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Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales

J. P. Day and R. C. Shattock
School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK (Fax: 01248 370731)

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Abstract

Mating type, *in vitro* sensitivity to the phenylamide fungicide metalaxyl, and mitochondrial (mtDNA) haplotype were determined in some or all of 618 isolates of *Phytophthora infestans* from the years between 1978 and 1995. A2 mating type occurred infrequently in most but not all years and insensitivity to metalaxyl increased over time. After 1982, the mtDNA Ib haplotype was largely replaced (except for one isolate in 1986 and one in 1995) by two new haplotypes, Ia and IIa. Type Ia was much more common than type IIa.

Approximately one quarter of these isolates (165) were compared using two components of fitness associated with aggressiveness (infection frequency × number of sporangia per lesion) on detached leaves of cultivars Maris Piper, Cara and Stirling, which were chosen as exhibiting increasing levels of race non-specific resistance. Isolates were compared with three 'standard' isolates of low, intermediate or high aggressiveness, and the data standardised for comparison between experiments. On cvs. Cara and Stirling, but not on Maris Piper, mtDNA Ia and IIa haplotypes were more aggressive than type Ib in several separate experiments. Similarly, metalaxyl sensitive phenotypes were more aggressive than insensitive phenotypes on Cara and Stirling but not on Maris Piper. The displacement of mtDNA type Ib by types Ia and IIa over this period may have been a result of the lower aggressiveness and lack of complete insensitivity to metalaxyl in type Ib isolates.

Abbreviations: FI = fitness index.

Introduction

The historical importance of the potato late-blight disease, caused by *Phytophthora infestans*, is well known. Attempts to control the disease through breeding resistant varieties have met with only limited success, resulting in the widespread use of fungicides. However, recent migrations of new and varied fungal strains, presumed to have originated from Mexico (Niederhauser, 1991; Goodwin, 1997), pose a potentially renewed threat to world potato production. Following the first report of A2 mating type strains in Switzerland (Hohl and Iselin, 1984), 'new' genotypes of both A1 and A2 mating type have been found in Asia, Africa, Europe and the Americas (Fry et al., 1993). The finding that the 'new' populations completely replaced the former population in only a few years, in some countries, suggests that the 'new' populations may be more fit. The population displacement of *P. infestans* isolates has therefore been compared with the similar situation with Dutch Elm Disease, in which morphologically distinct aggressive strains displaced the existing non-aggressive strain (Spielman et al., 1991). Data from the USA has recently indicated that 'new' isolates of *P. infestans* may be more aggressive on potato leaves than 'old' isolates (Kato and Fry, 1995).

A further factor which might contribute to increased fitness of 'new' isolates is their insensitivity to fungicides. Considerable data have been collected on the occurrence of insensitivity to phenylamide fungicides, with insensitive strains now found as a large proportion of the pathogen population in many

European countries (Gisi and Ohl, 1994). The first phenylamide to be introduced was metalaxyl, in 1979, and metalaxyl insensitivity was first recorded in 1980 in Switzerland, The Netherlands and Ireland. Since insensitivity was generally found in isolates of 'new' genotype, it is thought that the migration of insensitive strains via infected plant material contributed to the rapid appearance of the metalaxyl-insensitive phenotype in Europe and the United States (Staub, 1994; Goodwin et al., 1996).

In this study, 'old' and 'new' isolates stored in liquid nitrogen from the years between 1978 and 1987, and collected from the field between 1993 and 1995, were compared using two components of fitness that affect aggressiveness and therefore pathogenicity, namely infection frequency and sporulation on detached leaves. These data were tested for effects of isolate mating type, sensitivity to metalaxyl *in vitro* and mitochondrial DNA type. 'Old' isolates are those which were present before A2 isolates were first detected outside Mexico, and are characterised by the di-locus allozyme genotype *86/100* and *92/100* for glucose-6-phosphate isomerase and peptidase respectively, and 15 band DNA fingerprint (Fry and Goodwin, 1995) and mitochondrial DNA haplotype (Carter et al., 1990). Of the available markers, the mitochondrial DNA types were chosen in order to screen large numbers of isolates. 'Old' isolates specifically refer to the single clonal lineage (Goodwin et al., 1994) characterised by mtDNA type Ib, and 'new' to the variety of more recent lineages which are types Ia and IIa (Carter et al., 1990).

Materials and methods

Isolates of Phytophthora infestans

Isolates were routinely maintained on rye A agar (Caten and Jinks, 1968), or for longer term storage or initial isolation on rye A agar supplemented with 1 ml RAN (250 mg rifamycin, 200 mg ampicillin, 500 mg nystatin, made up in 10 ml dimethyl sulphoxide) per 500 ml agar. Samples were either extracted from storage in liquid nitrogen, in the case of older isolates, or isolated from infected leaves, stems, tubers or fruits of potato or tomato. These were either collected locally in North Wales, or supplied by ADAS, the MAFF Plant Health Inspectorate, the Potato Marketing Board, and also several amateur growers across England and Wales. Dependence upon such sources meant that sampling was not highly structured; however, a reasonable geographical spread of isolates was obtained in most years.

For isolation, small pieces of tissue from the edge of the lesion were placed onto rye agar supplemented with RAN, in 9 cm Petri dishes, and transferred to fresh medium when growing colonies of *P. infestans* could be seen, usually after 3–5 days.

Potato plants

Seed tubers of cultivars Home Guard, Maris Piper and Cara were obtained from commercial suppliers. Stirling was obtained from the National Institute of Agricultural Botany, Cambridge, CB3 0LE. Plants were grown in 5 L pots with John Innes no. 1 compost, and were fertilised weekly with 1:1:1 N:P:K fertiliser (Vitax Ltd, Colville, Leics., LE67 3DE). The glasshouse was maintained at a minimum of 16 °C, with mean recorded temperatures of 17–18 °C in the winter months and 19–21 °C in the summer months, reaching as high as 29 °C on a few days. Supplementary lighting from 400 W high pressure sodium lamps was provided, with a 16 h photoperiod. Compound leaves for aggressiveness tests were harvested from 7–8 week old plants (at the flower bud stage) using only fully expanded, unblemished leaves.

