Project Report

Variation in *Phytophthora infestans*: determination of mating types and pathogenicity

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Executive Summary

This report describes studies carried out at the Sárvári Research Trust (SRT) during 2004 and 2005. In both seasons, the researchers determined the frequency of the two blight mating types in samples collected as part of the BPC's Fight Against Blight campaign. In addition, some of the isolates were studied in more detail to provide information on the variability in virulence and pathogenicity among the isolates. In 2004, 93 isolates were tested for their ability to overcome the resistance of five potato clones, each carrying a different R-gene for immunity. Nineteen different types of virulence were distinguished. A selection of isolates including those with low, intermediate and high virulence in these initial tests was then assessed under laboratory conditions. They showed considerable variation in the characters studied. As a result a field experiment was carried out in 2005, to determine if differences observed under laboratory conditions resulted in differences in the ability of the strains to colonise foliage and tubers in the field.

In the field experiment, three strains of *P. infestans* were introduced into a growing crop of cv Nicola (0.5ha) just before a full Smith Period. Initial spread of each strain from the inoculation points within the field was similar and there was no significant difference detected in lesion growth rate or in sporulation density within the lesions. The primary foci developed rapidly so that all plants became infected although the "in-crop" weather station did not record further Smith Periods over this time interval.

The secondary spread of each strain to fully blight the crop was detected by collecting samples of lesions throughout the field and typing these with newly developed Simple Sequence Repeat (microsatellite) markers. The marker analysis showed that each strain was able to spread from its inoculation point to all parts of the field, but there was an indication that one of the strains produced more disease. Similar marker identification of strains infecting tubers at harvest showed that all three strains infected tubers. The small sample showed that the strain that predominated on foliage also infected more tubers than did the other two strains.

In 2005, *P. infestans* was isolated from samples taken from a total of 99 sites from throughout GB. The A2 mating type was detected in samples from a total of 38 sites (38.4%). This relatively high frequency of A2 has increased from 10.2% in 2004 and 5.3% in 2003. Molecular typing of 22 of the A2 isolates collected in 2005 showed that variation among A2 isolates has increased since the 1990s. More than half of the 2005 A2 isolates showed some resistance to metalaxyl, again showing that the situation has changed since the 1990s when A2 isolates were all found to be sensitive to that fungicide.

Experimental Section

Mating Types

Introduction

There is a common perception in western Europe that late-blight of potato, caused by *Phytophthora infestans*, has become more difficult to control, following the introduction of new strains of the pathogen during the 1970s. The detection of the A2 mating type alerted pathologists to population change and the new potential for sexual reproduction by interaction of A1 strains with A2 strains leading to the production of oospores. Oospores can survive for long periods in the soil in the absence of the host, germinate and infect new crops. Every germinated oospore generates several new and unique recombinant genotypes of pathogen. There is evidence that the low frequencies of strains of A2 mating type detected in most countries in the early 1980s have increased, rapidly in some countries (The Netherlands and Nordic countries) and more slowly in others (UK, Ireland, France). Studies of variation using molecular markers confirmed that variation was also increasing (Cooke *et al.,* 2003; Day *et al*., 2004). Populations were dominated by a few genotypes, mainly of A1 mating type, which were distributed widely and occurred every year. But a minority of infections were caused by genotypes confined to a single field or small area; these genotypes were not detected in the following years. It was hypothesised that unique genotypes were probably a product of sexual reproduction via oospores and that common genotypes were clonal lineages able to overwinter in tubers and initiate infection in spring from dumps, seed potatoes or volunteers.

In GB, A2 isolates from West Wales were detected in 1981 and 1982 and from various sites in Scotland, England and Wales in 1983 and 1985 (Shaw, 1987). An extensive survey in 1995-98 showed that A2 was widespread in GB and was detected at 34 sites (9.6%) (Day *et al.,* 2004). Following the collection of samples by BPC blight scouts in the 2003 Fight Against Blight (FAB) campaign, A2 was detected at 6 of 114 commercial sites (5.3%). Since only a single isolate was established from each site, sampling error means that the proportion of sites with A2 could have been substantially higher than 5.3%.

One of the aims of the present study was to determine if the A2 mating type had increased in frequency. The same methods were used as in 2003 although in some sites a larger number of isolates were recovered to determine if sites with the A2 mating type also had the A1 mating type. In 2005, the study was extended after the detection of high frequencies of the A2 mating type. Previous characterisation of GB isolates in the 1995-8 period showed that the majority of A2 isolates belonged to a single clonal lineage: RG57 fingerprint RF040, mitochondrial haplotype Ia; sensitive to metalaxyl. To determine if the A2 isolates in 2005 were of that same clonal lineage or not, A2s isolated from samples sent to the Central Science Laboratory (CSL) by Blight Scouts were characterised for sensitivity to metalaxyl and for molecular genotype.

Materials and methods

Single-lesion samples of leaflet or stem material were prepared at CSL from confirmed samples of late-blight disease. Most samples were sandwiched within a split potato tuber and mailed in padded envelopes to SRT at Henfaes Research Centre, Wales. Leaf and stem lesions were removed to a moist chamber and allowed to sporulate (overnight). Slices 1cm thick were also cut from each half of the split tuber and incubated in Petri dishes for several days to allow sporulation. Sporangia from lesions or from tuber slices were removed using an agar wedge and transferred to a plate of rye agar containing selective antibiotics. Pure cultures obtained were mated to tester isolates of known A1 and A2 mating type and cultures were examined for oospores one week later. All pure cultures of *P. infestans* were grown in an incubator at 18°C.

Farms and smallholdings in NW Wales and elsewhere were sampled more intensively. Samples of infected foliage from different parts of a field and/or from different cultivars on a holding were collected or sent directly to Henfaes and allowed to sporulate overnight. Multiple pure cultures were established and mated as above. Additional samples were obtained from gardeners and allotment holders.

In 2005, sensitivity to metalaxyl of 22 A2 isolates 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 100µg/ml metalaxyl for each isolate. The hyphal extension over 8 days' incubation at 18°C was measured and the mean growth on 100 µg/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, of 20 - 40% were classed as intermediate and of 40 - >100% as resistant. Mitochondrial haplotypes were determined by the PCR method (Griffith and Shaw, 1998). RG57 fingerprints were determined as in Day *et al.*, (2004).

Results

Mating Types

In both 2004 and 2005, isolates sent to SRT by CSL arrived in good condition and leaflets or tuber slices almost always produced sporangia of *P. infestans*. In 2004, 144 samples were received from CSL and 44 were collected locally. Pure cultures were established from 95 samples and were stored in vials with rye grains in water. Replicates of all cultures were sent to Dr D Cooke at SCRI. 10.2% of the samples received from the Fight Against Blight campaign, via CSL, contained the A2 mating type.

In 2005, 70 samples were received from CSL and 29 were received from other sources. The A2 mating type was detected in 23 of the CSL samples (32.8%; Table 1). Nine sites were in the West Country (6 Cornwall, 2 Somerset and 1 Devon). The other sites were scattered, with only 2 in Scotland (Borders and Banffshire), 3 Suffolk, 2 Norfolk, 2 Shropshire, 1 Cumbria and 4 South and West Wales (Appendix; Figs. A2-A3). Table 2 shows the results of the mating type tests for the other 29

samples. In these samples the number of lesion collected per site varied from 1 to 16. The A1 mating type was detected at 14 sites; A2 only was detected at nine sites and both mating types at six sites (Appendix; Fig. A4). Three sites were sampled on more than one occasion. At one site, (Benllech, samples 2-5) only A1 was detected each time (A1:A2 = 1:0, 5:0, 7:0, 23:0). At another (Bethel, N. Wales, samples 6 and 7) only A2 was detected on both occasions (0:4) and (0:16) and at the third site (Llangwbi, N. Wales, samples 23 and 24) A2 was detected (0:2) early in the season and both mating types (2:8) later in the season.

Characterisation of the A2 isolates from CSL samples

Seven distinct genotypes were found when the DNA fingerprints of the samples were studied using the probe RG57. One genotype occurred at eight sites, another at seven sites and a third at three sites. The other four genotypes were each found at a single site. Sixteen isolates were of haplotype Ia and six were of haplotype IIa. Twelve isolates were resistant to metalaxyl, eight were sensitive and two showed intermediate resistance (Table 1).

Postcode	County	Date of receipt	mt DNA	RG57 Fingerprint	Metalaxyl*	Metalaxyl**	Comment
PE31	Norfolk	19/07/2005	Ia	1,2, , , ,8, ,10,13,14,17,19,20,21,22,24,25	53	R	Morene
TD11	ScottishBorders	22/08/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	57	R	Maris Piper
TA13	Somerset	19/07/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	41	R	Estima
TA12	Somerset	27/07/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	43	R	Estima
IP12	Suffolk	18/08/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	43	R	Carlingford, 2nd crop
IP12	Suffolk	23/08/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	41	R	Maris Peer, 2nd crop
IP12	Suffolk	31/08/2005	Ia	1,2, , , , ,8, ,10,13,14,17,19,20,21,22,24,25	48	R	Carlingford, 2nd crop
TR12	Cornwall	23/05/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	65	R	Dump, Charlotte
TR12	Cornwall	21/06/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	110	R	Allotment, Desiree
TR2	Cornwall	04/07/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	71	R	Estima
TR27	Cornwall	07/07/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	73	R	Maris Peer
PL11	Cornwall	26/08/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	95	R	Lady Rosetta
TQ7	Devon	20/07/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	23	I	Estima
SY23	Ceredigion	12/07/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	21	I	Organic, K. Edward
SA73	Pembrokeshire	08/06/2005	Ia	1, ,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	20	S	Allozo
SA48	Ceredigion	21/07/2005	**IIa**	1,2,3,5,7, ,9,10,13,14,17,19,20,21,22,24,25	5	S	Organic, Minerva
PL30	Cornwall	21/07/2005	**IIa**	1,2,3,5,7, ,9,10,13,14,17,19,20,21,22,24,25	7	S	K Edward, severe
SY4	Shropshire	04/07/2005	**IIa**	1,2,3,5,7, ,9,10,13,14,17,19,20,21,22,24,25	5	S	Organic, Cosmos
SY4	Shropshire	04/07/2005	**IIa**	1,2,3,5,7, , ,10,13,14,17,19,20,21,22,24,25	7	S	Organic, Princess
NR11	Norfolk	04/07/2005	**IIa**	1, ,3,5,7, , , 10,13,14, ,19,20,21,22,24,25	0	S	Headland, Asterix
CA6	Cumbria	14/07/2005	**IIa**	1, , , 5, , , 10,13,14,17,19,20,21,22,24,25	7	S	Maris Piper
SA73	Pembrokeshire	22/06/2005	Ia	1, , , 5, , , ,,10,13,14,17, , ,20,21, , 24,	0	S	Charlotte
AB45	Banffshire	21/09/2005	***	****	***	***	Cara

TABLE 1. ISOLATES OF A2 MATING TYPE FROM CSL SAMPLES, 2005

* Growth made on 100µg/ml metalaxyl as a percentage of that made on 0µg/ml. ** R = resistant (growth 40% - >100%), I = intermediate (growth 20% - 40%) and S = sensitive (growth 0 – 20%). *** Isolate acquired too late to be characterised

Sample	Location	Date	A1 isolates	A2 isolates	Total isolates
1	Aberystwyth mid-Wales	14/07/2005	4	0	4
2	Benllech, Anglesey	02/07/2005	1	0	1
3	Benllech, Anglesey	25/06/2005	5	0	5
4	Benllech, Anglesey	01/09/2005	7	0	7
5	Benllech, Anglesey	22/09/2005	23	0	23
6	Bethel, N Wales	12/09/2005	0	4	4
7	Bethel, N Wales	25/07/2005	0	16	16
8	Blaen Camel, mid-Wales	05/08/2005	1	9	10
9	Brighton	05/08/2005	0	1	1
10	Cardigan, mid-Wales	11/08/2005	0	2	2
11	Cresswell, SW Wales	15/07/2005	7	4	11
12	Duchy College, Cornwall	15/07/2005	1	2	3
13	Horsham, Sussex	22/08/2005	2	0	2
14	Gaerwen, Anglesey	26/08/2005	6	0	6
15	Helston, Cornwall	18/05/2005	1	7	8
16	Henfaes, N Wales	01/09/2005	1	0	1
17	Hull, N Yorks	05/09/2005	1	0	1
18	Kirkcudbright, Galloway	30/06/2005	6	0	6
19	Leswalt, Galloway	22/08/2005	1	0	1
20	Llandygai, N Wales	23/09/2005	2	3	5
21	Llanerchaeron, mid-Wales	15/07/2005	4	0	4
22	Llanfairfechan, N. Wales	07/08/2005	0	6	6
23	Llangwbi, NW Wales	28/07/2005	2	8	10
24	Llangwbi, NW Wales	12/07/2005	0	2	2
25	Llanrhystud, mid-Wales	15/07/2005	0	5	5
26	Plantsystems, Jersey	19/04/2005	12	0	12
27	Plantsystems, Lincs	08/11/2005	8	0	8
28	Radstock, Somerset	12/08/2005	0	2	2
29	Saffron Walden, Cambridgeshire	07/07/2005	1	0	1
30	Shepton Mallet, Somerset	24/06/2005	0	1	1
31	Tavistock, Devon	09/07/2005	0	4	4
32	Tunbridge Wells, Kent	27/06/2005	4	0	4
33	Tyn Rhelyg, mid-Wales	05/08/2005	0	10	10
34	York	11/08/2005	10	0	10

TABLE 2. ISOLATES OF A2 MATING TYPE FROM NON-CSL SAMPLES, 2005.

Discussion

Even although only one isolate was made from each of the CSL samples, A2 mating type was detected at 23 of 70 sites (32.8%) in 2005. Comparable sampling via CSL in 2004 yielded 10.2% of sites with A2 and 5.3% of sites with A2 in 2003. In non-CSL samples in 2005, many of which were represented by more than one isolate, A2 was detected at 52% of sites and both mating types at 21% of sites. Taken together, these results indicate that the frequency of the A2 mating type in GB has increased. It is possible that both mating types could now be present in most, if not all, crops. If so, the potential for oospore formation and for the presence of infectious oospores in soils must have increased.

There is some indication that A2 was more common in coastal sites, particularly in the west. The highest concentration of CSL A2 sites was in Cornwall, Devon and Somerset where nine of 12 sites had A2 and SW Wales where all four sites had A2. The lowest concentration was in Scotland (2 A2 sites). The higher frequency of A2 in the non-CSL samples could be due in part to the larger sample sizes and in part to the location of most of the sites sampled being coastal and in the west of GB where the A2 was found to be more common in CSL samples.

The increase in the frequency of the A2 mating type detected here is consistent with the increase that has taken place in some other European countries. The increase in The Netherlands and in the Nordic countries took place in the 1990s (e.g. Drenth *et al.*, 1993; Hermansen *et al.*, 2000). In contrast, the increase in N. France was, as in GB, more recently detected (S. Duvauchelle, pers. comm.). In N. France, the A2 mating type was detected in 6% of fields in 2003, 20% in 2004 and 37% in 2005. This increase was accompanied by an increase in resistance to metalaxyl and resistance was detected even where little or no application of metalaxyl had been made. Outbreaks were earlier and more severe in 2005 than in the last 20 years and were more difficult to control. The most frequent phenotype characterized was A2 and metalaxyl resistant (S. Duvauchelle, pers. comm.). It is not known if isolates of this phenotype belonged to a clonal lineage or consisted of a range of unique genotypes or to both. Recent surveys in Hungary (Nagy, 2006) have detected roughly equal frequencies of each mating type and a high level of variation such that almost all molecular genotypes detected were unique to a single site.

