20,21,24,25 | II | 50 | R | A1 |
| 16282 | NG23 | Ia | 1,3,5,6,7,8,9,10,13,14,16,20,21,24,25 | II | 80 | R | A1 |
| 13420 | PE22 | Ia | 1,3,5,6,7,8,9,10,13,14,17,20,24,25 | | 19 | S | A1 |
| 14419 | DD11 | Ia | 1,3,5,6,7,8,9,10,13,14,20,24,25 | | 100 | R | A1 |
| M13 | | Ia | 1,3,5,6,7,9,10,13,14,16,17,20,21,25 | | 88 | R | A1 |
| 16120 | CT3 | Ia | 1,3,5,6,7,9,10,13,14,16,20,21,24,25 | | 71 | R | A1 |
| M22 | | Ia | 1,3,5,7,10,13,14,16,17,20,21,24,25 | VII | 20 | S | A2 |
| M30 | | Ia | 1,3,5,7,10,13,14,16,17,20,21,24,25 | VII | 51 | R | A2 |
| M23-1 | | Ia | 1,3,5,7,10,13,14,17,19,20,21,24,25 | III | 96 | R | A1 |
| M23-2 | | Ia | 1,3,5,7,10,13,14,17,19,20,21,24,25 | III | 73 | R | A2 |
| M33 | | Ia | 1,3,5,7,10,13,14,17,19,20,21,24,25 | III | 34 | I | A2 |
| M20 | | Ia | 1,3,5,7,10,13,14,17,20,21,24,25 | IV | 43 | R | A2 |
| M25-2 | | Ia | 1,3,5,7,10,13,14,17,20,21,24,25 | IV | 78 | R | A2 |
| M7 | | Ia | 1,3,5,7,10,13,14,17,20,21,24,25 | IV | 49 | R | A2 |
| M25-3 | | Ia | 1,3,5,7,10,13,14,20,21,22,24,25 | | 83 | R | A2 |
| M8-1 | | Ia | 1,3,5,7,9,10,13,14,16,19,20,21,25 | | 204 | R | A1 |
| M10 | | IIa | 1,3,5,7,9,10,13,14,17,20,21,22,24,25 | | 62 | R | A2 |
| 13447 | PH2 | IIa | 1,5,10,13,14,16,20,21,24,25 | IX | 11 | S | A1 |
| 13933 | DD10 | IIa | 1,5,10,13,14,16,20,21,24,25 | IX | 32 | I | A1 |
| 13852 | NR17 | IIa | 1,5,13,14,17,20,21,24,25 | | 10 | S | A1 |
| M11-1 | | Ia | 1,5,6,9,10,13,14,17,20,21,24,25 | | 11 | S | A1 |
| 14878 | YO61 | IIa | 1,5,9,10,13,14,16,20,21,22,24,25 | | 56 | R | A1 |
| 12502 | NR16 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 66 | R | A1 |
| 12529 | IP12 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 18 | S | A1 |
| 12610 | DG9 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 16 | S | A1 |
| 12772 | 1P12 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 13 | S | A1 |
| 12880 | LL76 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 37 | I | A1 |
| 12907 | NR16 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 17 | S | A1 |
| 12961 | DD6 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 53 | R | A1 |
| 13069 | SA73 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 26 | I | A1 |
| 13258 | NR10 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 24 | I | A1 |
| 13393 | NG32 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 26 | I | A1 |
| 13474 | CA22 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 36 | I | A1 |
| 13690 | PE32 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 55 | R | A1 |
| 14068 | DG7 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 31 | I | A1 |
| 14203 | DD2 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 38 | I | A1 |
| 14365 | CA4 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 15 | S | A1 |
| 14716 | YO7 | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 30 | I | A1 |
| M1 | | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 39 | I | A1 |
| M16 | | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 21 | I | A1 |
| M19 | | IIa | 1,5,9,10,13,14,16,20,21,24,25 | I | 33 | I | A1 |
| 15904 | KY10 | IIa | 1,5,9,10,13,15,17,20,21,24,25 | | 11 | S | A1 |

Table 1. Genotypes and phenotypes of FAB A1 isolates (five-digit number, highlighted green) and Miscellaneous isolates of both mating types (M followed by number as in BPC report, Shaw et al., 2005).

**Type** is an arbitrary Roman numeral assigned to fingerprints occurring more than once. Identical fingerprints have the same background fill – either grey or white. Metalaxyl * is the sensitivity as calculated from the growth on metalaxyl relative to the growth without metalaxyl, expressed as a percentage. ** R = resistant, I = intermediate resistance, S = sensitive (see Materials and Methods). Locations of miscellaneous isolates can be found in Fig. 5.