Tests for mating type

These were carried out on rye A agar in 9 cm Petri dishes. A strip of approx. 5 $\times$ 15 mm was taken from the unknown colony and placed between 5 mm square agar plugs of known A1 and A2 isolates. After incubation for 7 days at 18 °C in the dark the colonies were scored by examining whether oospores were formed with the A1 or the A2 tester isolate.

Tests for metalaxyl insensitivity

Isolates were plated onto rye A agar supplemented with 10 μg ml^{-1} technical grade metalaxyl, and onto unamended rye A agar, in 9 cm Petri dishes. Three unknown isolates were used for each dish, together with an isolate known to be metalaxyl insensitive. After 7 days the diameter of the colonies was recorded. Growth was compared on metalaxyl and control plates. Isolates which showed growth on metalaxyl which was 60% or more of that on control plates were scored as insensitive, while those with less than 5% of

normal growth on metalaxyl were scored as sensitive, and those from 5 to 60% were scored as intermediate.

Mitochondrial DNA type

DNA was extracted using the following method (G.W. Griffith, pers. comm., adapted from method of Sahgai-Maroof et al., 1984), which used cubes (0.5 cm^2) of mycelia-covered agar from colonies on rye agar or rye agar with RAN. The age of these colonies was found to be immaterial, as good results were obtained with cultures several months old. The agar pieces were snap frozen with liquid nitrogen, then incubated in 800 μl CTAB extraction buffer (2% CTAB [hexadecyltrimethylammonium bromide]; 1.4 M NaCl; 100 mM Tris-HCl, pH 8.0; 20 mM EDTA) at 65 °C for 1 h, with agitation every 15 min. The samples were extracted once with chloroform. Isopropanol (0.6 volumes) was added to the resulting aqueous sample, the DNA pellet was collected after centrifugation and was rinsed in 70% ethanol, dried, and resuspended in 100 μl TE (10 mM Tris, 1 mM EDTA, pH 8).

The resulting DNA samples were exposed to a two-stage process, using a pair of specific polymerase chain reaction (PCR) primers designated PiMtF2 and PiMtR2 (G.W. Griffith and D.S. Shaw, pers. comm.) to produce a 1070 bp product which, when cut with the restriction enzyme *Msp* I, showed characteristic patterns for each of the three mtDNA genotypes Ia, Ib and IIa described by Carter et al. (1990). For PCR amplification, the following 'master mix' was made up (final concentrations): dNTPs, 200 μM each; $MgCl_2$, 2.75 mM; primers, 0.325 μM; bovine serum albumin, 160 $\mu g\ ml^{-1}$; 1 × Thermo buffer; *Taq* DNA polymerase 1U. Primers were synthesised by Cruachem Ltd., Glasgow, G20 0UA; all other reagents were supplied by Promega Corporation Ltd., Southampton, SO16 7NS. 1 μl of the DNA prepared as above was added to 19 μl of the master mix in 0.5 ml microfuge tubes and overlaid with a drop of mineral oil. The tubes were then subjected to the following conditions, in a Techne PHC-2 thermal cycler (Techne Ltd., Cambridge, CB2 4PZ): 1 cycle of 94 °C for 90 s; 40 cycles of 94 °C for 40 s, 55 °C for 60 s, 72 °C for 90 s.

5 μl of the amplified product was digested in a volume of 20 μl for 2–3 h (or overnight) with restriction enzyme *Msp* I (Promega), and the DNA fragments separated by electrophoresis through a 2% agarose gel (Gibco BRL Ltd., Uxbridge, UB8 2YG) in 1 × TAE electrophoresis buffer (for 50 × TAE, 2 M Tris HCl, 50 mM EDTA, pH 8.0).

Leaf inoculations

Detached leaves (3 replicates) of each of the three 'test cultivars' (cvs. Maris Piper, Cara and Stirling) were inoculated on each of five leaflets with a 20 μl droplet of a sporangial suspension (2–3 × 10^4 sporangia per ml) which had been harvested from previously infected leaves of cv. Home Guard, a universally susceptible cultivar. Infected leaves were incubated in plastic salad boxes (Ashwood Plastics Ltd, London E14 0LN), which contained a double layer of blue paper tissue above a damp J-cloth (retail suppliers). They were incubated for 6 days at 18 °C, with a 16 h photoperiod of light supplied by eight 120 cm 36 W white fluorescent tubes, which provided 120–140 $\mu moles.m^{-2}.s^{-1}$ of photosynthetically active radiation.

Infection frequency was assessed by the proportion of inoculated leaflets which developed lesions, i.e. infection frequency was 0.6 if three of the five inoculated leaflets were infected. The number of spores per lesion was then automatically determined using a Coulter Counter (Coulter Scientific Instruments, Luton, LU3 3RH). A 'fitness index' (FI; = infection frequency × mean number of sporangia per lesion) was used as a measure of aggressiveness.

Statistical analysis

The FI data from aggressiveness trials were subjected to square root transformation before one- and two-factor Analysis of Variance (ANOVA), and least significant differences (LSD) were used for further comparison. The normality of the data was checked by calculating the correlation coefficient ($P < 0.05$) between residuals and normal scores. Where a large proportion of zero scores were obtained, particularly on the most resistant cultivar, Stirling, the data were not normal and non-parametric tests (Mann-Whitney or Kruskal-Wallis) were used instead of ANOVA. To allow comparison between separate experiments, three 'standard' isolates were used in each. These consistently showed the same ranking, even though the actual FI values varied between experiments. For each experiment, individual means (×) were transformed to a scale on which standard isolate I was 0.0 and standard III was 1.0, using the following formula:

$$\text{Transformed FI value} = \frac{\text{x} - \text{FI standard I}}{\text{FI standard III} - \text{FI standard I}}$$

The various means were then displayed graphically to allow comparison of isolates with respect to mat-

Table 1. Mating type, response to metalaxyl and mitochondrial DNA (mtDNA) type for isolates of *P. infestans* collected in England and Wales between 1978 and 1995