Collections of isolates from a large number of sites in GB over the period 1982 - 1998 were characterized for molecular fingerprint (RG57 and mitochondrial DNA haplotype) (Day *et al.*, 2004). The study showed that most isolates belonged to one or other of four common genotypes which were widespread every year. A minority of isolates had unique genotypes and were only detected at one site and only in one year. This suggested that the recurring genotypes were clonal lineages, surviving overwinter in tubers and that the unique genotypes were generated by sexual reproduction and were either unable to survive in tubers or did not increase in frequency so that they could be detected in the following seasons. The majority of the A2 isolates detected belonged to a single clonal lineage (RF040; mtDNA haplotype Ia). One isolate of this genotype was detected in 1981 and another three different A2 genotypes were characterized from A2 isolates from 1982-85. In the period 1995-8,

when A2 was detected at 37 sites, isolates from 29 sites were RF040, haplotype Ia; a single isolate of RF040 haplotype IIa was found at one site. Five other A2 genotypes were detected among the other six sites. This earlier work indicated that A2s were mainly clonal and were surviving asexually but were possibly leaving sexual progeny (the unique genotypes) after mating with various A1 genotypes.

The results of the analysis of the 2005 samples indicate that the situation has changed. Amongst the 22 A2 isolates characterized, this old clonal lineage was not detected although one of the isolates (13582) was RF040 but haplotype IIa (red in Table 1). This genotype was detected previously only in one isolate from Northern England in 1998. The other isolates belonged to one of six genotypes, none of which had been detected before. One of the genotypes (yellow in Table 1) is also IIa and differs from RF040 by the presence of band 9. This suggests that genotypes red and yellow might be related and belong to the same or related clonal lineages, derived from the old A2 lineage. Supporting this hypothesis is the fact that 13528 (yellow) was recovered from the same postcode area as 13582 (red).

One of the new genotypes (green in Table 1) was present at eight sites. This could be a new clonal lineage or could be related to RF040 to which it is identical but for the absence of band 2. However all the isolates of this genotype had the different mtDNA haplotype (Ia) and not haplotype IIa found in the red and yellow genotypes. Isolates of this green genotype appear to cluster: six isolates in Cornwall/Devon and two isolates in SW Wales. The commonest molecular genotype (blue in Table 1 and haplotype Ia) appears to be a new A2 clonal lineage and was detected at seven of the 22 sites with a cluster in E. Anglia. The remaining three genotypes were represented once each in the collection. These could be unique genotypes resulting from sexual reproduction or they could be members of less common clonal lineages, not detected more than once in this small sample.

Tests of the sensitivity of the A2 isolates to metalaxyl clearly showed that isolates varied widely. Twelve isolates able to make good growth with 100µg/ml metalaxyl were clearly resistant and eight isolates making no growth at this concentration were clearly sensitive to metalaxyl. Two isolates appeared to have intermediate sensitivity. This result is in contrast to results of tests on A2 isolates from the 1990s when none of the A2 isolates were wholly resistant to the fungicide (Day *et al.*, 2004). This means that it is no longer likely that use of metalaxyl will select against all A2 mating types in the population. The resistance phenotype showed a striking association with the molecular genotypes of the isolates. All blue isolates were resistant. All green isolates were either resistant or intermediate with the exception of isolate 12637; although classed as sensitive, this isolate was on the borderline of being intermediate (20% relative growth on 100 µg/ml and very close to the other two intermediates at 21% and 23%). All three yellow isolates and the possibly related red isolate were sensitive. This last result is more evidence that yellow and red are related to the old RG040 clone which was sensitive. It is notable that without exception, all the isolates of IIa haplotype were highly sensitive to metalaxyl and that all isolates of mtDNA haplotype Ia showed some resistance to metalaxyl with the exception of isolate 13096 which was sensitive. These metalaxyl phenotypes are consistent with the molecular data and together indicate the presence of clonal lineages.

Determination of the virulence and pathogenicity of isolates under laboratory conditions

Introduction

Although molecular variation in *P. infestans* in GB is well documented, little is known about the pathogenicity of recent strains of the pathogen. Day and Shattock (1997) have shown variation in infection frequency and sporulation among a collection of isolates collected between 1978 and 1995. Flier *et al*., (2003) have shown that there is specificity among isolates collected in the 1990s and different partially resistant cultivars of potato in the Netherlands. There is also recent anecdotal evidence (e.g. S. Alexander, pers. comm.) that new genotypes of the pathogen are more pathogenic and less easy to predict and control using DSS models. These models are based on characteristics of the disease based on work done in the 1950s and 1960s when the population of the pathogen in GB was genetically monomorphic. This was before migration of new genotypes of A1 and A2 mating type and generation of variation via the sexual cycle.

In the 2004 season, samples collected by BPC Blight Scouts were screened for pathogenic variation by SRT. Eleven of the isolates were selected for further study based on their ability to overcome R-gene resistance (virulence) and polygenic resistance (aggressiveness). They were compared in the laboratory for their ability to grow and sporulate at different temperatures and to infect and colonise potato leaf tissue *in vitro*.

Materials and methods

Virulence

Single R-gene differential potato clones, Black's differentials obtained from SASA (A. Cameron), were raised in 17l pots of compost (peat, sand and Chempak potting base fertiliser) in natural light in a greenhouse. Eleven clones comprised R1 up to R11, each reputed to carry a single R-gene. As a test of virulence of all isolates on all 11 clones would have been too time consuming, a simplified test was carried out on most isolates. Only differentials known from previous studies to discriminate amongst a collection of isolates were used in the simplified test. Accordingly in most tests, R2, R3, R5, R8 and R10 were used. Isolates avirulent on R3 were sent to Dr Steve Wisson, SCRI, who is attempting to clone the R3 avirulence gene of *P. infestans*. Five isolates were tested on all 11 differentials.

Virulence was assessed using sporangia harvested from lesions growing on detached leaves of cv Maris Piper. Spore number was estimated by counting the spores present in a 3µl droplet deposited on a microscope slide. The concentration of spores was adjusted to give 1000 spores per 30µl and diluted further to give 100 spores per 30µl. Leaflets of each differential being tested were inoculated as follows: one droplet of 1000 spores was placed on either side of the midrib near the tip of the leaflet and one droplet of 100 spores was placed on either side of the midrib near the base of the leaflet. Leaflets were incubated in the usual way and symptoms scored (necrosis, lesion extension and sporulation) after 72h and daily thereafter up until the seventh

day. An isolate was deemed to be virulent if spreading lesions with sporulation were formed. The reaction to infection of R10 was often less clear as the resistance gene does not confer immunity but results in a slow-blighting phenotype that was scored as avirulent. Rapid blighting was scored as virulent. During the virulence testing, isolates that colonised the leaflets of susceptible differentials and leaflets of cv Nicola particularly rapidly or particularly slowly compared to other virulent isolates were noted. Eleven of these isolates were selected for further study as described below.

The effect of temperature on hyphal growth of eleven isolates

Isolates were established on Rye Agar then incubated in a range of temperatures within a temperature gradient incubator. The incubator, constructed of aluminium, had an electrical heating coil embedded in the top plate and a cooling channel cut from the bottom plate. The space between these plates was occupied by 20 shelves, each able to accommodate three 90mm Petri dishes or three Repli-dishes (Sterilin Ltd). The incubator was enclosed in an insulated jacket. In operation, antifreeze at subzero temperatures was circulated through the bottom plate and the top plate was heated. The range of temperatures provided was adjusted by varying the amounts of heating and cooling applied.

Cultures of each of the eleven isolates were established on plates of Rye Agar. Discs of culture (4mm diam.) were cut from the edge of each colony and transferred to the edge of plates of Rye Agar. As only three plates could be accommodated at each temperature, four isolates were inoculated onto two of the plates and three isolates on the third plate. All sixty plates were incubated at 20°C for 72h before transfer to the gradient which had been stabilised to give temperatures from 1.4°C on the bottom shelf to 26.4°C on the top shelf. The position of the colony edge was marked on the bottom of the plate using a blunt scalpel when plates were first transferred and then at 24h intervals. The linear growth achieved was measured over one, two or three days according to the rate of growth. At optimal temperatures, growth made over the first 24h was enough to be measured accurately but at extreme temperatures where growth was slow, the accumulated growth over two or three days was measured. Care was taken to avoid measuring where colonies had grown close together as in such situations growth rates decrease.

The effect of temperature on the release of zoospores from sporangia of eleven isolates.

Experiment 1: sporangia from agar culture

The eleven isolates were grown on plates of Rye Agar by transferring inoculum from storage vials. Plates were incubated at 20°C for seven days when 10ml water was added to each plate to harvest sporangia. Sporangial suspensions were collected in 20ml glass vials; sporangia were allowed to settle and the supernatant was removed. Water was added and spore concentration was adjusted to 2,000ml^{-1}. Droplets of 30µg were pipetted onto empty Petri dishes using three or four isolates per plate and four replicate droplets per isolate. Plates were immediately placed in the gradient incubator set to give 4.2°C on the bottom shelf and 20.4°C on the top shelf. After 2h, plates were removed to 20°C and incubated for 15 min when droplets were drained with a pipette and fixed by adding 30µl of 50% ethanol. Numbers of empty and full sporangia were counted in a sample of 100 sporangia from each droplet.

Experiment 2: sporangia from leaflet lesions

The experiment was repeated using the same protocol but sporangia were harvested from sporulating lesions on leaf material. Sporangia from Rye Agar plates (see above) were used to inoculate detached leaflets of cv Nicola. Leaflets were incubated in moist chambers in the light for 5d when sporangia were washed off the leaflet. Leaflets were dried on absorbent paper and incubated for a further 24h when the newly formed sporangia were collected in water. A suspension of sporangia (1.0ml and 2000 spores ml^{-1}) of each isolate was placed in cells of a Repli-dish. Four replicate cells were inoculated for each isolate at each temperature. The temperatures on the gradient were extended to 26.1°C and to -1.4°C. and fixation was achieved by adding 0.5ml of the sterilant Jet 5 to each cell, used at the manufacturer's recommended dilution.

A comparison of the latent period and sporulation of ten isolates.

Ten isolates were grown on plates of Rye Agar by transferring inoculum from storage vials. Plates of isolate 11179 were contaminated and were not used in this experiment. After 7d, sporangia were harvested in water, counted and the concentration adjusted to 1,000/30µl. Detached leaflets of cv Nicola were inoculated with one 30µl droplet of spores in the middle of the leaflet, to the right of the midrib and three replicate leaflets were inoculated per isolate. Leaflets were incubated in moist chambers at 20°C and under a light bank of fluorescent tubes delivering 160µmoles/m^2/sec of Photosynthetically Active Radiation. After 24h, the inoculum droplet was blotted dry and the leaflet was inverted so that the adaxial surface was uppermost. Leaflets were observed microscopically 44.5; 47; 49; 51.75; 56.75; 66.25; 69; 72; 75; and 92h after inoculation. Presence or absence of sporangiophores was recorded, and amount of necrosis around the point of inoculation and lesion size noted. After 6days, leaflets were sprayed with water and the run-off containing detached sporangia was collected. The volume of run-off was noted and the number of sporangia was counted so that the yield of sporangia per leaflet could be determined. Leaflets were dried on absorbent paper and replaced in their moist chambers and incubated for a further 24h when the yield of sporangia was again determined.

A comparison of tuber infection by the eleven isolates.

Tubers of Nicola, Lady Balfour and Axona were scrubbed free of soil and dried on paper towelling. Tubers of cv Nicola were bought in the supermarket in prepacks whereas those of Lady Balfour and Axona were grown in the field, harvested two months previously and stored until mid December at approx. 8°C. The eleven isolates were grown on plates of Rye Agar by transferring inoculum from storage vials. Plates were incubated at 20°C for 7d when 10ml water was added to each plate to harvest sporangia. Sporangial suspensions were collected in 20ml glass vials; sporangia were allowed to settle and the supernatant was removed. Water was added and spore concentration was adjusted to 1,000/30µl. Eight tubers of each cultivar were inoculated with each isolate. Tubers were placed rose-end up in seed trays and supported with paper towelling. One droplet of 30µl was placed on the rose end of each tuber. The paper was moistened and a plastic lid was placed over the tray to maintain 100% RH. After 24h the plastic lids were removed and the inoculum droplet was blotted dry from each tuber. Trays were stored at 5 – 8.5°C for 12 weeks. At

weekly intervals the tubers were scored for symptoms of tuber blight and the amount of blight on the tuber surface was estimated. After 12 weeks the tubers were photographed then sliced longitudinally and each half was photographed. Blight symptoms on cut surfaces were recorded. A slice approx. 4mm thick was taken form one half of each tuber and incubated in dry Petri dishes for up to 5 days. White mycelium on the surface was examined microscopically and any evidence of typical sporangia of *P. infestans* was recorded.

Results

Virulence

Before storage and while isolates were actively growing on leaflets, sporangia from 90 isolates were inoculated on a partial set of differentials to determine their virulence phenotype on these differentials. Five of these isolates were also inoculated onto the full set of 11 differentials. Table 3 shows the virulence phenotypes obtained.

The partial set of differentials distinguished 19 virulence phenotypes. One isolate, RF1, was identified which was avirulent on all the differentials of the partial test (R2, R3, R5, R8 and R10) but produced a sporulating lesion on cv Nicola (Table 3). Nine isolates were virulent on a single differential. Of the 27 isolates virulent on two differentials, 13 were phenotype 3,10. Of the 41 isolates virulent on three differentials, 23 were of virulence 3,8,10. Thirteen isolates attacked four differentials and only three isolates were virulent on all five differentials (virulence phenotype 2, 3, 5, 8, 10). Ability to infect R10 was present in 88 of the 93 isolates whereas 66 isolates infected R3, 41 isolates infected R8, 30 isolates infected R5 and 27 isolates infected R2. Isolate 19 was observed to be a particularly aggressive isolate on cv Nicola and on susceptible differentials on the basis of its rapid lesion growth rate. In contrast, 7372 showed slow colonisation and isolate 8074 showed very slow colonisation.

The selection of isolates for further testing.

Eleven isolates were selected to represent a range of variation in virulence phenotype from widely scattered sites in the UK including the main areas of ware production. This selection included nine A1 isolates, one A2 isolate and one self-fertile isolate. The origins and characteristics of these isolates is shown in Table 4. Isolate RF1 was chosen to represent strains with particularly low virulence. Isolates 10315 and 11854 represent strains with high virulence, having virulence to all differentials tested; 10315 was also of A2 mating type. Isolate 11179, tested on all differentials lacked virulence on R8 and R9 only. Isolates 7372 and 10639 had intermediate virulence and were of the two most common virulence phenotypes. The other isolates chosen had a variety of intermediate virulence.