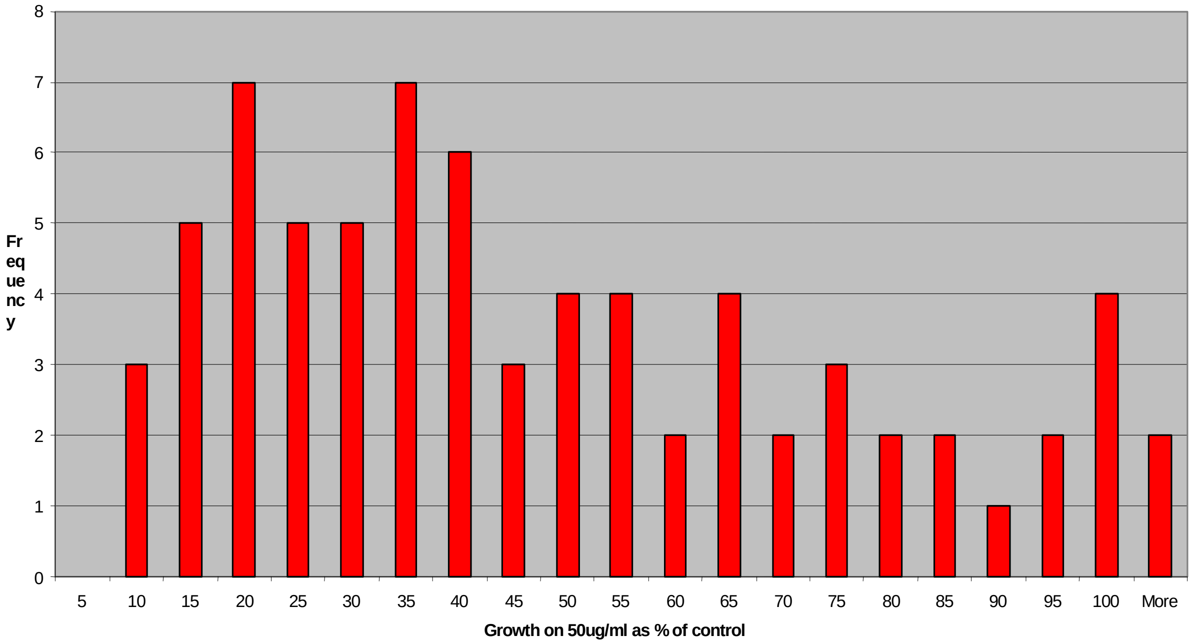

FIG. 1. THE FREQUENCIES OF SENSITIVITY CLASSES TO METALAXYL IN 5% INTERVALS.