Year	Mating type			Response to metalaxyl			mtDNA type		
	A1	SF[3]	A2	Sensitive	Intermediate	Insensitive	Ib	Ia	IIa
1978	4 (100)[1]	0 (0)	0 (0)	3 (75)	1 (25)	0 (0)	4 (100)	0 (0)	0 (0)
1981	3 (75)	0 (0)	1 (25)	4 (100)	0 (0)	0 (0)	1 (25)	3 (75)	0 (0)
1982[2]	27 (79)	0 (0)	7 (21)	29 (85)	5 (15)	0 (0)	22 (67)	9 (27)	2 (6)
1983	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
1985	21 (81)	1 (4)	4 (15)	12 (46)	11 (42)	3 (12)	0 (0)	22 (85)	4 (15)
1986	11 (61)	0 (0)	7 (39)	10 (56)	1 (6)	7 (39)	1 (5)	14 (74)	4 (21)
1987	7 (50)	0 (0)	7 (50)	11 (79)	0 (0)	3 (21)	0 (0)	10 (71)	4 (29)
1993	201 (97)	0 (0)	6 (3)	164 (79)	19 (9)	24 (12)	0 (0)	70 (92)	6 (8)
1994	138 (95)	4 (3)	3 (2)	63 (43)	20 (14)	62 (43)	0 (0)	75 (93)	6 (7)
1995	162 (99)	0 (0)	2 (1)	56 (34)	66 (40)	42 (26)	1 (2)	43 (96)	1 (2)

[1] Figures in parentheses are percentages.
[2] Samples from 1982 were collected exclusively from Anglesey, North Wales.
[3] Self-fertile phenotypes producing oospores in the absence of A1 or A2 testers.

ing type, mtDNA type and metalaxyl phenotype. The 'intermediate' standard isolate II was shown in the graphs to give an indication of the variation between experiments. Not all of the experiments have results plotted for all three of the cultivars, because, where the standards I and III were not significantly different from each other (e.g. experiments 3, 8, 9 and 11 on cv. Maris Piper) the data were discarded.

Results

Mating type, metalaxyl insensitivity, mitochondrial DNA type

The data shown in Table 1 for England and Wales were obtained from isolates in liquid nitrogen storage (1978 to 1987), and from isolates collected from blighted tissue between 1993 and 1995. Isolates with the mitochondrial DNA haplotype Ib were not recorded after 1982 except for one isolate in 1986 and another in 1995. In this period, type Ia predominated, with a smaller proportion of type IIa isolates. Mixtures of the two types occurred on several occasions. For example, in 1993 three out of four leaf samples taken from a single field in Gwynedd, North Wales, yielded type Ia isolates, whilst isolates from the fourth were type IIa. In a second field, also in Gwynedd, sampled in 1994, six leaf samples contained type Ia isolates, one contained type IIa isolates, and one contained both types Ia and IIa. On other occasions, both type Ia and type IIa isolates were found together in a tomato leaf sample from Gwynedd, in a potato leaf sample from Cambridgeshire and in a single potato tuber from Gwynedd. However, for each of the 23 other samples from England and Wales (between 1993 and 1995) where more than one isolate was tested, only type Ia was recorded. Type IIa isolates occurred at a low frequency without being confined to any specific sampling area, and their presence was also unrelated to potato cultivar, tissue (stem, leaf or tuber) or month of sampling, and they were as likely to be found in tomato as potato.

The proportion of A2 isolates (1 to 3%) found from 1993 to 1995 (Table 1) was considerably lower than that reported in the surveys of 1985 to 1988, where the percentage A2 varied between 6.7 and 15.1 (Shattock et al., 1990). The mitochondrial DNA type of isolates (Ia or IIa) was not significantly correlated with mating type ($P < 0.05$); although none of the 'old' type Ib isolates was A2 mating type.

All three mtDNA types differed in their response to metalaxyl (Table 2). Type Ia isolates constituted the highest proportion of insensitive isolates, whilst fully insensitive phenotypes were not observed in type Ib isolates. Type IIa isolates differed significantly from type Ia isolates ($P < 0.05$) in having a smaller proportion of insensitive phenotypes, although their proportion of intermediate phenotypes was larger. Overall, the proportion of metalaxyl-insensitive isolates increased from 1982 onwards (Table 1).

Table 2. Chi-square contingency table for distribution of metalaxyl phenotypes in *P. infestans* isolates of mtDNA type Ia, Ib and IIa, collected from England and Wales between 1978 and 1995

mtDNA type	Response to metalaxyl			
	Sensitive	Intermediate	Insensitive	Totals
Ib	19	7	0	26
	(73)[1]	(27)	(0)	(100)
Ia	123	34	71	228
	(54)	(15)	(31)	(100)
IIa	14	9	3	26
	(54)	(35)	(11)	(100)
Totals	156	50	74	280
	(56)	(18)	(26)	(100)

$\chi^2 = 18.9$, df = 4, $P < 0.001$.
[1] Figures in parentheses are percentages.

Aggressiveness of isolates

The data for the first of the experiments are shown in full in Table 3. FI readings were generally lower on cv. Cara than on cv. Maris Piper, and much lower on cv. Stirling than on either of the other two cultivars. This was as expected from the differing race non-specific resistance of the three cultivars, upon which basis they had originally been chosen for study. However, a significant interaction ($P < 0.05$) was observed between isolate and cultivar effects when 2-factor ANOVA was carried out, for this and for ten out of the 12 subsequent experiments summarised in Figures 1, 2 and 3 (experiments 3 and 11 being the exceptions). Thus many isolates, whilst showing high levels of sporulation on cv. Maris Piper, were unable to infect cvs. Cara and/or Stirling, whilst others of similarly high aggressiveness on Maris Piper were able to infect both the other cultivars (though usually with reduced FI).

Figures 1, 2 and 3 illustrate that there was an overall trend for isolates from more recent years to show higher FI relative to the standard isolates. This is likely to be related to the source of the isolates: those from the years before 1993 had been recovered from liquid nitrogen storage, which may have affected their pathogenicity. Thus only isolates of comparable age and origin were compared within each experiment (Table 4). Single-factor ANOVAs were used to investigate possible effects of isolate mating type, mtDNA type and metalaxyl phenotype (shown in Figures 1, 2 and 3 respectively). Effects of origin of host material (potato leaf, tuber, or tomato) were also tested in separate comparisons; however, no consistent, significant effects ($P < 0.05$) were observed (most of the samples were from potato leaves).