Virulence phenotype using 5 differentials	Number of isolates
0	1
8	1
10	7
2,10	2
3,10	13
2,3	1
5,10	3
8,10	6
3,8	2
2,3,10	5
2,8,10	1
3,8,10	23
3,5,10	6
5,8,10	1
2,5,10	5
2,3,5,10	9
2,3,8,10	1
3,5,8,10	3
2,3,5,8,10	3
Virulence phenotype using 11 differentials	
1,3,4,7,8,10,11	1
1,2,3,4,7,8,10,11	1
1,2,3,4,6,7,10,11	1
1,2,3,4,5,6,7,10,11	2

TABLE 3. VIRULENCE PHENOTYPES OF 90 ISOLATES TESTED ON FIVE DIFFERENTIALS AND FIVE OF THESE ISOLATES TESTED ON 11 DIFFERENTIALS

Isolate number	Date collected	Cultivar	Postcode	Virulence phenotype	Lesion growth rate	Growth rate/heat tolerance	Hatching temperature Expt 1	Expt 2	Tuber LB	Mating type
RF1 8506	29 July	Mandel	LL76	0*		slow	high % release; optimum 8.8°C	optimum 8.8°C		A1
19	15 June	M. Piper	DY7	1,3,4,7,8, 10,11**	fast		high release	optimum 7.3°C		A1
7372	21 June	M. Piper	WV15	3,8,10*	slow			variable	3 cv	A1
8074	26 July	Santé	IP21	8,10*	very slow	heat tolerant		optimum 5.8°C		A1
8182	28 July	Charlotte	LL57	8*		fast	low % release	optimum 5.8°C		Self fertile
9640	10 August	M. Piper	PH1	2,8,10*				low % release	0 cv	A1
10315	17 August	M. Piper	HR9	2,3,5,8,10*		slow	10.5°C		0 cv	A2
10639	18 August	M. Peer	AB42	3,10*			8.8°C			A1
10747	20 August	P. Dell	YO25	2, 3*				low % release		A1
11179	26 August	P. Dell	PE23	1,2,3,4,5,67,10,11**			Narrow range	low % release		A1
11854	15 Sept.	unknown	LD3	2,3,5,8,10*				Low % release		A1

TABLE 4. THE ORIGINS OF THE 11 ISOLATES SELECTED FOR FURTHER STUDY AND A SUMMARY OF THEIR PHENOTYPES.

Only extreme characters are shown. Blank cells indicate an average result. Virulence was tested on R-gene differentials: *R2,R3,R5,R8,R10: **R1,R2,R3,R4,R5,R6,R7,r8,R9,R10,R11

The effect of temperature on hyphal growth of eleven isolates

The growth rate, as measured by the linear extension of hyphae per hour of the eleven isolates at each temperature is shown in Figure 1. Although the response to temperature of all isolates was similar, isolates varied in their maximum rate of growth which occurred between 12.9°C and 18.9°C. For example, 8182 showed a consistently high rate of growth over this range whereas RF1 and 10315 show a consistently lower rate of growth. There is clear evidence of a slowing of growth at 20.4°C and above and all isolates showed minimal growth at 26.4°C. At the other extreme, all isolates showed a minimum growth at 3.1°C and a clear increase in growth rate at 4.6°C. It is not known if the slow growth recorded at 1.6°C and below is an artefact or real. When plates which had been incubated at 26.4°C for 4d were transferred to 18.9°C, ten isolates failed to initiate growth but one isolate, 8074, recovered and grew normally over the following 24h period.

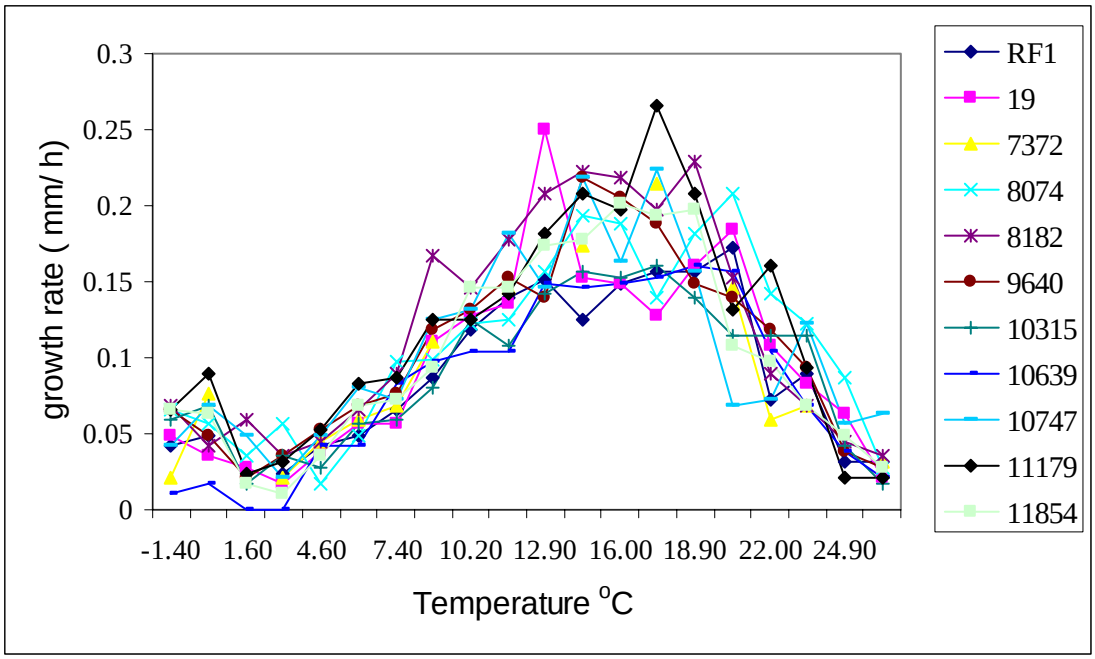


FIGURE 1. GROWTH RATE OF COLONIES OF ELEVEN ISOLATES OVER A RANGE OF TEMPERATURES

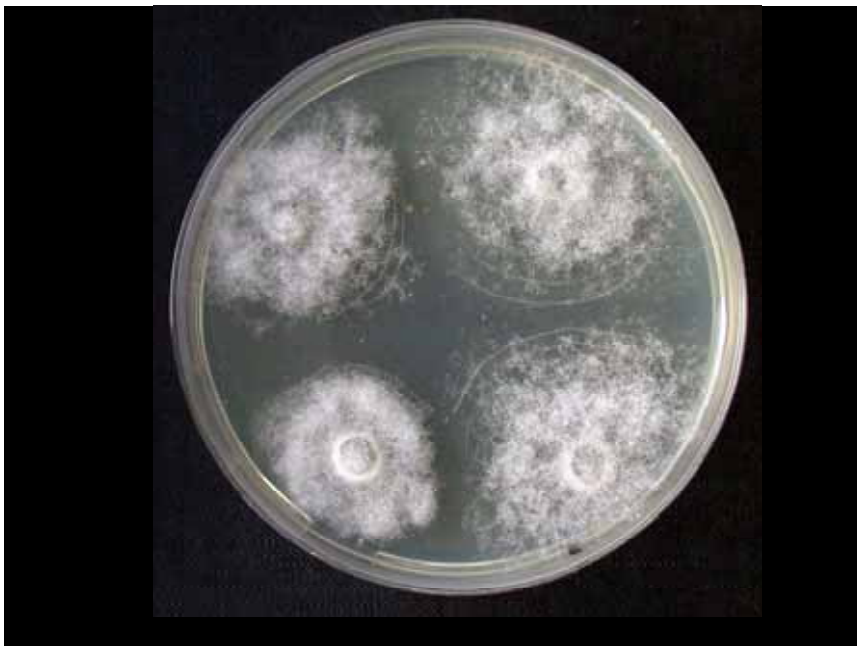


FIGURE 2. FOUR ISOLATES INCUBATED AT 22ºC. NOTE GROWTH INCREMENTS MARKED ON UNDERSIDE OF PLATE

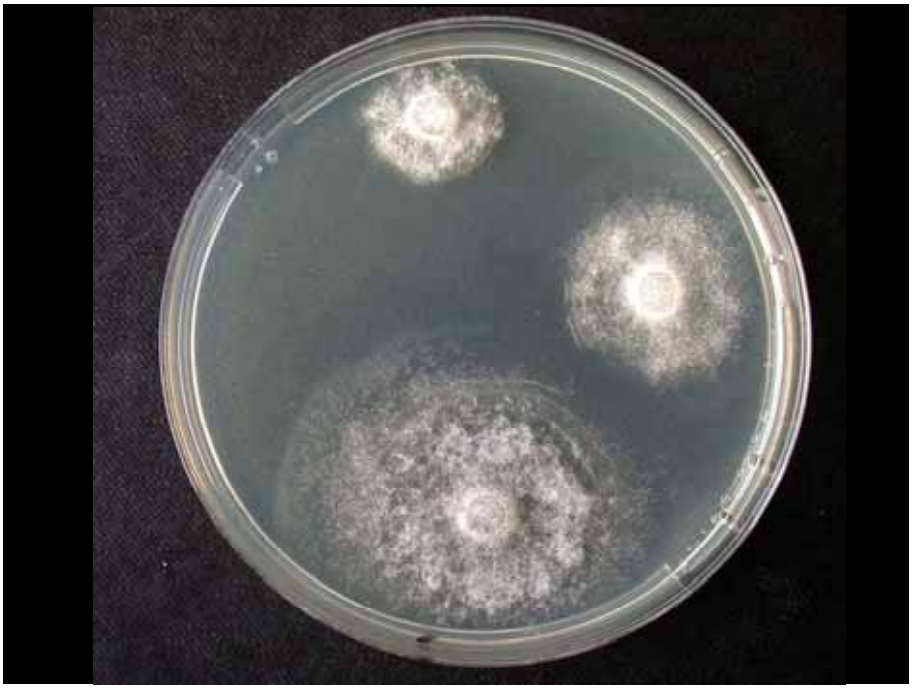


FIGURE 3. PLATE INCUBATED AT 26.4°C THEN TRANSFERRED TO 18°C FOR 24H.

Upper two isolates (RF1 at 11 o'clock and 19 at 3 o'clock) did not recommence growth. Lower isolate, 8074, resumed normal growth.

The effect of temperature on the release of zoospores from sporangia of eleven isolates.

Experiment 1: sporangia from agar culture.

Figure 4 shows the percentage of sporangia which were able to release zoospores at different temperatures. In all isolates, release increased with increasing temperature and reached a maximum of 50 – 90% within the temperature range 8.85 – 13.4°C. At

higher temperatures release declined until at 20.4°C it was less than 15% and close to zero for some isolates. There is an indication that isolates varied in their response to temperature: two isolates, RF1 and 19, showed a higher percentage release at most temperature and showed >40% even at the lowest temperature tested (4.25°C) whereas other isolates showed around 10% release at this temperature. Release in isolates 8182 and 11179 declined to 15% at 16.0°C whereas other isolates still showed higher levels of release (35 – 75%) at this temperature.

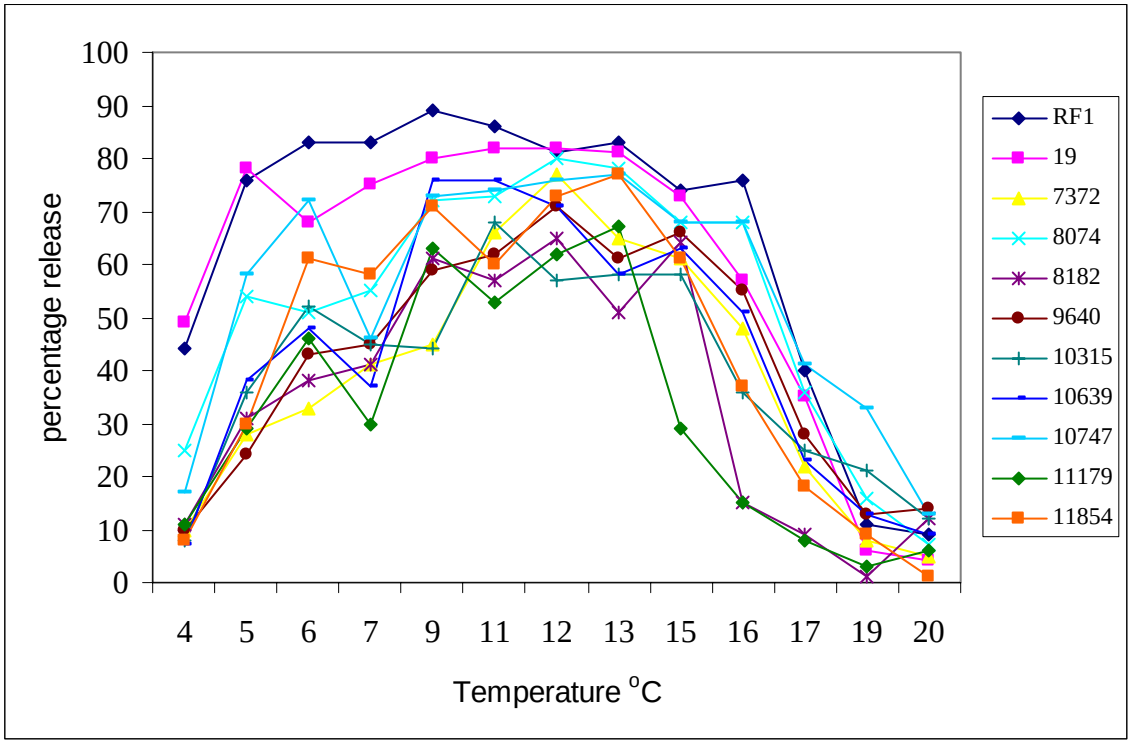


FIGURE 4. ZOOSPORE RELEASE FROM SPORANGIA OF ELEVEN ISOLATES OVER A RANGE OF TEMPERATURES (EXPERIMENT 1).

Experiment 2: sporangia from leaflet lesions.

Figure 5 shows the percentage of sporangia that were able to release zoospores over a wider range of temperatures. As in Experiment 1, release increased with temperature then progressively decreased as the temperature continued to increase. However, in this experiment, the range of temperatures giving maximum release was between 4.5 and 8.8°C. Isolates varied in the maximum percentage release achieved in this temperature range. Four isolates (11854, 11179, 10747 and 9640) showed low maximum levels of release of below 25%. As in the first experiment, isolate 19 showed a high level of release of >60% at the lowest temperature of –1.2°C but another two isolates, 8074 and 10639, showed similar high release at this temperature.

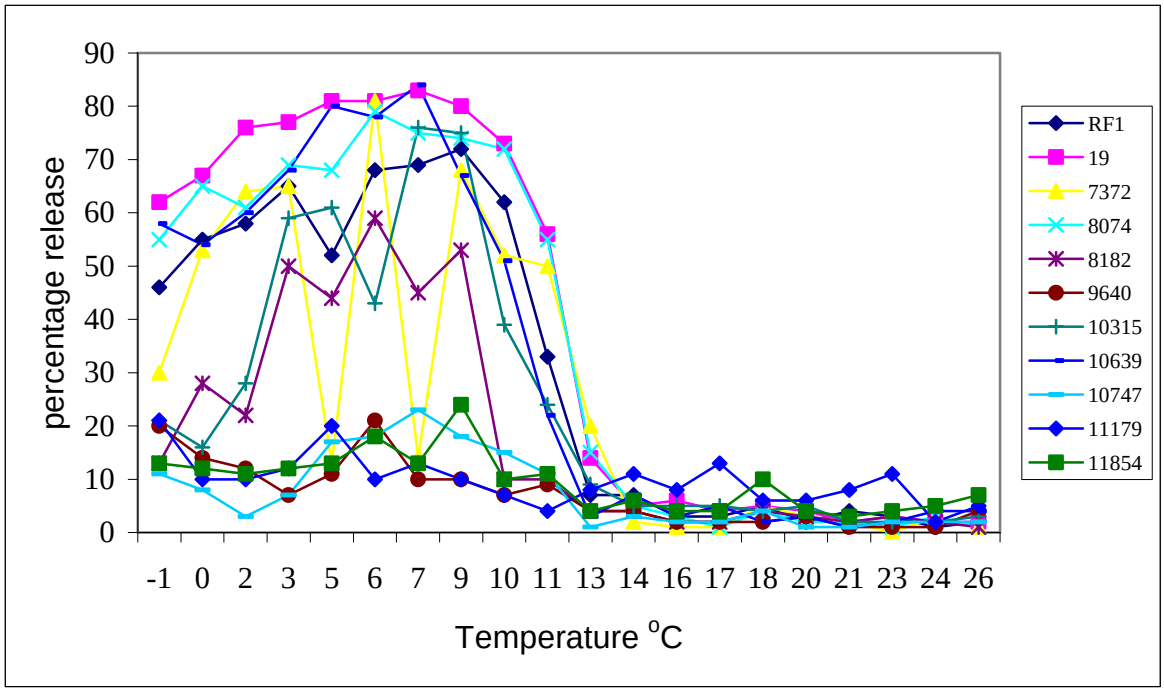


FIGURE 5. ZOOSPORE RELEASE FROM SPORANGIA OF ELEVEN ISOLATES OVER A RANGE OF TEMPERATURES (EXPERIMENT 2).