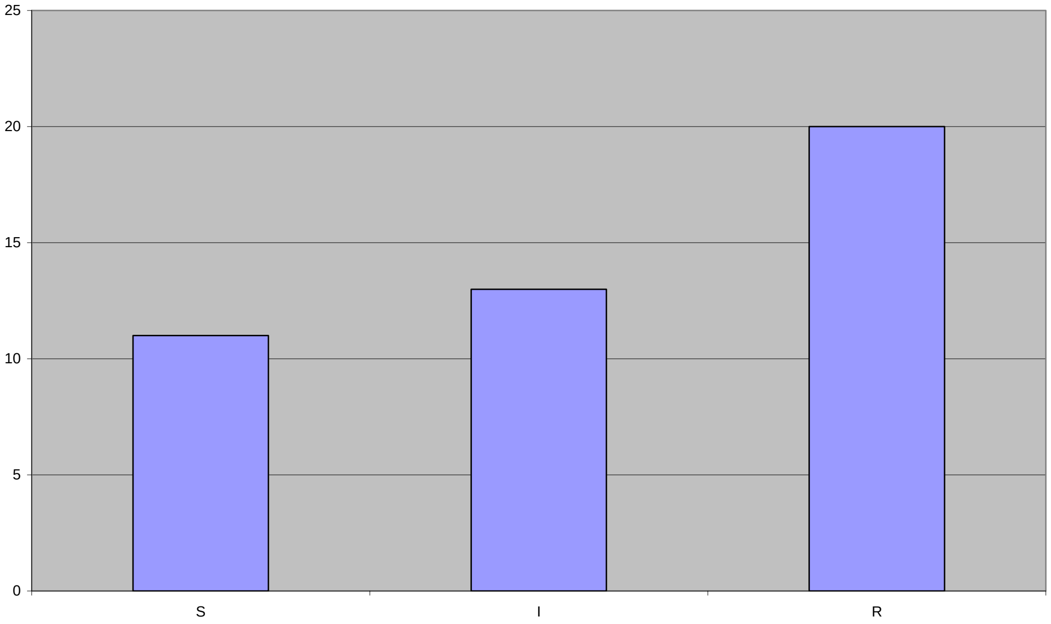

FIG. 2. FREQUENCIES OF FAB A1 ISOLATES S, SENSITIVE; I, INTERMEDIATE AND R, RESISTANT TO METALAXYL

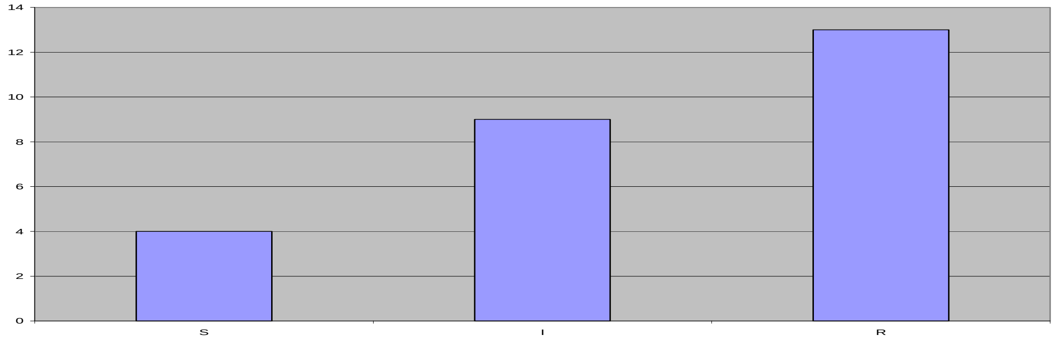

FIG. 3. FREQUENCIES OF MISCELLANEOUS