A significant effect ($P < 0.05$) of mating type was observed in several experiments and on all three test cultivars, but was not consistent between cultivars. On cv. Maris Piper, A1 isolates from 1982 showed significantly higher FI than A2 isolates from the same year (experiments 1 and 2); although the single A2 isolate (from 1985) tested in experiment 4 was of higher FI than the A1 isolates (Figure 1). No significant effects of mating type were found in subsequent years, in which only a few A2 isolates were available for testing. In the case of cv. Cara, by contrast, A2 isolates from 1982 and 1986/87 were of significantly higher FI than A1 isolates from the corresponding years (experiments 1, 2 and 5; Figure 1). Mating type effects could not be statistically determined on cv. Stirling, because of the large number of zero scores obtained (for both A1 and A2 isolates).

Isolates of different mitochondrial DNA type also showed differing relative FI on the three test cultivars. Results for cv. Maris Piper were inconsistent: 'old' type Ib isolates from 1978, 1981 and 1983 (Table 3) were found to have lower FI than 'new' type Ia or IIa isolates, but the reverse was found when the same comparison was carried out with isolates from 1982 (experiments 1 and 2; Figure 2a). Clearer differences were observed on cv. Cara, where type Ib isolates showed significantly lower FI than 'new' type Ia or IIa isolates in every experiment where significant differences were observed (Table 3; Figure 2b, experiments 1, 2, 4 and 5). On cv. Stirling, FI scores for type Ib isolates were uniformly zero (except for one isolate from 1981; Table 3), preventing statistical analysis from being carried out (zero scores on cv. Stirling were only observed in 16 of the 36 type Ia and IIa isolates tested from the same years).

Differences were also observed in some experiments between the two 'new' mtDNA types, Ia and IIa. On cv. Maris Piper, the FI of type IIa isolates from 1985 was significantly higher than the FI of type Ia isolates (experiment 4, Figure 2a); and this was also the case for isolates from 1982 on cv. Cara (experiment 2, Figure 2b). However, the reverse was true (type Ia isolates showing higher FI than type IIa isolates) when the 1985 isolates were tested on cv. Cara (experiment 4, Figure 2b), and also with isolates from 1993 tested on cv. Stirling (experiment 6, Figure 2c). However, the

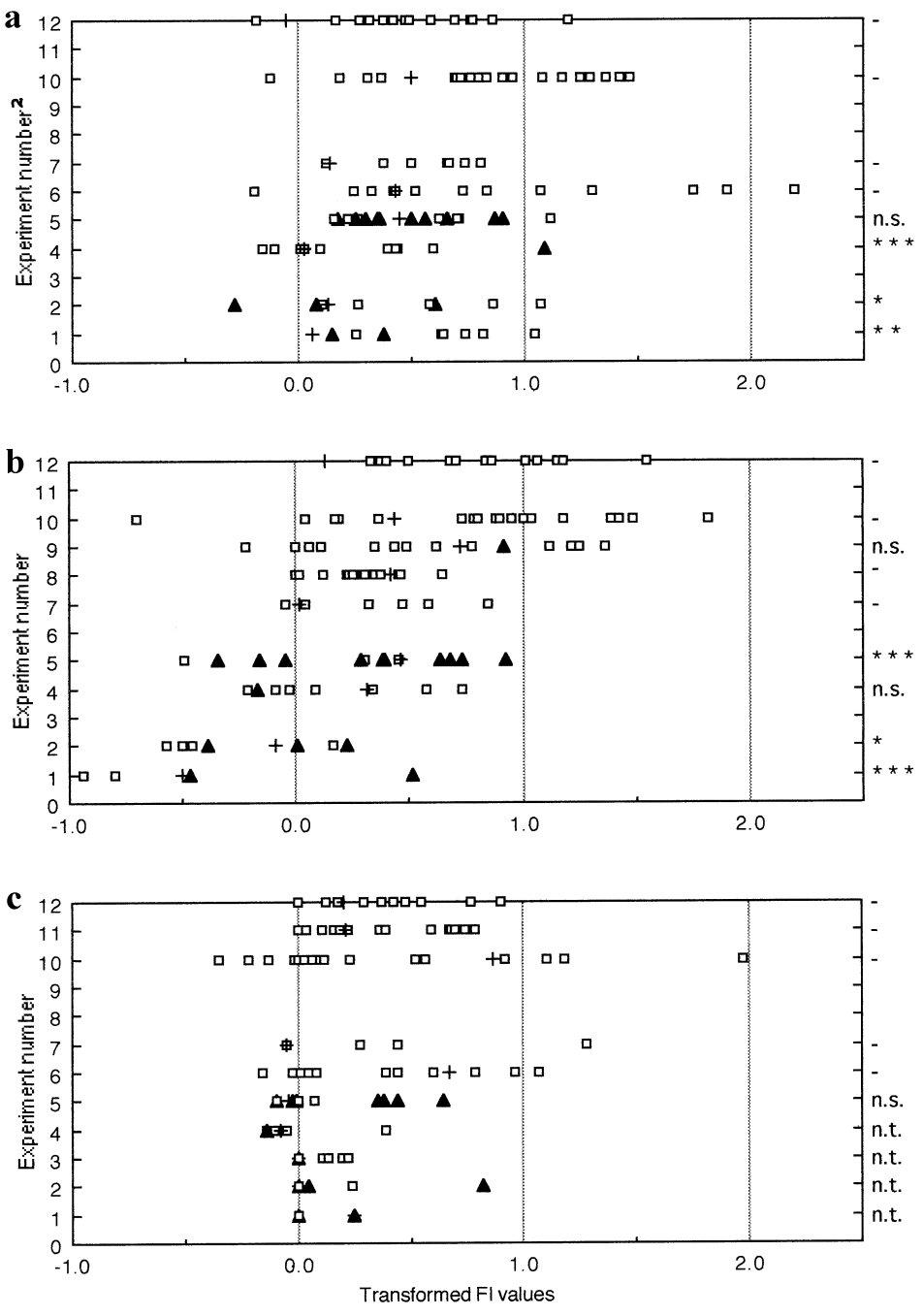

Figure 1. Summary of mean transformed 'fitness index' (FI) values[1] for A1 and A2 mating type isolates of *P. infestans* on detached leaves of cvs. (a) Maris Piper, (b) Cara and (c) Stirling. [1]Individual means were transformed as described in text, to a scale on which 'standard' isolate I (low aggressiveness) was 0.0 and 'standard' isolate III (high aggressiveness) was 1.0. 'Standard' isolate II (intermediate aggressiveness) is shown by the symbol + for each experiment. [2]Number of experiment as listed in Table 4. □ = A1 mating type; ▲ = A2 mating type. ∗, ∗∗, ∗∗∗ indicate significant differences of P<0.05, P<0.01 and P<0.001 respectively between isolates of A1 and A2 mating type. n.s. = not significant at P<0.05. Calculated using one-factor ANOVA for all experiments except (b) 1 and (c) 5, where Kruskal-Wallis test was used. n.t. indicates effects of mtDNA or mating type could not be tested by either ANOVA or non-parametric tests, because of large number of zero values. – indicates isolates not tested because only one type was included in experiment.