A comparison of the latent period and sporulation of ten isolates.

Table 5 shows the number of replicates out of 3 of each isolate which produced sporangiophores on the adaxial surface of the developing lesions on cv Nicola. Only one replicate of one isolate (7372) formed sporangiophores at 51.75h after inoculation. After 56.75h, one or two replicates of all ten isolates had formed sporangiophores. All three replicates of nine isolates formed sporangiophores by 66.25h; the tenth isolate, 11854, formed sporangiophores on all replicates by 69h

Hours from infection	RF1	19	7372	8074	8182	9640	10315	10639	10747	11854
51.75	0	0	1	0	0	0	0	0	0	0
56.75	1	1	1	0	1	2	2	1	1	1
66.25	3	3	3	3	3	3	3	3	3	2
69.0	3	3	3	3	3	3	3	3	3	3

TABLE 5. LATENT PERIOD OF TEN ISOLATES WHEN INOCULATED ON CV NICOLA. Formation of sporangiophores was observed on 0, 1, 2 or 3 replicates

Sporangia were harvested from each leaflet 6d after inoculation and again after a further 24h. Yields of sporangia from each leaflet are shown in Figure 6. The mean yields of sporangia which had formed 6d after leaflet inoculation ranged from 39,000 (isolate 11854) to 327,000 (isolate 10747) and over the following 24h period from 59,000 (isolate 10639) to 210,000 (isolate 7372). However, due to the large errors within replicates, the mean yields for each isolate are not significantly different at 6d or on the seventh day ($P>0.05$).

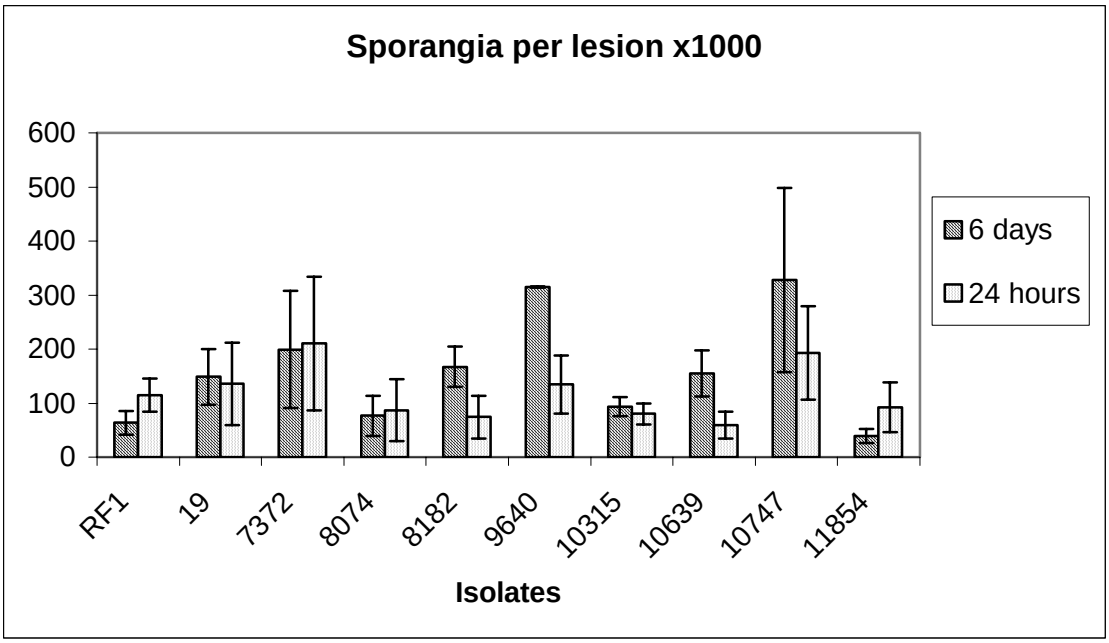


FIGURE 6. INFECTION OF CV NICOLA WITH ELEVEN ISOLATES: YIELD OF SPORANGIA FROM SINGLE LESIONS. ERROR BARS SHOW STANDARD ERROR OF MEANS OF THREE REPLICATES.

A comparison of tuber infection by the eleven isolates

Most of the tubers showing symptoms of tuber blight on the surface showed typical symptoms of tuber blight when cut in half (Apendix; Figs. 5-7) and when slices were incubated. Several tubers that looked healthy on the outside were identified as having blight when cut open and incubated. Some tubers with blight-like symptoms on the outside were blight-free when cut and did not produce sporulating mycelium on incubated slices.

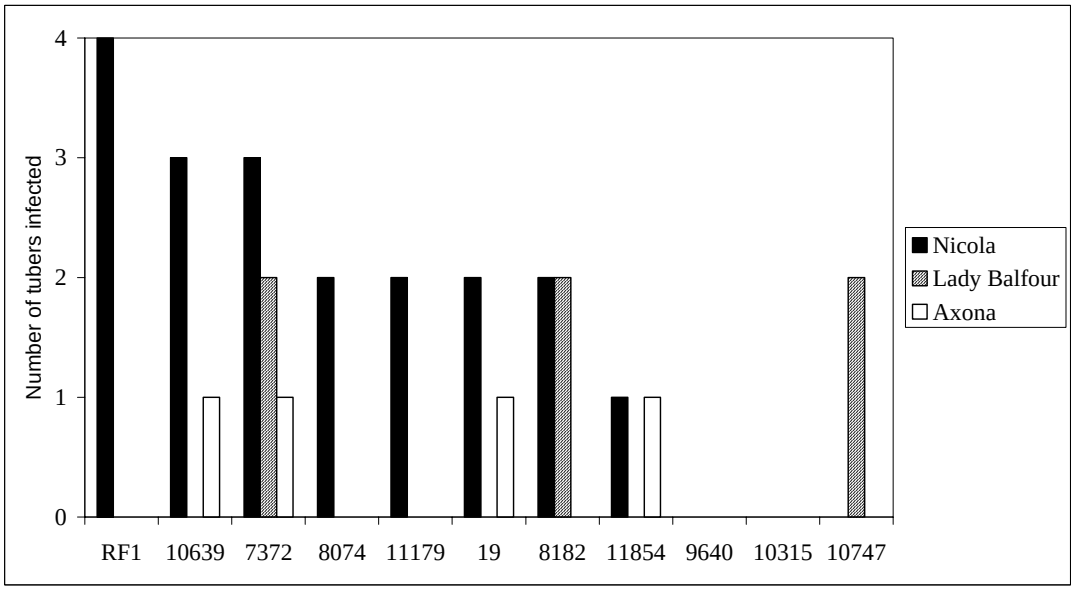


FIGURE 7. NUMBERS OF TUBERS OF THREE CULTIVARS THAT BECAME INFECTED AFTER INOCULATION WITH ELEVEN ISOLATES OF *P. INFESTANS*.

Of the eight tubers of each cultivar inoculated with spores of each of the eleven isolates, most remained healthy over the three-month period without developing detectable symptoms. Figure 7 shows that more of the tubers of cv Nicola became infected (19 of 88) than that of cv Lady Balfour (6 of 88) or of cv Axona (4 of 88). Eight of the isolates produced tuber blight on cv Nicola, four isolates produced tuber blight on cv Axona and three isolates produced tuber blight on cv Lady Balfour. Isolates which infected cvs Lady Balfour or Axona were also able to infect cv Nicola except isolate 10747 which infected cv Lady Balfour but not cv Nicola. Two isolates did not induce tuber blight on any cultivar.

Discussion

Virulence

Although only a partial set of differentials was employed for most of the testing, virulence to the chosen differentials was variable as expected. Virulences to R2 and R5 were least common amongst the isolates but almost half of the isolates could attack R8. The next most common virulence was to R3 followed by virulence to R10, possessed by all but five isolates. Most workers worldwide agree that virulence to R9 is never found; this may be because more than one resistance gene is present. However, a stem lesion was observed on a field-grown plant of R9 in Anglesey in 2004 and an isolate was recovered which sporulated when inoculated onto R9 leaflets. A2 mating type was identified in isolates from this site. About one third of the isolates belonged to just two virulence phenotypes (3, 8, 10 and 3, 10) and as expected from previous work, extreme isolates with few or many virulences were uncommon.

The effect of temperature on hyphal growth of eleven isolates

The temperature gradient incubator provided an excellent stable source of 20 temperatures over an adjustable range. As space within the incubator is strictly limited, replication of cultures at each temperature was not possible but since adjacent temperatures were closely spaced, this provided a substitute for true replication. Where the graph of the results for any one isolate shows wider variation in growth rates among adjacent temperatures, this could have been a result of measurement error or error due to non-linearity of the gradient or a combination of these factors. The response curves of Figure 1 show a gradual increase in growth rate of all isolates as temperatures increased from 3.1°C to 12.9°C and a similar decrease in growth rate as temperature increased from 20.4°C to 26.4°C. The growth rates in the interval between 12.9°C and 20.4°C were fairly constant and over this range there is evidence for some isolates having a faster maximum rate of growth than others. Differences between the fastest (e.g. 8182) and slowest isolates (e.g. 19) in this range were in the order of 0.1mm/h. It is remarkable that growth of colonies and, by implication, growth of leaf lesions was near optimal at temperatures as low as 12.9°C and was approximately half of the maximum rate at as low a temperature as 8.8°C. Although there was little detectable variation in response to temperature amongst the eleven isolates, more substantial variation might have been detected if a larger sample of isolates had be assessed.

Ten of the isolates were inactivated and probably killed by a temperature of 26.4°C as they did not commence growth when transferred to an optimal temperature. These ten

isolates did commence growth when transferred from 24.9°C to an optimal temperature. One isolate, 8074, was not killed at 26.4°C and must have a higher thermal death temperature. There is clearly variation among isolates in their thermal death point and this may affect how successful a strain of the pathogen is in periods of sustained hot weather. More accurate assessments of thermal death point could be made with the gradient incubator set to a higher temperature range. Of course it must be stated that the response of hyphal growth to temperature may be different within leaf, stem and tuber tissue but in these situations, growth is much more difficult to measure.

The effect of temperature on the release of zoospores from sporangia of eleven isolates.

Again, the temperature gradient incubator provided a useful range of temperatures for the investigation of zoospore release. From the results of Experiment 1 (Fig. 4) it can be seen that the response of release of zoospores to temperature is very similar among the eleven isolates. The largest difference between isolates is in the maximum percentage of sporangia able to release zoospores. This varied from nearly 90% in isolate RF1 to 65% in isolate 8182. RF1 and another isolate, 19, which showed a high maximum release, were able to release zoospores from more than 40% of sporangia even at 4.25°C. The temperature resulting in maximum release for each isolate is less clear and accurate determination would require replication and possibly the sampling of more sporangia to increase the accuracy of measurement. However, there is an indication that some isolates have a lower temperature optimum for hatching. Most isolates show maximum release at 11.7 °C or 13.4 °C but RF1 and perhaps 10639 show maximum release at 8.85°C. There is an indication that isolate 11179 had a narrower response curve than the other isolates, particularly at higher temperatures where release had fallen rapidly at temperatures above 13.4°C. Further work would be required to confirm these suggestions.

In Experiment 2 where sporangia were harvested from detached leaflets, the response to temperature of hatching was clearly different (Fig. 5). For all isolates, hatching was minimal, usually <5%, at temperatures of 13°C and above. The equivalent temperature in Experiment 1 was 20.4 ºC. Differences from Experiment 1 were also apparent in the lower temperature range where release was still substantial at 4.5°C and continued to decrease as temperature decreased to –1.2°C. Even at this temperature, four isolates showed >40% hatching on return to 20°C. As in Experiment 1, the optimal temperature for release of each isolates is not clearly defined but it is certainly lower, around 6°C instead of around 12°C in Experiment 1. Differences among isolates in this respect are again difficult to assess but any differences must be small. For example, perhaps 8074 has a lower optimum of 5.8°C, 19 has an optimum of 7.3°C and RF1 has a higher optimum of 8.8°C. A striking feature of the results in this experiment is that several isolates show little or no stimulation of hatching at low temperature. It is not known why this should be and until both experiments are repeated it is not possible to say if this lack of release is typical of these isolates when sporangia are harvested from foliage. Most workers would agree that the percentage of release for any particular isolate varies widely over a period of time; the cause of this variation has yet to be pinned down. It is not known why certain isolates and particularly 7372 gave highly variable release values

in the range of temperatures which gave optimal release. There was no evidence that any isolate was unable to form zoospores. Some recent results from The Netherlands (W. Flier, pers. comm.) indicated that such isolates may have evolved recently.

There is little published work on the detailed response to temperature of zoospore release. Experiments in the 1980s (D Shaw, unpublished) with one isolate from Wales, using a similar gradient incubator, showed that release from sporangia grown on Rye Agar had a clear temperature optimum of 10°C for sporangia produced in agar culture. The results here are consistent with this finding indicating that the optimum for zoospore release has not changed to any large extent in recent isolates.

A comparison of the latent period and sporulation of ten isolates.

All ten isolates on detached leavers of cv Nicola produced sporangiophores on at least one replicate by 56.75h after inoculation (Table 5). There was an indication that isolate 7372 might be precocious as one replicate had signs of sporulation after only 51.75h and that isolate 11854 might be slower than the rest as one replicate did not sporulate until 69h. Convincing evidence of small differences among isolates might have been detected had many more replicates been used and if cultures had been observed over shorter intervals of time.

Yields of spores over the first 6d and over the following 24h were variable (Fig. 6) but because of the large variation in yield within replicates, due at least in part to variation in leaflet quality, ANOVA indicated no significant differences. The yield at 6d recorded spores formed from about 66h until the sixth day. For most isolates this yield is slightly larger than that in the next 24h. The substantial yield over the 24h is not unexpected as the increment in lesion diameter at this stage was substantial and the second crop of spores was produced around the edge of the lesion. There is some evidence that isolates 7372, 9640 and 10747 produced more spores over the total period of 7d than the other isolates.

A comparison of tuber infection by the eleven isolates.

As expected, tubers of cv Nicola (NIAB rating 3) were more susceptible to tuber blight than cv Lady Balfour (NIAB 7) or the Sárpo cv, Axona (NIAB 5). Given the small number of tubers inoculated with each isolate, namely eight, and thus the large sampling error involved, it is not possible to measure infectivity of each isolate with accuracy and thus distinguish among the isolates for their ability to cause tuber blight. However, there is an indication that isolate RF1 could cause tuber blight on a susceptible cv but was unable to overcome the resistance of the other two cvs. Isolate 7372 showed an ability to cause tuber blight on all three cvs. There was no evidence for ability to cause tuber blight in isolates 9640 and 10315. Tuber halves remaining at the end of this experiment will be planted in pots; when foliage is growing actively, plants will be subjected to 100%RH to determine if any isolate is able to survive in the tuber of any cv and transmit blight to the foliage.