ISOLATES S, SENSITIVE; I, INTERMEDIATE AND R, RESISTANT TO METALAXYL

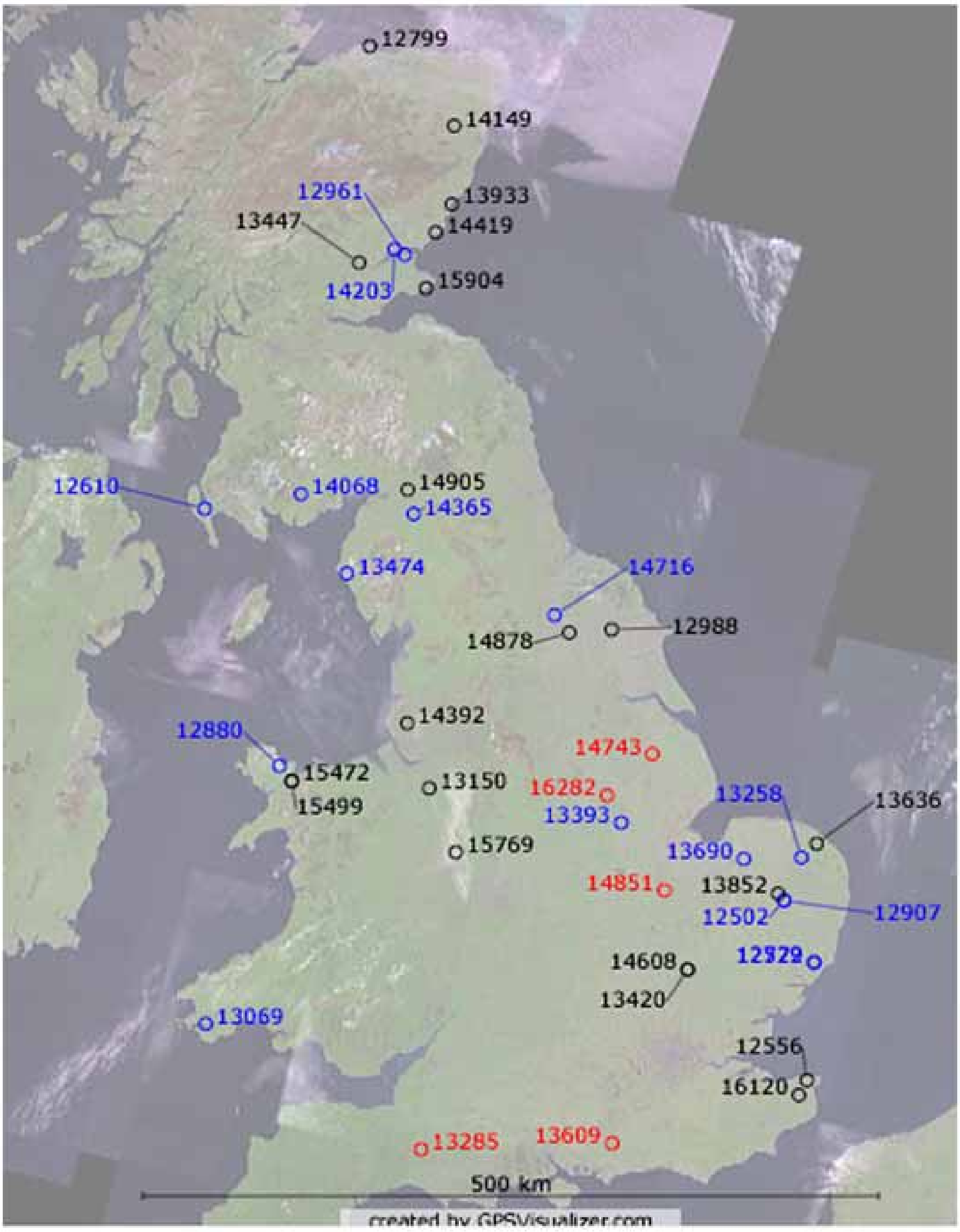

Fig. 4. Location map for sites of FAB A1 isolates. Type I sites are labelled in blue, type II in red.