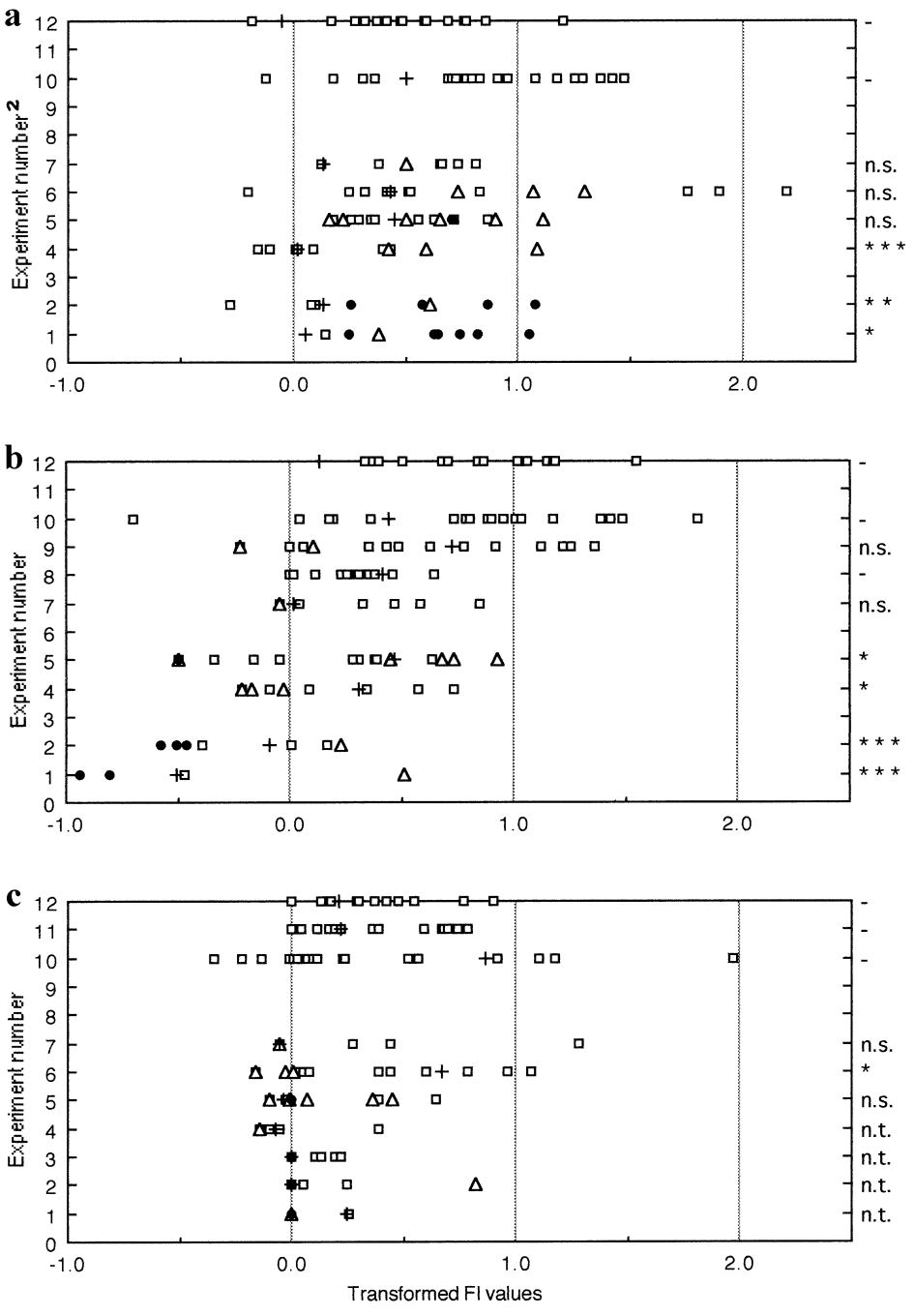

Figure 2. Summary of mean transformed 'fitness index' (FI) values[1] for 'old' (mtDNA type Ib) and 'new' (mtDNA type Ia and IIa) isolates of *P. infestans* on detached leaves of cvs. (a) Maris Piper, (b) Cara and (c) Stirling. [1]Individual means were transformed as described in text, to a scale on which 'standard' isolate I (low aggressiveness) was 0.0 and 'standard' isolate III (high aggressiveness) was 1.0. 'Standard' isolate II (intermediate aggressiveness) is shown by the symbol + for each experiment. [2]Number of experiment as listed in Table 4. ● = mtDNA type Ib; □ = mtDNA type Ia; △ = mtDNA type IIa. ∗, ∗∗, ∗∗∗ indicate significant differences of P<0.05, P<0.01 and P<0.001 respectively, between isolates of different mtDNA type. n.s. = not significant at P<0.05. Calculated using one-factor ANOVA for all experiments except (b) 1 and (c) 5, where Kruskal-Wallis test was used. n.t. indicates effects of mtDNA or mating type could not be tested by either ANOVA or non-parametric tests, because of large number of zero values. – indicates isolates not tested because only one type was included in experiment.