The total phenotype of the eleven isolates

Table 4 shows how each of the eleven isolates performed in the various assessments. RF1 was an early season isolate not able to overcome R-genes 2,3,5,8 or 10. It proved to be slow growing in culture and released zoospores optimally at 8.8°C in both experiments. Most other isolates released optimally at higher temperatures in Experiment 1 and at lower temperatures in Experiment 2. Isolate 19 had intermediate virulence, a fast growth rate in culture and showed high zoospore-release ability. Isolate 7372, infected tubers of all three cvs and had shorter latent period although greater sporulation than most isolates. Isolate 8074 formed very slow-growing lesions on leaflets but was heat tolerant and released zoospores at lower temperatures in Experiment 1. Isolate 8182 was unusual in being the only self-fertile isolate detected in 2004. It had a fast growth rate and an ability to release zoospores at low temperatures but poorly and maybe because it was self fertile. 9640 did not infect tubers of any cv but sporulated well on leaflets. 10315 was A2 mating type, did not infect tubers of any cv and had a high optimum for zoospore release in experiment 2. Isolate 10639 had a low temperature optimum for release of zoospores in experiment 1. Isolate 10747 sporulated well on leaflets and isolate 11179 showed a narrower temperature range for optimal release of zoospores. 11854 was one of the most virulent isolates but was otherwise undistinguished. In conclusion, the eleven isolates showed considerable variation in characters which are likely to affect their success in reproducing to cause epiphytotics in the field.

Comparison of pathogenicity of three strains (isolates) of P. infestans *in the field*

Introduction

In an agro-ecosystem, a highly pathogenic isolate is one which destroys the crop most rapidly and most efficiently. As late blight is a polycyclic disease, the pathogenicity of an isolate or strain will depend on a large number of properties amongst which are: how efficiently the isolate is able to penetrate the host; how it spreads within the tissues of the foliage; how soon it sporulates; how many sporangia are produced; how it infects tubers; how it colonises tubers. In 2005, some of the isolates that had been characterised in the laboratory in 2004 were studied under field conditions to determine if differences in pathogenicity were detectable between the isolates.

Materials and methods

An organic smallholding was selected where the farmer agreed to host a crop which would be infected and would be left untreated for the duration of the experiment until the foliage throughout the field was completely destroyed by blight. The holding was at Trigonos, Nantlle nr. Caernarfon. This is a high-rainfall/high humidity area with steep mountain slopes surrounding the village of Nantlle. A crop of approximately 0.5 ha was grown in a field between the steeply sloping slate quarry to the north and the lake (Llyn Nantlle Uchaf) to the south.

The land was ploughed from permanent pasture and cultivated. Certified seed of cv Nicola, a second-early susceptible to foliage and tuber blight, was planted by machine on May 6th. The 84 rows were spaced at 70 cm and within row spacing of seed was 30cm. A transmitting weather station, supplied by Plantsystems Ltd, was erected on the northern headland as close to the crop as the transmission signal would allow. Data recorded were temperature, humidity, precipitation, wind speed and direction. Plants had closed over the drills in most of the field by the date of inoculation although there were some patches where plants grew more slowly or were more widely spaced; in these patches, a complete canopy was slow to develop.

Three strains (isolates) of the pathogen were used for inoculation. Strains (isolates) 19 and 11179 were chosen from those collected by Blight Scouts in 2004. Strain 19 was isolated from an early sample collected on 15th June from cv Maris Piper grown near Dudley, W Midlands. Strain 11179 was isolated from a late sample, collected on 26th August form cv Pentland Dell, grown near Spilsby, Lincs. These two strains were stored as pure cultures in plastic vials containing water and one sterile grain of rye. They were subcultured on rye agar then cycled twice on susceptible leaflets of potato before use. Strain 16 was isolated from a sample collected on 2nd July, 2005 from cv Charlotte growing on an early potato farm near Benllech, Anglesey. This strain was subcultured on potato leaf material before use. Phenotypes of strains 19 and 11179, determined in 2004, are shown in Table 6.

Thirty-two-day-old plants, cv Nicola, grown in 1l pots of compost in the greenhouse, were inoculated by spraying with suspensions of sporangia to run-off. For each of the

three strains three replicate plants were inoculated. The plants were each then covered with a plastic sack overnight to maintain leaf wetness. After removing the sacks, the plants were incubated in the laboratory at room temperature for two more days when lesions of 4 – 5 mm in diameter had developed over all leaf and stem surfaces. Plants were then incubated within plastics sacks overnight when abundant sporulation had occurred and were transported to the experimental site on 30th July, 2005. Each bagged plant was positioned within the crop and the pot was inserted firmly between adjacent ridges. The sack was cut off, taking care not to contaminate the operator.

Pheno- and genotypic features	**Strain number**		
	16	**19**	**11179**
Mating type	A1	A1	A1
Virulence	1, 2, 3, 4, 5, 6, 7, 10	1, 3, 4, 7, 8, 10, 11	1, 2, 3, 4, 5, 6, 7, 10, 11
Response to metalaxyl	Sensitive	Resistant	Sensitive
Isozyme Gpi	100/100	100/100	100/100
Isozyme Pep	100/100	83/100	83/100
Mt DNA haplotype	IIa	Ia	IIa
Lesion growth rate	Not tested	Fast	Average
% zoospore release	Not tested	High	Low
Temperatures for zoospore release	Not tested	Average optimum	Narrow range
Date of isolation	2nd July, 2005	15th June, 2004	26th August 2004

TABLE 6: SUMMARY OF PHENOTYPIC AND GENOTYPIC FEATURES OF THE THREE STRAINS USED.

Measurement of growth of lesions in the field

Ten leaflets, each with single lesions 1.0 – 1.5cm in diameter sited around each of the 9 inoculation sites, were selected 18 days after inoculation. The infected leaflets had a similar exposure within the crop canopy. Lesions were photographed and again, three days later. Lesion area was calculated from the photographs using the ImageJ 1.35i Image Analyser Program (Rasband 2004). Lesion growth was expressed as the new lesion area formed over 72h divided by the size on the first day.

Measurement of sporulation of lesions in the field

Another ten leaflets similar to the above with a lesion area of *ca*. 5 squ.cm. were selected near each of the 9 inoculation sites. Sporangia were harvested from the leaflets into a vial by shaking the leaflets together with 5–10 ml water. To preserve the suspension for later counting, domestic bleach solution was added to each vial (one volume of bleach per 9 volumes of suspension).

Measurement of sporulation of lesions in the laboratory

Lateral leaflets of potato cv. Nicola grown in a greenhouse were inoculated with 10, 100 and 1000 sporangia of each strain. Each inoculation test of the three strains and three concentrations of sporangia consisted of six replicates. Leaflets were inoculated and incubated at 18°C in the light. After one week, the area of the lesions and the number of sporangia formed in the lesions were determined as was described for the test of sporulation in the field (see above). A t-test was used for assessing the differences in the number of sporangia produced.

Disease development in the field

The field was screened on the 4th, 7th, 9th, 11th and 13th day after inoculation to locate possible new outbreaks of late blight and assess the development of the disease from the inoculum plants. Care was taken not to spread blight spores on clothes of the observer by reducing entry to the crop to a minimum and by wearing a different boiler suit to visit the replicates of each strain. Twenty three days after inoculation, when massive infection was present around the inoculated plants the extent of foci with more than 75% foliage loss (Dowley et al, 1999) was measured. Four directions (N–S, E–W, NE–SW and SE–NW) were chosen and distance along these axes were determined from the inoculated plants to the edge of the focus.

Sampling of diseased foliage

Leaf samples were collected on each screen date and their location noted. Leaflets with single lesion of late blight were collected at the edge of the primary focus around each inoculation point to determine the extent of the spread. On day 21 after infection, when lesions were well scattered over the field, another sample collection method was used. Single lesions of the same size were collected from every fourth row and from plants spaced at 1.4 m intervals.

Sampling of diseased tubers

The blighted foliage was removed mechanically 52 days after inoculation. The crop was harvested 56 - 62 days after inoculation, using a single-row, web harvester which deposited tubers on the soil surface. The same rows (every fifth row) were chosen for tuber sampling as those sampled for blighted lesions, 21 days after inoculation. One blighted tuber was sampled at random from each interval of 5m within each sampled row throughout the field; no samples were taken from rows 1 and 5. Green tubers that had grown on the soil surface were not chosen.

Identification of the leaf samples using molecular markers

For identification of the strains, microsatellite markers (simple sequence repeats, SSR) were chosen. Microsatellites are repetitions of 1–6 base pair long DNA motifs scattered abundantly in the genome of eucaryotic nuclei. These markers have great variability in their length. Specific primers of polymerase chain reaction (PCR) can be designed for each microsatellite locus and once it is known, detection of microsatellites are easy by means of a polymerase chain reaction. For *P. infestans*, 12 microsatellite loci can be detected in the genome. In a preliminary study, all of these 12 loci were tested for the three strains used in this study. Of the 12 loci, two were completely monomorphic ie. they were not different in the three strains. Eight SSR loci made distinction between one isolate and the other two. Only two SSR loci (G11 and Pi02) distinguished between the three strains. On this basis, two multiple PCRs were set up with three and five SSR primer pairs in the same reaction.

DNA for PCR was extracted from dried leaf material. Leaflets with a single, well-developed lesion collected from the field were pressed and dried between regularly-changed paper towels. To minimise cross contamination, all the samples were handled with forceps flamed or wiped with ethanol between different samples.

Two discs of 2 mm diameter were punched from the dried leaflets and ground with an infinitesimal amount of sterile sand in an Eppendorf tube. Grinding was continued with 25 µl NaOH (0.5 M). The tubes were then centrifuged at 13000 RPM for 5 minutes. Ten µl of the DNA extract was then diluted with 85 µl Tris-HCl buffer (0.01 M). RNase-A was also added to the DNA extract to digest RNA from the sample at 37°C for 30 minutes. One µl of this DNA extract were used as template for PCR. Mixture of reagents for the PCRs for each samples was as follows: reaction one 1 µl DNA template, 0.1 µl *Taq* polymerase (10 U/µl), 2 x 0.475 µl of 10 µM primer Pi4B (for each forward and reverse), 2 x0.275 µl of 10 µM primer Pi89, 2 x 0.1 µl of 10 µM primer Pi02, 0.75 µl MgCl (25 µM), 1.25 µl 10×BSA, 1.25 µl dNTPs (2.5 mM each), 1.25 µl 10 x buffer, 5.2 µl water. For reaction two: one 1 µl DNA template, 0.1 µl *Taq* polymerase (10 U/µl), 2 x 0.3 µl of 10 µM primer Pi63, 2 x0.3 µl of 10 µM primer Pi56, 2 x 0.375 µl of 10 µM primer Pi70, 2 x 0.1 µl of 10 µM primer Pi04, 2 x 0.375 µl of 10 µM primer G11, 0.75 µl MgCl (25 mM), 1.25 µl 10×BSA, 1.25 µl dNTPs (2.5 mM each), 1.25 µl 10 x buffer, 4.0 µl water. The PCR conditions were 2 min at 95 °C followed by the thermal cycle of 20 s at 95°C, 20 s at 58 °C, 1 min at 72 °C. The cycle was repeated 30 times and followed by the final chain elongation for 20 min at 72 °C. PCR products were then separated on an ABI3730 capillary electrophoresis system and scored with the program Genotyper. Sample identification was considered to be successful if SSR markers of all loci were identical to any of the three isolates and at least five loci of the eight gave a result.

Results

Disease development in the field

No visible blight symptoms were present within the field before inocula were introduced. At the time of inoculation, 30th July, weather conditions were favourable for late blight (Full Smith Period on 31st July/1st August) allowing the pathogen to spread rapidly. Late blight disease was monitored in the days following inoculation when four half Smith Periods were recorded (on 3rd, 5th, 12th and 15th August) and only one full Smith period on 16th August (Fig. 8). Late blight disease development was traced in the field by mapping lesions until 12th August when lesions began to appear not only next to the inoculated plants but also at some distance, suggesting that either alien strains were present or that more distant transmission from inocula had taken place.

On the fourth day after inoculation, only three plants showed symptoms of late blight (Fig. 9) two of them were either in physical contact with the inoculated plant or sporangia may have been washed by the rainfall during that period. However, the third lesion appeared at a distance of 1.4 m from the original potted plant inoculated with strain 19. Several new lesions were observed seven days after inoculation. Two to five lesions were detected around the inoculated plants except plant B3 (Fig. 9). The distances from the inoculated plants ranged from 60 to 280 cm (Table 7). Leaf lesions of late blight were visible around all the nine inoculated plants by day 9 after inoculation. The first lesions farther away from inoculated plants appeared on 11th and 13th day after inoculation. Following this date (12th August) it was not possible to trace the most likely origin of all lesions. Distance of lesions from each inoculated plant increased gradually (Fig. 9). On the eleventh day after inoculation, two lesions

appeared, far from any other lesions and nearly halfway between the inoculated plants B2 – C3 and C1 – A2. These new infections may have resulted from a wild strain but this could not be confirmed when isolates were tested for SSR markers; there were insufficient PCR products. Seventeen days after inoculation another full Smith Period allowed the pathogen to spread widely. A systematic inspection revealed that every potato plant was blighted by the 18th day after inoculation. Following this inspection, samples were collected systematically from plants in evenly spaced intervals over the whole field. Lesions of the same diameter were chosen to ensure that they originate from the same period of infection. The SSR genotype of these lesions was determined to identify the distribution of the three strains on the field at this time (Fig. 10). Twenty three days after inoculation, areas where the foliage was more than 75% blighted were assessed. Their locations are illustrated in Fig. 10. Along with the nine inoculated primary foci, there were another four areas where the infection had progressed to a similar extent. They may have originated from an alien strain; unfortunately, no SSR profiles are available.

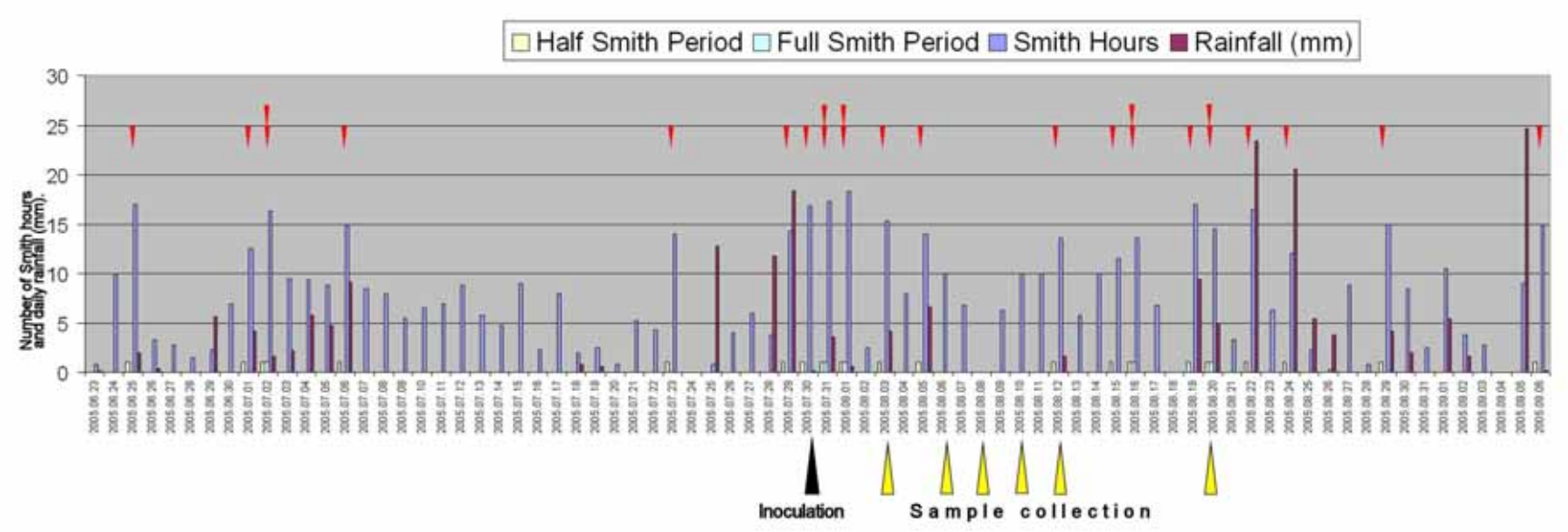


FIGURE 8: WEATHER DATA WITH SMITH PERIODS AND RAINFALL DURING THE CROP GROWTH IN THE EXPERIMENTAL FIELD IN NANTLLE.