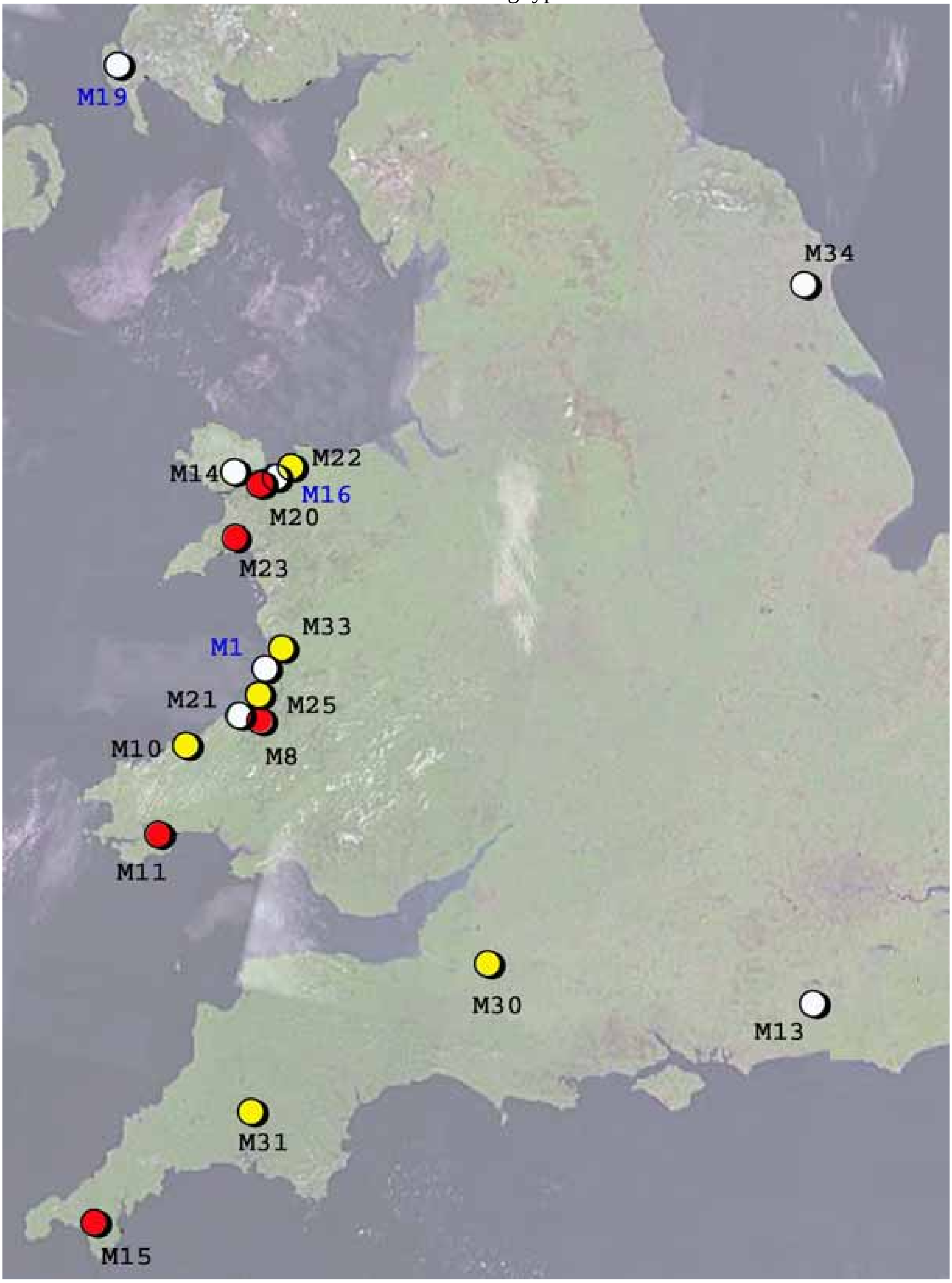

FIG. 5. LOCATIONS OF MISCELLANEOUS ISOLATES; TYPE 1 SITES ARE LABELLED IN BLUE. WHITE CIRCLES INDICATE A1 ISOLATES, YELLOW, A2 ISOLATES AND RED, BOTH MATING TYPES.

## *2.4 Discussion*

Previous work had shown that the FAB A2 isolates from 2005 were variable, belonging to one or other of seven fingerprints; sensitivity to metalaxyl was highly variable and included fully resistant phenotypes (Shaw *et al.*, BPC report, 2005). In contrast, A2 isolates from the 1990s were monomorphic and never fully resistant to metalaxyl. The present work shows that the additional A2 isolates from miscellaneous sites were also variable in fingerprint (eight types) and metalaxyl sensitivity. Thus the total number of A2 types detected in 2005 was 15. None of these eight fingerprints were detected in collections made in the 1990s nor were they detected in the A2 fingerprints from the FAB collection in 2005.

The A1 isolates of the FAB and miscellaneous collections are also highly variable. The most obvious result is that the variant (fingerprint type I, haplotype IIa) is the commonest variant by far, having been detected at 16 of the 41 sites (39%). This variant (RF006, Day *et al*., 2004) was the third most common clone in GB in the 1990s when it was found at 15% of sites sampled. Small samples from Northern Ireland (1995/6) showed that this variant was present at all of 29 sites (Carlisle *et al.*, 2001). Later sampling both in Eire (Griffin *et al.*, 2002) and Northern Ireland (Cooke *et al*., 2006) indicated that this variant had become less widespread in recent years.

The commonest variant in the 1990s was RF039 (Day *et al.,* 2004) when it was detected in >50% of sites; this variant was not detected in the present study. None of the other variants found here at more than one site was detected in the 1990s study. The second most common A1 variant (type II) was found in the FAB collection and was detected at 12% of sites. Because the sample size of other isolates with a common fingerprint was small, all that can be said is that these variants can be detected at more than a single site but could be more widespread.

As expected, two mitochondrial haplotypes, Ia and IIa, were detected among both FAB A1 isolates and miscellaneous isolates. When the frequency of each haplotype among FAB A2 isolates is included, 60.5% of 2005 isolates were haplotype Ia and 39.5% were IIa. The proportion of these in the 1990s in GB was 91% and 9% respectively. The increase of haplotype IIa in 2005 can be seen to be due to the success of the common type I variant with haplotype IIa. As in the 1990s, the number of fingerprints detected in 2005 with haplotype Ia (28) is still much higher than the number with IIa (12). One fingerprint (type III) was represented by one isolate of each mating type occurring at one site; this was found occasionally in the 1990s and could have been the result of recombination of nucleus and mitochondria following a somatic fusion between different variants.

## *2.5 Conclusions*

Our results show that the GB population of the pathogen has evolved over the last 10 years. The basic structure of the pathogen population has remained similar in that the GB population still consists of common and less common clones and a large variety of rare genotypes, some of which could be less successful clones and others could be sexually produced variants which do not survive from one season to the next. Evidence has been produced to show that both mating types are now common, providing the potential for frequent sexual reproduction and that variation within the A2 mating type is now more comparable to that found within the A1 mating type. Clones which were less frequent 10 years ago have been selected while others which predominated before are now not detectable at the level of sampling used. Also, the spectrum of both clonal and rare genotypes found in 2005 has totally changed from 10 years ago. The selective forces driving the changes are at present not identifiable but could involve factors like migration from other countries, climate change and changes in agronomic practice. Further work with larger sample sizes analysed by more easily characterised markers will shed more light on the new population, its composition and structure.

## 2.6 References

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