Table 3. 'Fitness index' (FI) for two of the three 'standard' isolates of *P. infestans* from 1993, together with eight isolates from 1978, 1981 and 1983, inoculated onto leaves of cvs. Maris Piper, Cara and Stirling and scored as described in text

Isolate[1]	Origin	mtDNA type	Mating type	Metalaxyl phenotype	FI[2] M. Piper	Cara	Stirling
93.100(I)	Cornwall	Ia	A2	Insensitive	n.t.[3]	n.t.	n.t.
93.28.2(II)	Gwynedd	"	A1	Sensitive	118	143	30
93.106.1(III)	Somerset	"	"	"	236	228	159
78.6	Gwynedd	Ib	"	"	127	0	0
78.18	Avon	"	"	Intermediate	26	0	0
78.24	Gwynedd	"	"	Sensitive	174	128	0
81.2	"	"	"	"	210	238	4
81.5	"	Ia	"	"	276	192	81
81.9	"	"	A2	"	214	192	0
83.2	"	"	A1	"	319	285	66
83.6	"	"	"	"	102	133	37
					LSD[4]	72.1	

[1] Isolates 93.28.2 (II) and 93.106.1 (III) were used in all subsequent experiments as 'standard' isolates of intermediate and high aggressiveness, respectively. Isolate 93.100 was selected later as standard I, of low aggressiveness, and was not included in this experiment.
[2] Square root transformation of FI (FI = infection frequency $\times$ mean number of sporangia per lesion).
[3] n.t. = not tested.
[4] Least significant difference (LSD; $P < 0.05$) for comparison of individual means, from 2-factor ANOVA; $P < 0.001$ for cultivar and isolate effects, and cultivar $\times$ isolate interaction.

Table 4. Summary of origin of isolates used in experiments 1 to 12

Experiment[1]	Year of collection	Locality	Number of isolates[2]
12	"	ware tubers, Egypt, Cyprus	15
11	"	" , Morocco, Israel, Cyprus	17
10	1995	2 sites, S. England & S. Wales	20
9	"	various sites, N. Wales	18
8	1994	" , N. & S. Wales	18
7	" , 1994	" , England & Wales	10
6	1993	N. Wales	14
5	1986, 1987	various sites, England & Wales	17
4	1985	"	10
3	" , 1985	single field, Anglesey, various counties England	10
2	"	single field, Anglesey (N. Wales)	8
1	1982	"	8

[1] Experiment number, corresponds to numbers on Y-axis of Figures 1, 2 and 3.
[2] Number of isolates tested, not including 'standard' isolates I, II and III which were tested in every experiment.

relatively small numbers of type IIa isolates included in any one experiment make these results inconclusive.

Metalaxyl-sensitive isolates were generally more aggressive than fully metalaxyl-insensitive isolates on all three cultivars (Figure 3a, experiment 7; Figure 3b, experiment 7, 8 and 9; Figure 3c, experiment 7). The large number of zero scores on cv. Stirling in experiments 1–5 (particularly for metalaxyl-insensitive and -intermediate isolates) prevented statistical analysis from being carried out; however, fewer zero scores were recorded for the sensitive isolates. Isolates of intermediate phenotype frequently showed higher FI than fully-insensitive isolates (Figure 3b, experiments 8, 9 and 10; Figure 3c, experiment 10). Interestingly, isolates of intermediate phenotype collected in 1995 (from imported ware tubers) were more aggres-

sive (higher FI) than metalaxyl-sensitive isolates from the same source. These isolates were also particularly aggressive in comparison with most isolates from England and Wales, particularly against cv. Stirling (Figure 1c, 2c and 3c).

Discussion

Analysis of the mitochondrial DNA types of both stored and recently collected isolates of *P. infestans* from England and Wales indicated that the present population consists mainly of isolates of mitochondrial DNA type Ia, along with a small proportion (less than 10%) of type IIa isolates. The 'old' type Ib isolate had constituted 100% of the (small) sample from 1978, and 67% of the sample from 1982. A single type Ib isolate was recorded in 1986, and just one (from tomato) in 1995. The 1986 isolate was obtained from CIBA Agriculture, Whittlesford, Cambridge, and may represent a tester strain used in fungicide trials. Other agrochemical companies are known to have retained such strains over many years and they are also type Ib isolates, i.e. relict forms (unpublished data). Analysis of a selection of isolates from Northern Ireland and the Republic of Ireland indicated that these populations also contained 'new' type Ia and type IIa isolates, although the sample size was too small to rule out the presence of the 'old' type Ib (unpublished data). Type Ib was, however, found to be present in Germany in 1994; of 12 isolates tested, 7 were type Ia, 2 were type IIa and 3 were type Ib. These findings are in agreement with the general consensus, based on other molecular markers such as DNA fingerprinting and isozymes, that the 'old' genotype isolates (i.e. those present before the first finding of A2 isolates outside Mexico) have been, or are being, displaced by 'new' genotypes (Spielman et al., 1991; Fry et al., 1993; Fry and Goodwin, 1995).

Overall, the results based upon infection frequency and sporulation of late-blight isolates on detached leaves indicated that the 'old' type Ib isolates were less pathogenic than the 'new' type Ia or IIa, on the more resistant cultivars Cara and Stirling. This does not necessarily indicate that the 'new' genotypes are better adapted to these cultivars; it is equally likely that differences in aggressiveness were less distinguishable on the susceptible cultivar Maris Piper (where results were less consistent). However, interpretation was complicated by the frequent discrepancy in results between the different cultivars, and the frequent occurrence of significant cultivar x isolate interactions when the data were tested by 2-factor ANOVA. This strongly suggests the presence of race-specific resistance. Although the three cultivars had been chosen for their differing race-non-specific resistance and absence of R-genes, it has since been confirmed that cv. Cara contains the major resistance gene R1 (Connolly et al., 1995). It is possible, therefore, that some of the pathogenicity differences on cv. Cara observed in this study may be due to differential gene-for-gene interactions (Al-Kherb et al., 1995). This would explain some of the differences in the results obtained on this cultivar, when compared with cvs. Maris Piper and Stirling.

An overall increase in FI in isolates from 1982 to 1995 relative to the 'standard' isolates was observed when the data were standardised and displayed graphically (Figures 1, 2 and 3). This may reflect a deterioration in the pathogenicity of isolates from the years before 1993, which had been stored in liquid nitrogen. It was found that such storage, even for only a few months, decreased the aggressiveness of recently collected isolates (data not shown). This finding shows the danger of comparison between isolates from different years, but does not affect the distinction between 'old' and 'new' isolates within sampling years.