Smith periods (at least two consecutive days when the minimum temperature is above 10 °C and the humidity is more than 90% for at least 11 hours) and half Smith periods are also indicated and highlighted with one and two red arrows, respectively.

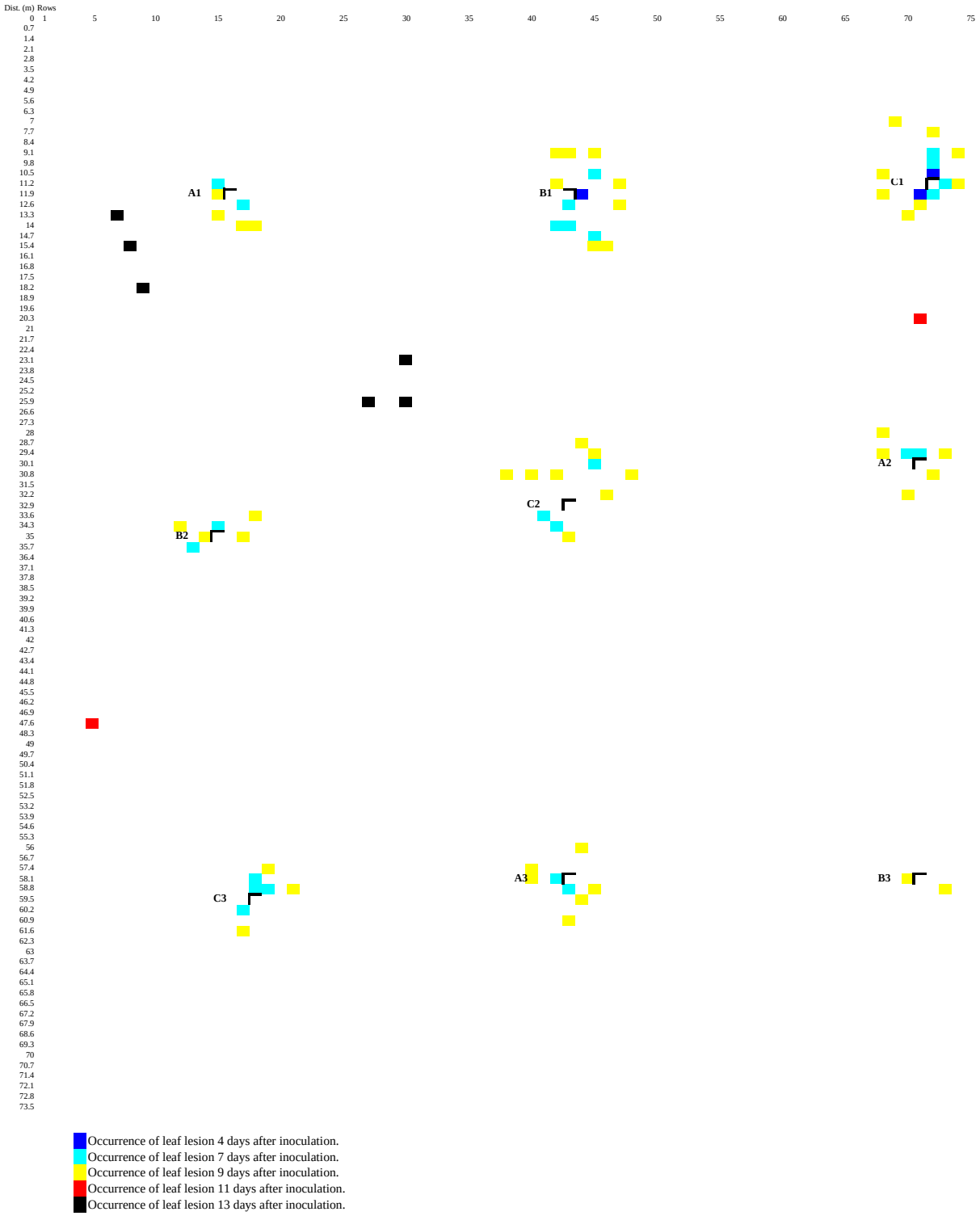


FIGURE 9: LOCATION OF THE FIRST LESIONS ON POTATO PLANTS IN THE FIELD.

The field was divided into contiguous lattices of 70 × 70 cm quadrats; their distance is indicated in the first column in metres. The edge of the quadrats are not indicated. Points of highlighted corners indicate the location of the inoculated plants (A: 11179, B: 16, C: 19).

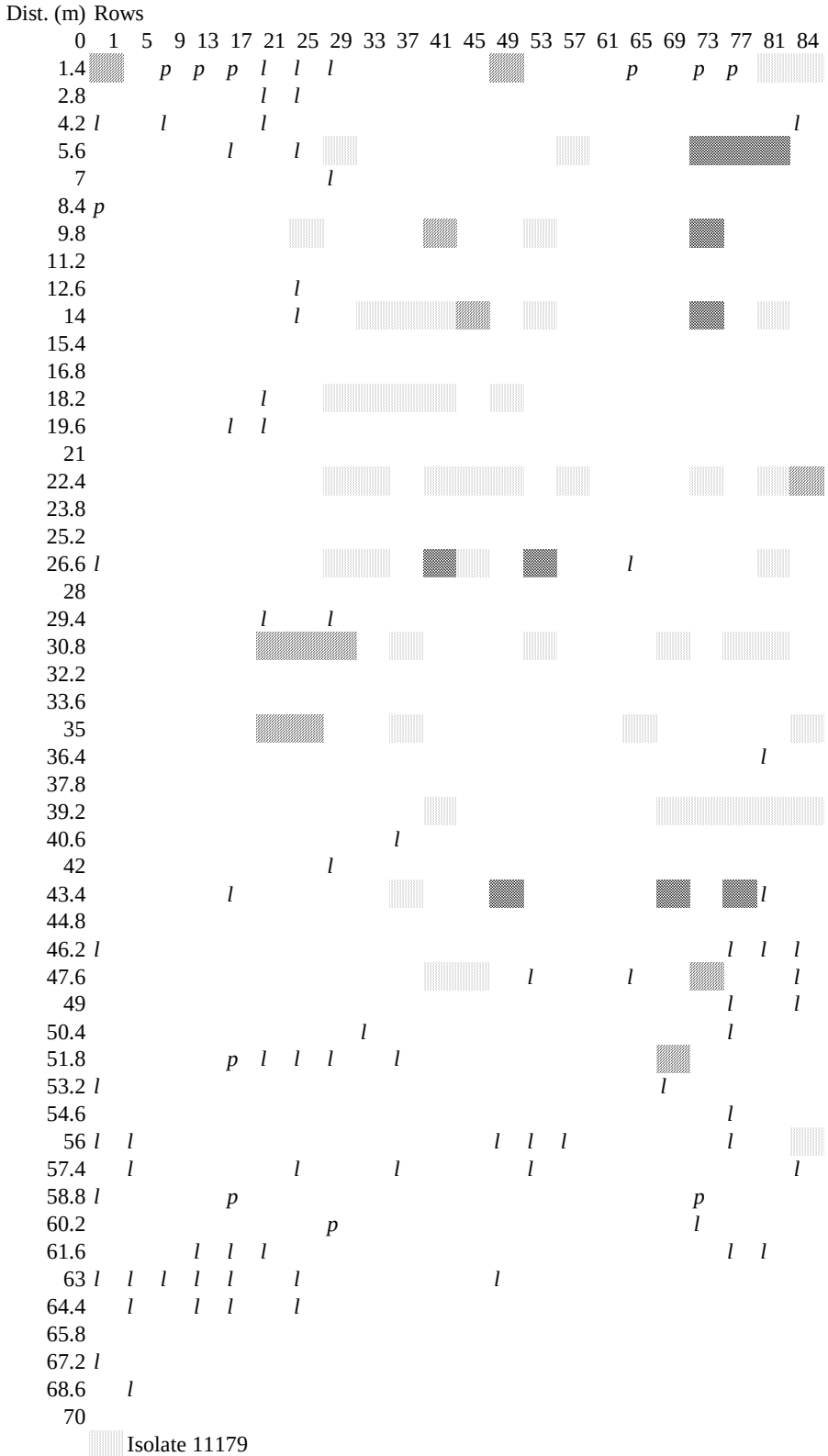


FIGURE 10. LOCATION OF THE THREE STRAINS IN THE FOLIAGE 21 DAYS AFTER INOCULATION.

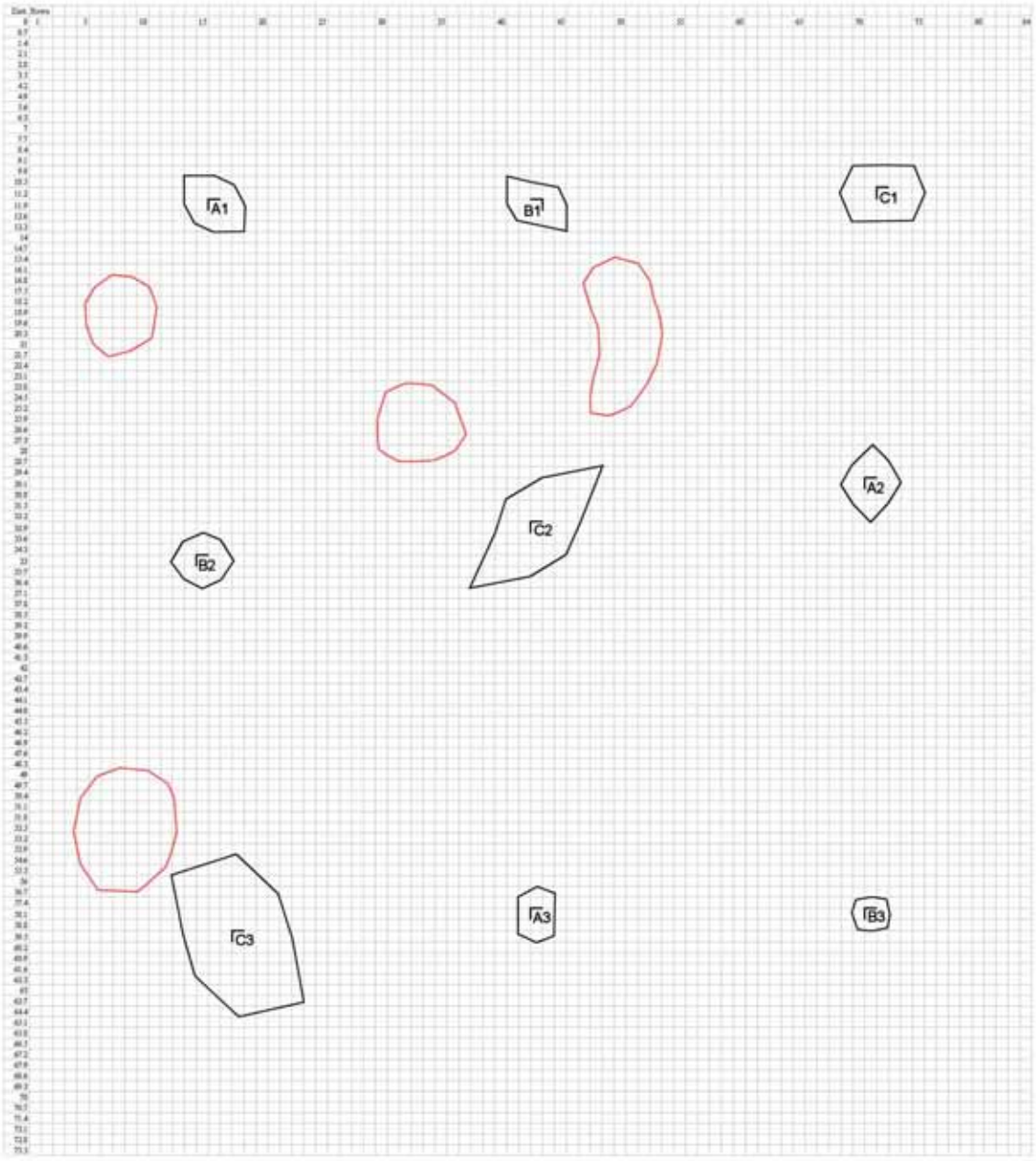


FIGURE 11: AREAS WHERE THE LATE BLIGHT DAMAGE WAS > 75% 23 DAYS AFTER INOCULATION.

The black polygons show the extent of infection around inoculated plants; the estimated location of other areas with the same foliage damage is outlined in red.

	Day after inoculation		
Inoculated plant	**4**	**7**	**9**
A1	no lesions	110 cm (n = 2)	220 cm (n = 4)
A2	no lesions	35 cm (n = 2)	158 cm (n = 5)
A3	no lesions	64 cm (n = 2)	180 cm (n = 6)
B1	contact with B1	116 cm (n = 4)	261 cm (n = 8)
B2	no lesions	50 cm (n = 2)	155 cm (n = 4)
B3	no lesions	no lesions	110 cm (n = 2)
C1	70 cm (n = 2)	114 cm (n = 4)	198 cm (n = 9)
C2	no lesions	137 cm (n = 3)	290 cm (n = 8)
C3	no lesions	112 cm (n = 4)	193 cm (n = 3)

TABLE 7**:** AVERAGE DISTANCE OF LESIONS FROM THE INOCULATED PLANTS AT DIFFERENT TIMES AFTER INOCULATION.

The lesions were collected from the edge of the primary foci containing infected plants around the inoculated plants. (A1, A1, A3: strain 11179; B1, B2, B3: strain 16; C1, C2, C3: strain 19.)

A comparison of lesion growth of the three strains in the field

On the eighteenth day after inoculation, ten leaflets were selected from plants at a distance of approximately 1.5 m from an inoculated plant of each strain. However, after SSR identification, only 2 and 5 isolates were identical with the SSR profile of the strains 16 and 19, respectively. In contrast, for strain 11179, eight leaflets were confirmed to be infected with this strain and another three of this strain were identified within the samples taken from the foci inoculated with the other two strains. Therefore 11 samples were tested in the case of strain 11179. Average lesion growth rates, (± standard deviation) calculated from the increment in lesion area divided by the area on the first day were:

Strain 16: 7.22 ± 4.48 (n = 2).
Strain 11179: 10.23 ± 8.98 (n = 11).
Strain 19: 11.25 ± 5.35 (n = 5).
The t-test showed that differences among the strains in lesion growth were not significant (P=0.05) due to large variation among replicates.

