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ORIGINAL PAPER

Distinguishing major-gene from field resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) and selecting for high levels of field resistance

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Abstract Potato cultivar Stirling, which has a *Solanum demissum* derived R-gene and quantitative field resistance to late blight, was crossed with the susceptible cultivar Maris Piper to produce an F1 population from which three genotypes (94B13A29, 57 and 61) were backcrossed to Maris Piper. The F1 and backcross populations were assessed for resistance to simple race 1,4 (incompatible with Stirling's R-gene) and complex race 1,2,3,4,6,7 of *Phytophthora infestans* (compatible with R-gene) in whole plant glasshouse tests. The segregation results in the F1 generation with the simple race confirmed the presence of a single copy of the R-gene in Stirling, and the results with the complex race were consistent with Stirling having a high level of quantitatively inherited field resistance. Comparisons of the results with the simple and complex races apparently enabled F1 clones to be classified for the presence or absence of the R-gene and to be assessed for their level of quantitative field resistance. However, two out of the three backcrosses done to check classifications revealed unexpected findings: 94B13A29 had two copies of the R-gene as a result of double reduction, but was, as expected, susceptible to the complex race; and 94B13A57 had the R-gene (one copy) and it, and its offspring with the R-gene, had some resistance to the complex race, whereas those offspring without the R-gene were susceptible. Clone 94B13A61, as expected, lacked the R-gene and had moderate quantitative field resistance to both races. The implications are discussed for breeding potatoes with durable resistance to late blight.

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Introduction

Late blight disease of potato (*Solanum tuberosum*, a tetraploid which displays tetrasomic inheritance) first made its impact outside of Mexico in 1845 and 1846 when severe epidemics swept through North America and Europe and resulted in the Irish potato famine (Large 1940). The epidemics were caused by the asexual reproduction of the A1 mating type of the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary. Since 1984, new populations of *P. infestans*, comprising both mating types, have been spreading from Mexico to the rest of the world (Goodwin and Drenth 1997). There is therefore the possibility of sexual reproduction resulting in oospores which can over-winter in the soil and start epidemics earlier each season. Sexual reproduction may also allow the faster evolution of more virulent and aggressive strains of the pathogen. Chemical control is available and necessary for the many popular but susceptible cultivars which are widely grown. However, spraying is expensive, not always effective, and the new populations of *P. infestans* are often resistant to the widely used systemic fungicide metalaxyl (GILB 1999). The need for resistant cultivars is as great today as it was after the epidemics of 1845 and 1846. The challenge for breeders remains that of combining high levels of durable resistance with the yield, early maturity and quality required for commercial success (Bradshaw and Birch 2006).

The breeding strategy during the first half of the 20th century was utilisation of the major dominant R-genes which had been discovered in the Mexican wild species *Solanum demissum* (Muller and Black 1951). However, by

1968 in Britain and elsewhere it was clear that these R-genes would not provide durable resistance, either singly or in combination, owing to the evolution of new races of *P. infestans* (Malcolmson 1969). Cultivar Pentland Dell (with genes *R1, R2* and *R3*) entered production in Britain in 1963 when race 4 was the prevalent race of *P. infestans*, but succumbed to blight in 1967. Furthermore, in 1967 and 1968 races capable of overcoming the more recently discovered R-genes (*R5–R11*) were widely distributed in Britain, despite these R-genes not being present in common commercial varieties. As a consequence, many breeders started to select for quantitative field resistance, either by using races of *P. infestans* compatible with the R-genes present in their material, or by creating R-gene free germplasm so that screening could be done with any race (Toxopeus 1964; Black 1970; Wastie 1991; Ortiz 2001).

The new populations of *P. infestans* which have been spreading from Mexico since 1984 have prompted fresh screenings of germplasm collections for new sources of resistance and new R-genes have been found, mapped and cloned in species other than *S. demissum* (Bradshaw et al. 2006). There is much interest and debate over whether or not these R-genes will be durable, or can be deployed durably, for example in a multiline, produced by map-based cloning of different R-genes and independent *Agrobacterium*-mediated transformation of a popular but susceptible cultivar (Niederhauser et al. 1996; Huang 2005; Smilde et al. 2005).

It can be argued that the best breeding strategy is to combine R-genes with high levels of field resistance, so that the near immunity conferred by R-genes is utilised until they are defeated, then the high levels of field resistance come into operation together with any residual resistance contributed by the defeated R-genes (Stewart et al. 2003). It therefore seemed timely to re-examine how easily major-gene resistance can be distinguished from field resistance, and how best to select for high levels of field resistance. The cross between cultivars Stirling and Maris Piper was chosen for this work because Stirling possesses both a single copy of an *S. demissum* derived R-gene and high levels of field resistance (Bradshaw et al. 2004), whereas Maris Piper lacks R-genes and is moderately susceptible to late blight. The offspring were expected to show segregation for the R-gene and for field resistance. The aim was to see if clones could be classified for the presence or absence of the R-gene and for their level of field resistance by assessment with a compatible (able to overcome R-gene) and an incompatible (not able to overcome R-gene) isolate of *P. infestans*. Confirmation of the classification was sought for three clones by genetic analysis in which they were backcrossed to the susceptible parent Maris Piper. Two clones were chosen because they were thought to have moderate to high field resistance without the R-gene and the third one was chosen because it was thought to have the R-gene but no field resistance, i.e. the combinations not present in Stirling and Maris Piper. Although the R-gene in Stirling is probably *R7* (Bradshaw et al. 2004), this has not been confirmed (and is not essential for this experiment) and a diagnostic molecular marker for the R-gene is not available. Hence, the R-gene can only be detected by its expression and segregation.