Although enhanced aggressiveness may be an important factor accounting for the displacement of 'old' by 'new' genotypes, the occurrence of complete insensitivity to metalaxyl in the latter suggested that agricultural selection is also important (Spielman et al., 1991). Although some 'old' type isolates from Europe have been reported as metalaxyl 'resistant' (Goodwin et al., 1996), the assay used was different from that employed in the present study. This finding may therefore be comparable with the 'intermediate' phenotype which was found here in six mtDNA type Ib isolates from 1978 and 1982. It is possible that these too would have been at a selective disadvantage if exposed to competition from 'new', immigrant isolates expressing full insensitivity to metalaxyl. Currently in North America, metalaxyl insensitivity is limited to a few 'new', immigrant clonal lineages which have largely and rapidly displaced the original, 'old' metalaxyl-sensitive strain (Goodwin et al., 1996).

The likely migration of 'new' genotypes to Europe in 1976, in potato tubers from Mexico (Niederhauser, 1991), occurred shortly before the commercial release of phenylamide fungicides. Phenylamide-insensitive isolates were first recorded in Europe in 1980, notably in The Netherlands (Davidse et al., 1981) and Ireland (Dowley and O'Sullivan, 1981). By 1985, the proportion of insensitivity in populations in England and

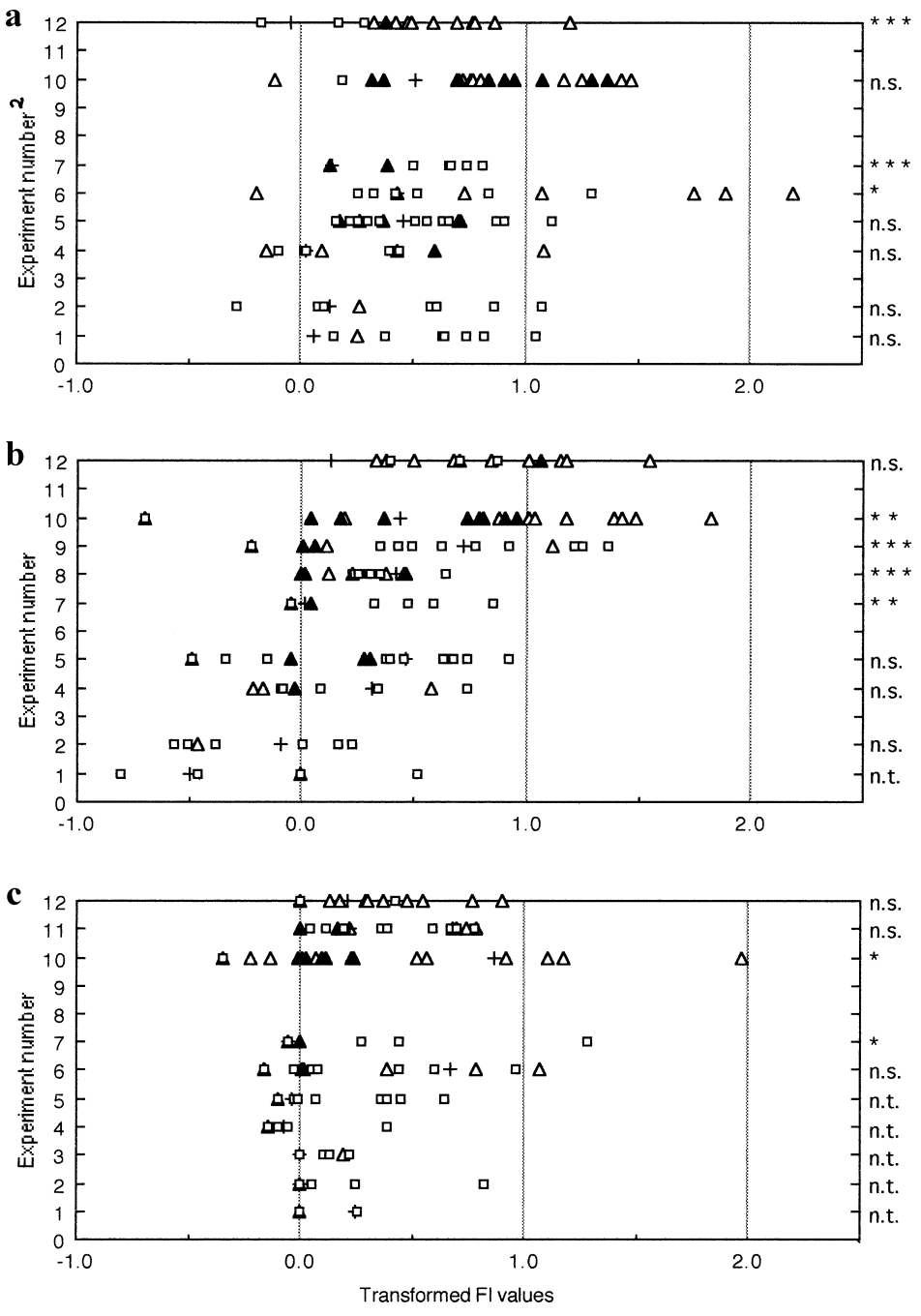

Figure 3. Summary of mean transformed 'fitness index' (FI) values[1] for metalaxyl-sensitive, intermediate and -insensitive isolates of *P. infestans* on detached leaves of cvs. (a) Maris Piper, (b) Cara and (c) Stirling. [1] Individual means were transformed as described in text, to a scale on which 'standard' isolate I (low aggressiveness) was 0.0 and 'standard' isolate III (high aggressiveness) was 1.0. 'Standard' isolate II (intermediate aggressiveness) is shown by the symbol + for each experiment. [2] Number of experiment as listed in Table 4. □ = metalaxyl-sensitive; △ = intermediate; ▲ = insensitive. ∗, ∗∗, ∗∗∗ indicates significant differences of $P<0.05$, $P<0.01$ and $P<0.001$ respectively, between isolates of different metalaxyl phenotype. n.s. = not significant at $P<0.05$. Calculated using one-factor ANOVA for all experiments except (b) 8 and 10 and (c) 7, 10 and 12, where Kruskal-Wallis test was used (or Mann-Whitney where only 2 factors present). n.t. indicates effect of metalaxyl phenotype could not be tested by either ANOVA or non-parametric tests, because of large number of zero values.

Wales was 41%, increasing to 60% in 1988 (Shattock et al., 1990). Since then, the occurrence of metalaxyl-insensitive isolates in England and Wales has remained high, such that 41%, 65% and 79% of samples collected in 1993, 1994 and 1995 respectively contained either fully insensitive or intermediate phenotypes. Over the same period (1993–1995), 51–58% of the ware crops in England and Wales have been treated annually with phenylamides (M.R. Thomas, pers. comm.).