A comparison of sporulation density of strains *in vitro*

An *in vitro* sporulation test was set up in October. All three strains had been maintained on susceptible potato (cv. Bintje) leaflets over the summer until that time. However strain 19 did not produce enough sporangia for inoculation. It was therefore excluded from the experiment. Numbers of sporangia harvested from the leaflets are shown in Table 8.

No. of sporangia in the inoculum	**Strain 16**	**Strain 11179**
1000	8539 ± 4710 (n = 6).	10267 ± 3780 (n = 6).
100	17647 ± 8513 (n = 6).	15492 ± 7299 (n = 6).
10	9560 ± 6139 (n = 4).	no sporulation

TABLE 8**:** AVERAGE DENSITY OF SPORANGIA (SPORANGIUM/CM^2 ± STANDARD DEVIATION) ON LESIONS OF TWO STRAINS FROM DETACHED LEAFLETS.

Counts were made seven days after inoculation. n = sample size.

No significant difference was observed if leaflets were inoculated with different numbers of sporangia from the same strain (P = 0.05). Also, in a comparison of strains 16 and 11179 the t-test did not show a significant difference among leaflets inoculated with the same number of sporangia at P = 0.05.

Production of sporangia by the three strains in the field

SSR analysis indicated that of the ten lesions sampled for spores for each isolate, only 4, 6 and 4 belonged to strain 11179, strain 19 and strain 16 respectively. The mean numbers of sporangia produced by each strain in the field, overnight were:

Strain 16: 8733 ± 5600 (n = 4).
Strain 11179: 15948 ± 5600 (n = 4).
Strain 19: 10572 ± 4877 (n = 6).
The differences were not significant at P = 0.05 in any of the comparisons among the three strains.

Blight symptoms on the tubers

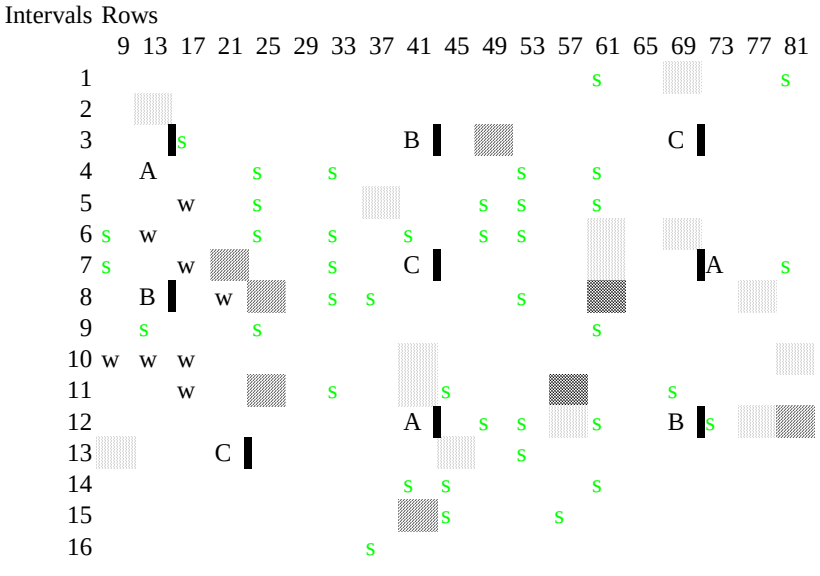
A total of 419 tubers showed late blight symptoms in the 19 rows of the field which were sampled for tuber blight. In those sampling intervals where more than one tuber had symptoms, one tuber was selected randomly for further testing making a total of 199 tubers. The location of the tubers is shown on Fig. 10. Because the majority of these 199 tubers had secondary infections caused by other pathogens, *P. infestans* was isolated from only 70 tubers. It was possible to identify the origin of the strain by SSR for 22 of these tubers; the PCR product in 40 samples was insufficient for identification. Eight tubers were infected with an alien strain, 14 with strain 11179, 6 with strain 16 and 2 with strain 19. The locations of those tuber samples that were infected by the three experimental strains are shown on Fig. 11.

Intervals Rows

	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	77	81
1	**2**		**1**	**1**		**2**		**2**				**1**		**1**		**1**	**1**		**1**
2	**2**	**1**	**1**		**1**				**1**			**1**		**1**	**1**	**1**	**2**		
3	**2**	**3**	**1**				**1**				**2**	**1**	**1**	**2**	**2**	**1**	**1**		
4	**1**	**3**		**3**	**2**	**4**	**2**		**1**	**1**	**2**	**2**		**1**		**1**	**3**		**1**
5	**1**	**8**	**2**		**3**	**1**	**1**	**2**	**3**		**2**	**4**	**2**	**4**		**4**	**7**	**2**	
6	**2**	**3**	**3**		**1**	**2**	**2**	**1**	**3**		**3**	**3**		**6**		**3**		**2**	**1**
7	**8**	**3**	**1**	**3**	**1**	**2**	**3**		**1**			**1**		**1**	**1**	**1**		**1**	**2**
8	**9**		**1**	**2**	**4**	**5**	**3**	**2**	**1**	**1**		**5**	**2**	**4**		**3**	**5**	**3**	**1**
9	**2**	**2**			**7**		**5**	**3**	**1**			**2**	**3**	**1**		**1**	**1**	**3**	**1**
10	**1**	**4**	**2**		**2**		**3**	**3**	**2**			**2**	**2**			**1**	**1**		**3**
11	**2**	**1**	**2**	**1**	**2**	**4**	**3**	**1**	**4**	**3**			**3**	**2**	**1**	**2**	**1**		**2**
12	**3**	**2**	**1**	**1**		**3**	**5**	**1**	**2**	**1**	**2**	**4**	**3**	**1**	**1**	**1**	**2**	**2**	**3**
13	**2**	**2**	**1**	**2**	**2**		**2**		**2**	**3**		**1**		**2**	**2**			**1**	
14	**2**	**2**			**1**		**1**		**2**	**2**		**1**	**2**	**5**	**1**				
15							**1**		**1**	**1**			**2**	**2**	**1**		**2**		**1**
16	**1**			**1**				**2**	**1**				**1**					**1**	

Red figures: succesful isolation.
Black figures: *P. infestans* not confirmed.

FIGURE 10: DISTRIBUTION OF TUBERS EXHIBITING LATE BLIGHT SYMPTOMS IN THE FIELD.
The number of tubers with symptoms in each different interval is shown.



Strain 11179 (A)
Strain 16 (B)
Strain 19 (C)
s: succesful isolation, but SSR identification failed.
Bars indicate the approximate position of the inoculated plants (see capital letters).

FIGURE 11: LOCATION OF TUBERS BLIGHTED BY THE THREE DIFFERENT STRAINS.
w indicates the location of an alien strain.

Discussion

The three strains used were all of A1 mating type and were derived from early, middle and late-season outbreaks on commercial farms in three regions of the country. As the multilocus genotype determined by probe RG57 has not been determined, it is not known if any of the three strains belong to one of the common clonal lineages identified in the 1980s (Day *et al.*, 2004), a newer clonal lineage or if any has arisen recently via sexual reproduction. Strain 11179 was virulent on all R-gene differentials except R8 and R9. Virulence on R8 is rare in GB and in most of Europe and R9 has only been attacked very late in the season in Eastern Europe but not in GB. This is therefore one of the most virulent isolates to be found in GB. Strain 16 was not tested on R11 but had the same phenotype as strain 11179 on the other differentials. As virulence to R11 is one of the commonest in the GB population, these two strains have a high probability of being the same complex virulence phenotype. Strain 19 was avirulent on R2, R5, and R6 as well as on R8 and R9. This is not an unusual virulence phenotype in Europe, according to the new EUCABLIGHT database at www.eucablight.org.

As shown in Table 6, strains 16 and 11179 share many characters and the SSR genotypes determined here indicate that they are more closely related than either is to strain 19. They can be distinguished from strain 19 on the basis of virulence, sensitivity to metalaxyl and mtDNA haplotype. Strains 16 and 11179 can be distinguished on the basis of peptidase phenotype. However, methods used to determine these markers are more time-consuming and expensive and these molecular markers depend on extracts from pure cultures to give dependable results. It was thus decided to use the more versatile and automated SSR marker system.

Identification of the strains was effective with SSRs. The method was robust and gave the opportunity to test *ca* 500 leaf samples over two weeks. It is possible to distinguish alien strains in the field along with the experimental strains within one laboratory procedure because a combination of eight SSR markers is an efficient way of detecting diversity of this kind. Although the multi-locus RFLP probe, RG57, can also detect this kind of diversity, the method requires relatively large amounts of pure DNA. Problems with the SSR diagnosis were experienced which meant that some samples could not be characterised from the dried material. In some samples, DNA may have degraded during drying of the leaflets; in others not enough DNA might have been extracted due to the problems of location of the young part of the lesions on dried leaflets. Both of these can prevent enough PCR product being replicated to allow detection. All samples giving no product, have been retained, could be re-extracted from an increased amount of leaf material to produce enough DNA template for PCR.

Early epidemic development

Weather data (Fig. 8) showed that conditions in the field prior to inoculation were unconducive for sporulation and infection. Only one Half Smith Period was recorded (23rd July) in the previous month. This was fortunate as it meant that the risk of infection from alien strains in the area was low and no lesions were observed in the field before inoculation. A Full Smith Period immediately after inoculation ensured

that the spores from the introduced plants were able to initiate primary foci of infection which were recorded and mapped from day 4. Eighteen days after inoculation, the disease had spread to every plant in the field and most plants had suffered a loss in leaf area of at least 70% by 21 days. During this early post-inoculation period, the weather was relatively dry and sunny with a heavy dew on most nights. Half Smith periods were recorded on days 13 and 16 and Full Smith Periods on days 17/18 and 21/22. The prediction from this weather data is that after the initial spread to give visible lesions on days 4 and 7, no further spread should have taken place until day 18. However results show that additional spread took place in this period (lesions appearing on days 7, 9, 11 and 13 (Fig. 11)), probably as a result of dew- induced sporulation and infection during Half Smith Periods. Plant Plus may be a better predictor of infection in cases like this. During this early phase of development, similar spread was recorded for each of the three strains in each of their three replicates.

After virtually all plants in the field showed symptoms of late blight on day 18, the frequency of each strain throughout the field was determined by systematic sampling on day 21 and SSR analysis of the dried lesions. Although more than 1000 leaflet samples were taken, it was only possible to analyse 290 in the time available. Of these only 67 yielded a sufficiently detailed SSR genotype to allow diagnosis of the strain. Notwithstanding, it was possible to map the locations of each strain amongst the samples (Fig. 11). All three strains were detected; 45 samples were strain 11179, 12 were strain 16 and 10 were strain 19. Although this is a small sample, it can be concluded that leaflet lesions of strain 11179 were more frequent than those of strains 16 and 19. The small sample also precludes the detection of any pattern in their distribution. Analysis of additional dried samples in store could give additional data on frequency and distribution of genotypes.

Isolations made from blighted tubers and analysis of SSR genotypes of isolates also suffered from technical problems. However, 14 isolates were confirmed as strain 11179, 6 as strain 16 and 2 as strain 19. It is noteworthy that all three strains were present in the small sample of tuber blight analysed. There is an indication that strain 11179 is more frequent (than strains 16 and 19). The three stains are scattered within the field with no obvious pattern. Again analysis of additional samples could yield a more detailed map.

SSR analysis of the leaflet samples detected one alien strain in one leaflet sample on the western margin of the field. This is not unexpected as plants with lower-stem lesions were detected soon after inoculum was introduced. Also the same genotype was detected in 8 tubers from the same part of the field as the plants with stem lesions and plant with leaflet lesion. It seems likely that the initial stem lesions were derived from blighted seed tubers as few other crops of potato were grown in the vicinity and all of these were observed to be healthy in late July.

Attempts were made to determine if the ability of the three strains to form lesions and to sporulate under uncontrolled field conditions varied. Although samples were taken for lesion growth 18 and 21 days after inoculation and for sporulation after 19 days after inoculation, that is while the primary foci were still small, SSR analysis revealed

that some lesions were not of the genotypes expected from their proximity to the inoculum. Unfortunately, this reduced the number of replicates from the chosen number of 10 in the majority of cases. Even although averages for both growth rate and for sporangium yield differed substantially among strains, the large variance and the small numbers of replicates resulted in no significant differences being detected. The additional test of sporulation *in vitro* at a later date also showed large variances and no significant differences in sporulation between isolates 11179 and 16.

This work has examined the spread of three strains of *P. infestans* in the field. This has been possible due to the very recent availability of suitable genetic markers for the identification of genotypes from samples of leaf and tuber lesion. SSR markers are sufficiently variable in the pathogen population to allow discrimination among isolates from different sites with a high probability. The method is based on the PCR reaction which can be used, as shown here, to amplify loci from crude DNA samples derived from dried infected potato leaflets or tubers. Many more samples were taken from the field than could be genotyped with the markers in the time available to complete the project. These samples could still be used to generate more data on spread and allow more detailed conclusions to be drawn. This is the first study of its kind, using SSR markers in epidemiology in the field.

There is increasing evidence that late blight is starting earlier in the season and is more difficult to control since the 1980s. It is often assumed that this is because new strains of *P. infestans* are more aggressive than those of the old clonal lineage. While there is convincing evidence that control is more difficult and fungicide use has increased steadily, evidence for more rapid epidemics in the field is lacking. For example, a recent analysis of blight epidemics in Finland (Hannukkala *et al.*, 2006) shows that outbreaks now start earlier than previously and are controlled with more fungicide. But comparisons of blight progression on foliage (Apparent Infection Rate and Area Under the Disease Progression Curve) show that these have not increased over this period. There was evidence in Finland that earlier infections were correlated with climatic change (early season increase in temperature and increase in rain days) and lack of any rotation. The latter was taken to imply that oospore infections were involved.

Conclusions

Characterisation of small and larger samples of blight collected from most areas of GB in 2005 has established that the frequency of the A2 mating type has increased compared to 2003 and 2004. The highest frequencies of A2 were detected in the west of GB. Limited molecular characterisation showed that variation in the A2 isolates had increased since the 1990s and that six of the seven genotypes were new and had not been detected in previous studies. Several genotypes which each occurred at more than one site could have been clonal lineages. These were present along with unique genotypes that could have been the result of sexual reproduction or less common clonal lineages. Metalaxyl resistance, not formerly detected in A2 isolates, was now common within the collection tested.

Laboratory studies of the 93 isolates collected in 2004 showed that the capacity to overcome most of the R-genes carried by R-gene-differential clones was not uncommon but that some isolates were much less virulent. There is an indication that the frequency of isolates able to overcome certain R genes (e.g. R8) has increased from a low level in previous years (A Cameron; D Shaw, unpublished). The detailed assessment of how the sample of 11 isolates responded to a range of temperatures showed clearly that all isolates were able to make substantial growth at temperatures under 10°C. Although it was clear that rate of hyphal extension at optimal temperatures varied among the isolates, response to the range of temperatures was remarkably uniform. There was no evidence of low or high temperature ecotypes. However one isolate showed a clear difference from the others in its ability to survive incubation at the highest temperature used (26.4°C). This genotype might be more successful in surviving periods of sustained high temperature in the field.

The experiments on temperature response of zoospore release from sporangia showed that all 11 isolates did not release zoospores at temperatures which promoted optimal hyphal growth but needed to be subjected to a temperature drop to initiate hatching. The bell-shaped curve of release with temperature obtained in both experiments showed that maximum release occurred at intermediate temperatures. The optimal temperature was distinctly higher when sporangia were derived from agar culture and lower when they were derived from detached leaflets. Small differences amongst isolates in temperature response were indicated but further work would be needed for confirmation.

Preliminary experiments on the ability of the 11 isolates to infect tubers showed that all but two isolates infected the susceptible cv Nicola. Three isolates caused tuber blight in the resistant cv Lady Balfour and four isolates blighted tubers of resistant cv Axona. These laboratory experiments show that today's strains of the blight pathogen are variable and are able to grow and form zoospores over a wide range of temperatures. The results suggest that prediction models (DSS) based on temperature and humidity may be accurate for some strains of the pathogen but not for others.

The development of foliar and tuber blight was studied in a growing crop of potatoes in NW Wales. Three strains of recently isolated *P. infestans* were used to inoculate

the crop and development of primary foci was followed. Samples were taken when the foliage blight was at an advanced stage and blighted tubers were sampled at harvest. An in-crop weather station was used to record temperature, humidity, rainfall and wind. New molecular markers (SSR) were used to distinguish each strain in the samples. All three strains formed primary foci at about the same rate and the markers showed that all strains spread to all parts of the field. All strains were detected within blighted tubers. Although the size of samples was small, there was an indication that one strain was more frequent than the other two on foliage and within tubers. The epidemic progressed rapidly even in the absence of Smith Periods. This indicates that Smith Periods may not predict infection events for new strains of the pathogen. Smith criteria may need to be updated or an alternative system adopted as an alternative.

Appendix

FIGURE A1 : ASPECTS OF THE POTATO CROP AT NANTLLE, CAERNARFON

a) Upper left: Site of potato crop sloping gently to the south between the slate quarry and the lake, Llyn Nantlle Uchaf, 28 August, 2005.
b) Upper right: late stage in blight development around inoculation point; 28 August, 2005.
c) Lower left: several plants showing >70% foliage blight on 28 August, 2005. d) Lower right: lower-stem lesion on western margin of crop, 12 August, 2005.

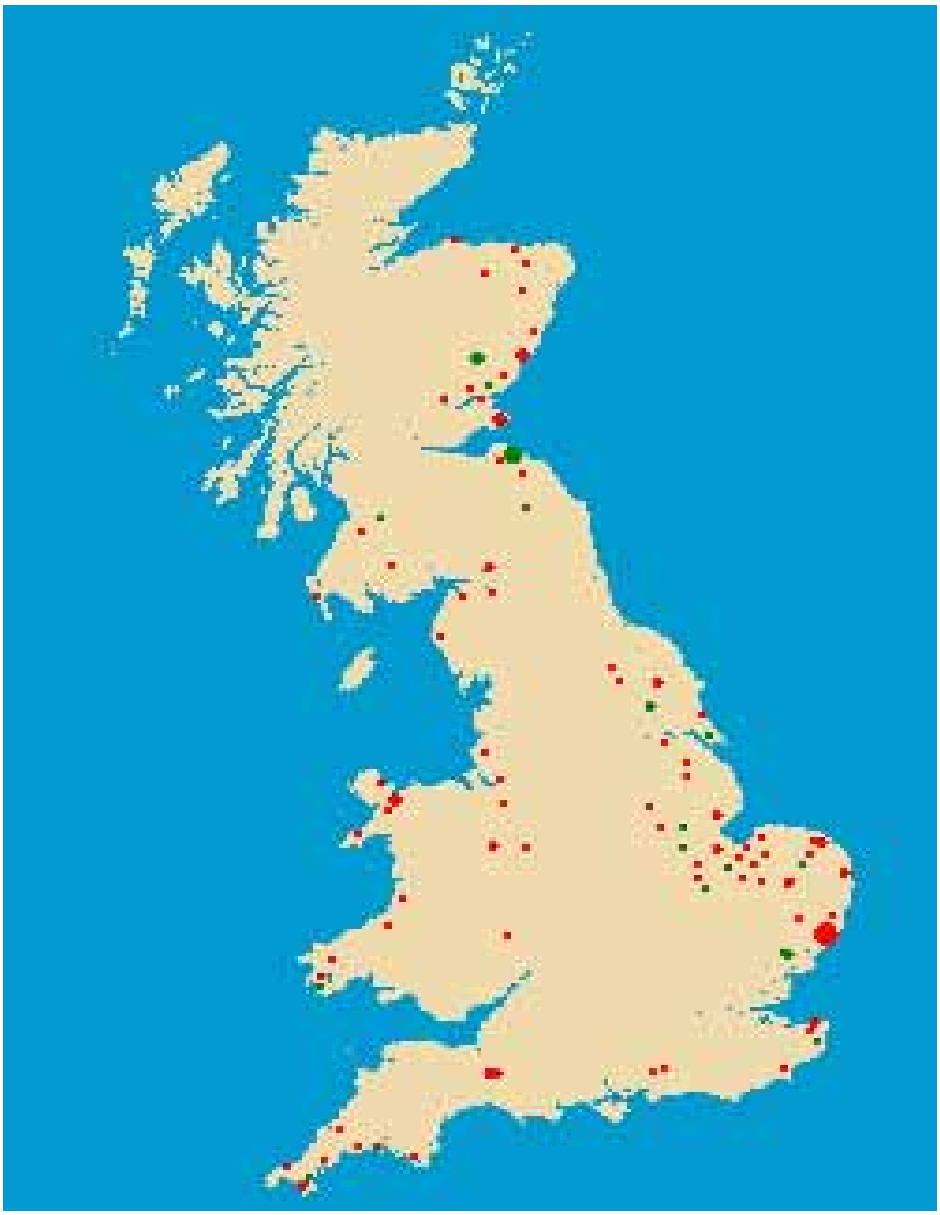


FIGURE A2: SITES OF LATE-BLIGHT SAMPLES COLLECTED BY FAB SCOUTS IN 2005 AND SENT TO HENFAES VIA CSL

Red spots show sites where blight was confirmed. The figure was downloaded from FAB pages of the BPC website.

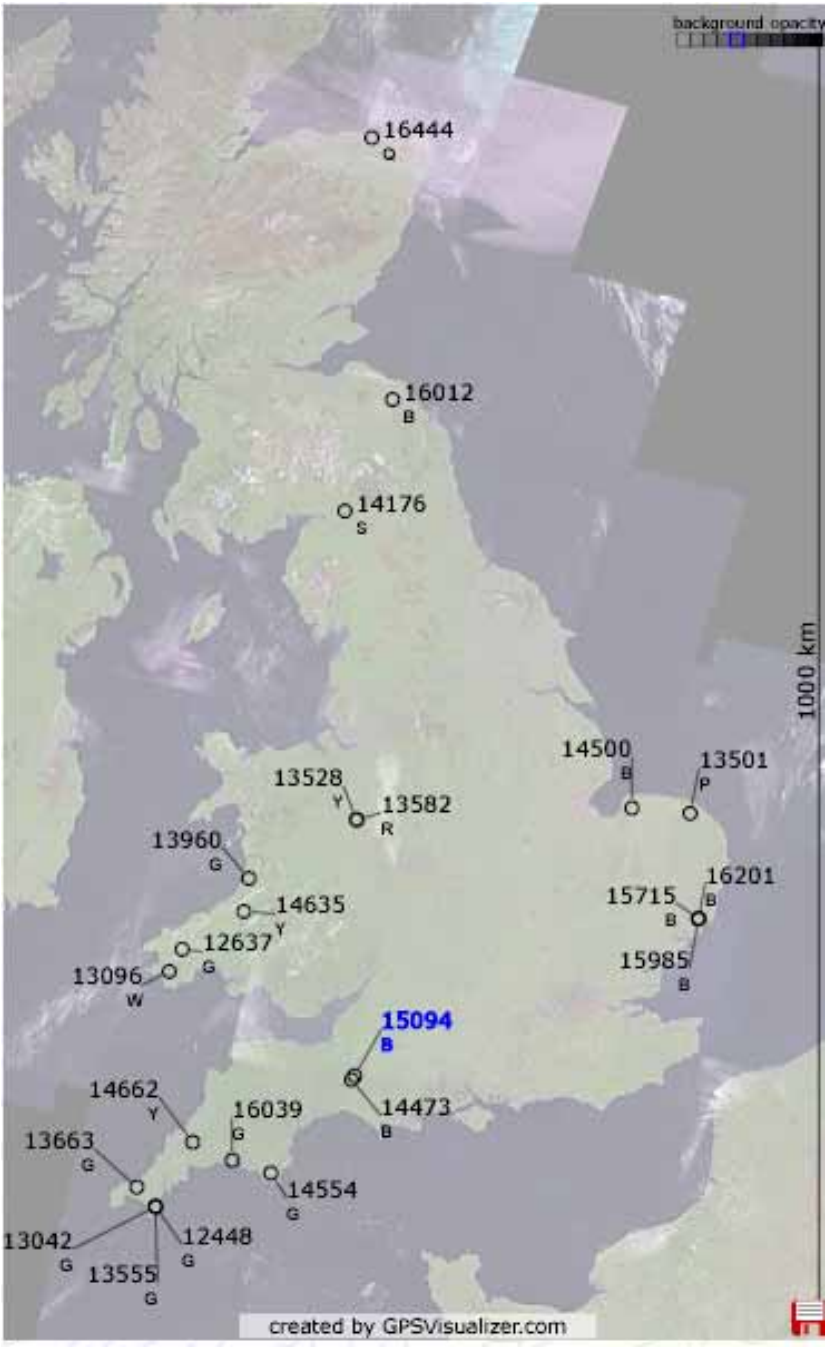


FIGURE A3: SITES OF BLIGHT SAMPLES (LABELLED WITH FAB ID NUMBER) FROM WHICH ISOLATES OF A2 MATING TYPE WERE ESTABLISHED IN 2005.

Single letter refers to RG57 fingerprint of isolate: G = green, B = blue, Y = yellow, R = red, P = purple, S = silver, G = grey, Q = unknown. Code relating colour to RG57 banding pattern is shown in Table A1.

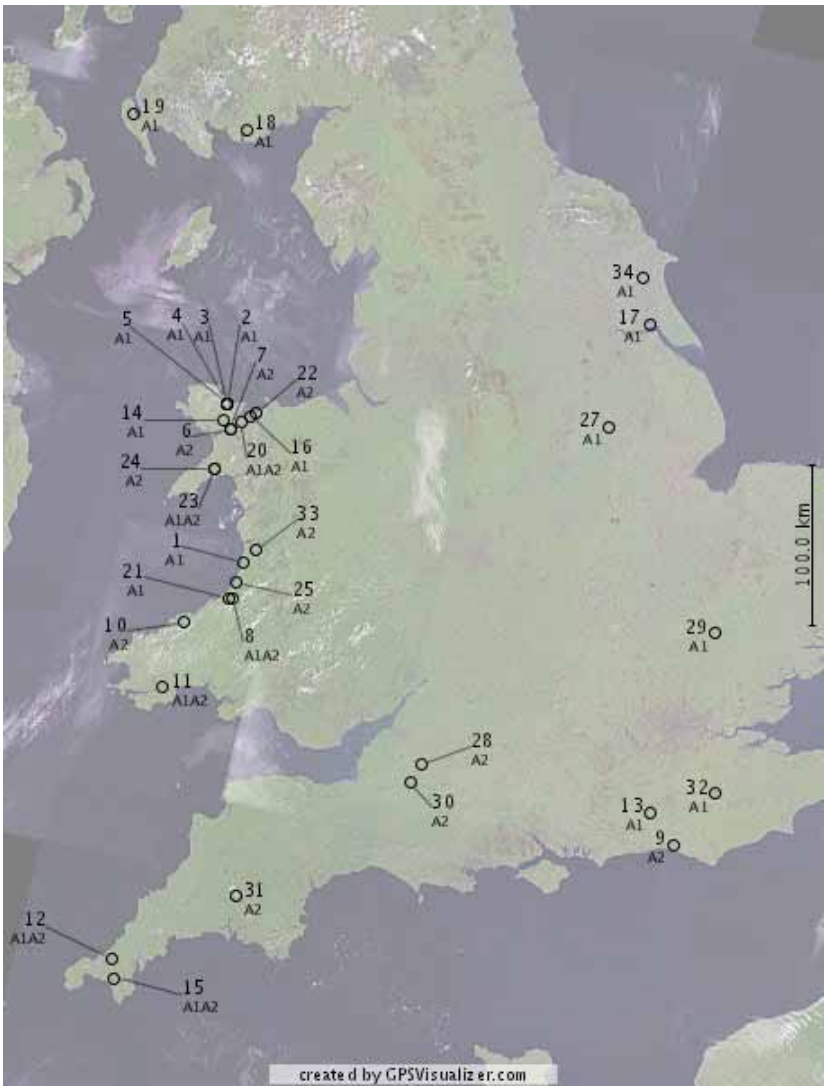


FIGURE A4: SITES OF NON-CSL BLIGHT SAMPLES IN 2005.

Sites are labelled with sample numbers as in Table A2; presence of A1, A2 or both mating types is indicated.

FIGURE A5. TUBERS OF CV NICOLA THREE MONTHS IN STORE AFTER INOCULATION WITH ISOLATE 10639.

Three tubers were blighted. Tuber on bottom left had soft rot.

FIGURE A6. EIGHT TUBER HALVES OF CV NICOLA THREE MONTHS IN STORE AFTER INOCULATION WITH ISOLATE RF1.

The four tubers on the right were blighted.

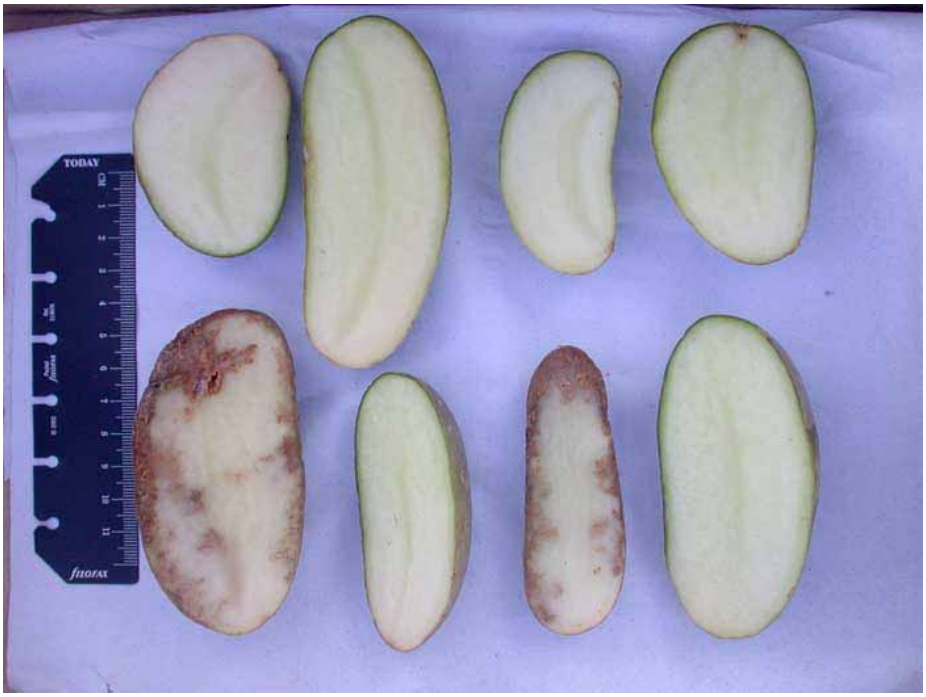


FIGURE A7. EIGHT TUBER HALVES OF CV LADY BALFOUR THREE MONTHS IN STORE AFTER INOCULATION WITH ISOLATE 7372.

Two tubers were blighted.

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