Amongst this 'new' population, metalaxyl-insensitive isolates were found to be less pathogenic (lower FI on detached leaves of all three test cultivars) than sensitive isolates. This suggests that, as long as phenylamides are restricted to their current, controlled use (i.e. a maximum of five applications, as pre-pack mixtures with non-systemic protectant compounds, ceasing in early August), it is unlikely that fully insensitive isolates will completely displace sensitive isolates. Indeed, several reports suggest that insensitivity is not stable in the population, but instead is selected anew each year, increasing steadily as the season progresses but declining again over winter (Williams and Gisi, 1992; Dowley et al., 1995). A lack of fitness for metalaxyl-insensitive isolates has also been suggested by observations from Ireland and The Netherlands, where their frequency in the population declines when the fungicide is withdrawn (Dowley and O'Sullivan, 1985; Davidse et al., 1989). For European isolates this has been attributed to decreased sporulation on leaves (Dowley, 1987), or to poor survival in tubers over winter (Walker and Cooke, 1990; Staub, 1994). Decreased survival in tubers for metalaxyl-insensitive isolates was also reported from a study in Israel, although in this case the insensitive isolates were more aggressive on foliage than the sensitive isolates (Kadish and Cohen, 1992). The finding in the present study that isolates of intermediate phenotype isolated from imported Mediterranean ware tubers were more pathogenic than metalaxyl-sensitive isolates, also indicates that decreased pathogenicity is not an inevitable consequence of insensitivity to phenylamides, even though it appears to be so in England and Wales at present.

The two 'new' mtDNA types, Ia and IIa, were found to be similar with regard to their proportion of A1 and A2 mating types. In respect to their metalaxyl phenotype, both had a similar proportion of sensitive phenotypes, although more type Ia isolates were insensitive phenotypes than type IIa, whereas intermediate phenotypes were more common in the latter. Some of the detached leaf inoculations on cvs. Cara and Stirling indicated that type IIa isolates were less pathogenic than type Ia, but the reverse was found in other experiments cvs. Maris Piper and Cara. However, the numbers of type IIa isolates tested were too small to draw any real conclusions. Although genotypic analysis was not carried out for all of the isolates tested, recent RG57 'fingerprint' analysis of a selection of them has indicated at least seven different clones amongst type Ia isolates, whilst only one has so far been identified in the few type IIa isolates tested (unpublished data). Type IIa isolates were not confined to any particular geographical location, nor were they preferentially isolated from any one type of host material. Indeed, they were frequently found in the same sample as type Ia isolates, and on one occasion even from the same infected tuber.

Isolates of different mating type were also found to be extremely similar with regard to all characteristics tested. Results for pathogenicity were again dependent on cultivar, with A1 isolates appearing more pathogenic than A2 on cv. Maris Piper, whilst the reverse was true for cv. Cara (where results were more consistent). In spite of the lack of any clear evidence for phenotypic differences between A1 and A2 isolates, it appears that the proportion of A2 isolates in the population in England and Wales has been declining in recent years, as has been observed in other European countries e.g. the Republic of Ireland (O'Sullivan et al., 1995) and Poland (Sujkowski et al., 1994). Thus far, A2 mating type isolates have not been recorded among the 'new' genotypes in France (Andrivon et al., 1994) and the adjacent Channel Islands (unpublished data).

Although sexual reproduction is theoretically possible wherever A1 and A2 isolates occur (Drenth et al., 1994; Sujkowski et al., 1994; Goodwin et al., 1995), mechanisms other than sexual reproduction can produce rapid displacement of particular genotypes (see review by Goodwin, 1997). Late-blight conforms to the 'metapopulation' model, in which local populations, each having a finite life expectancy, are continually being replaced by migration from neighbouring populations (Burdon, 1993). The disappearance of such local populations need not necessarily reflect on their fitness, as populations of all pathogens will increase in favourable conditions, and decrease in adverse conditions. Thus the expansion of *P. infestans* populations by asexual reproduction during epiphytotics in the growing season, followed by the winter 'bottleneck', may mean that each year, even if envi-

ronmental conditions are identical, the pathogen populations that occur will vary enormously and erratically, depending entirely upon which few 'founder' isolates happened to survive the winter (Shaw, 1994). Such conditions will also, in the absence of sexual reproduction, tend to decrease the variability of populations. This may account for the observed decline in the frequency of A2 isolates in England, Wales and the Republic of Ireland, and may also mean that the rarer mitochondrial DNA type, IIa, will also decline in future years. Indeed, it may be that the genetic uniformity of the 'old' type Ib isolates came about not as a consequence of the uniformity of the founder population, but because the original population, however varied, lost its genetic variability and fitness over successive asexual generations (Goodwin, 1997).

Despite this, evidence has been found to suggest that some of the 'old' type Ib isolates may have recombined, either sexually or somatically, with the 'new' migrant strains. RG57 fingerprinting of a selection of type Ib isolates from 1978 and 1982 indicated (unpublished data) that they were identical with the 'old' clonal lineage designated elsewhere as US-1 or PO-1 (Goodwin et al., 1994; Sujkowski et al., 1994). In contrast, the single type Ib isolate collected in 1995 showed a completely different fingerprint pattern, more similar to that found in 'new' mtDNA type Ia isolates. Further sampling, and characterisation using a variety of molecular techniques, should reveal whether such combinations are a significant feature of the 'post-migration' population.

Local and long-distance migration of *P. infestans* populations, and their transport by human activity, is likely to continue to affect the structure of pathogen populations. In the present study, isolates from imported ware tubers from various Mediterranean countries were found to be highly aggressive, even against the high race-non-specific resistance of cv. Stirling. Although these infected tubers had been intercepted at the ports by Ministry Plant Health Inspectors, it is unlikely (however desirable) that all infected potato ware could ever be excluded by such means. The intra- and inter- continental movements of potato seed and ware tubers, and tomatoes, have introduced a constant, potential source of new inoculum. For example, the severe epidemics of recent years in North America are acknowledged to be the result of immigration of a succession of new clonal lineages (probably on tomato) from Mexico (Fry and Goodwin, 1995).

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