Materials and methods

Crosses and plant material

Cultivars Stirling (female parent) and Maris Piper were crossed to produce an F1 population which was assessed for its resistance to foliage blight. Three F1 clones (94B13A29, apparently with R-gene but no field resistance, and 94B13A57 and 94B13A61, apparently with field resistance but no R-gene) were then backcrossed to Maris Piper (male parent) to produce three populations which were also assessed for their resistance to foliage blight. Seedlings of each population were raised in a glasshouse to provide seed tubers for establishing the populations in clonal form. The populations were maintained by planting three tubers of each clone in the glasshouse in subsequent years. Blight tests were done on 58 clones of the F1 population and 112, 149 and 88 clones, respectively, of the backcross populations with 94B13A29, 94B13A57 and 94B13A61 as parents. The whole of the F1 population was assessed for late blight resistance in 1999. Twenty F1 clones were then chosen for another research project and this allowed the repeatability of the assessment to be checked in tests done in 2000 and 2002. The first two backcross populations were assessed in 2003 and the third in 2004.

Experimental design

For each assessment, in spring or early summer, plants of each clone were raised in a glasshouse in a randomised complete block design with four replicates (one plant per clone in each replicate). Two plants (three where possible) of each parent and Stirling were included in each replicate. When the majority of plants were at the flower bud stage of growth (6 weeks after planting), two replicates were inoculated with an isolate of *P. infestans* which is compatible with Stirling (i.e. can overcome its R-gene) and the other two replicates with an isolate which is incompatible with Stirling.

Phytophthora infestans isolates

The Stirling compatible isolates (both ‘complex’ race 1,2,3,4,6,7) were 36.4.3 in 1999 and 99/23 in subsequent

years. The incompatible isolates (both 'simple' race 1,4) were 16.5.2 in 1999 and 2000 and 15.5.1 in subsequent years. All isolates were naturally occurring in Scotland. They were passaged through potato tubers to select for aggressiveness before use in the resistance tests. The reaction of each isolate with Stirling and Maris Piper was checked before use and its virulence characteristics were determined in a whole plant test using Black's differential series of R-gene-bearing clones (Black et al. 1953; Malcolmson and Black 1966; Malcolmson 1969).

Late blight resistance testing

The whole-plant glasshouse test developed by Stewart et al. (1983) was used for all assessments of resistance to late blight. *P. infestans* inoculum was prepared as described by Malcolmson (1976), using the zoospore suspension resulting from a sporangial suspension at an initial concentration of 14×10^3 sporangia/ml. A hand sprayer was used to apply the inoculum and the spray was allowed to fall evenly on the plants from above. The plants were dampened, inoculated and incubated for 24 h in a misted controlled environment cabinet at 15°C and 95–100% humidity, then kept at 15°C in a cooled glasshouse. Seven days after inoculation each plant was scored using Malcolmson's 1–9 scale of increasing resistance as illustrated by Cruickshank et al. (1982). Analyses of variance of the disease scores were done using GENSTAT 5 Release 3 (GENSTAT 5 Committee 1993).

In addition to the blight scores with the simple and complex races, an attempt was made to confirm which clones had inherited Stirling's major R-gene by comparing their reactions to these races. Clones were considered to be R-gene-free if there was no difference in response to the two races with all plants bearing spreading lesions. They were considered to have inherited the R-gene if the plants inoculated with the simple race were symptomless, or had only isolated necrotic lesions or spreading lesions on the oldest leaves alone, whilst those inoculated with the complex race had spreading lesions on all foliage.

Results

The F1 generation

Analysis of variance of the late blight scores in 1999 confirmed a large race × clone interaction ($P < 0.001$). With the simple race, 33 clones had a disease score of 8 or higher compared with 9 for cultivar Stirling and 25 clones had a range of scores from 2 to 7.5 (mean 5.34) compared with 3.5 for cultivar Maris Piper (Fig. 1a). The ratio of 33–25 clones was consistent with the segregation of a single copy of an R-gene as it is not significantly different from a 1–1 ratio (Chi-square = 1.10, $P > 0.05$), nor from a ratio of 27–31 (Chi-square = 2.49, $P > 0.05$) which would be expected with a coefficient of double reduction of one sixth (see Sect. "Discussion"). With the complex race, the disease scores were approximately normally distributed with a range from 1 to 7 (Fig. 1b). The range was 1–7 (mean 4.30) for the 33 clones thought to have the R-gene and 2–6.5 (mean 4.00) for the 25 clones thought to lack the R-gene. The means of the two groups were not statistically different ($P > 0.05$). Five clones (including 94B13A29) with the R-gene were as susceptible as Maris Piper (score 2.25) when inoculated with the complex race, and five clones (including 94B13A57 and A61) thought to lack the R-gene were moderately resistant (5.5–6.5 compared with 7.25 for Stirling).

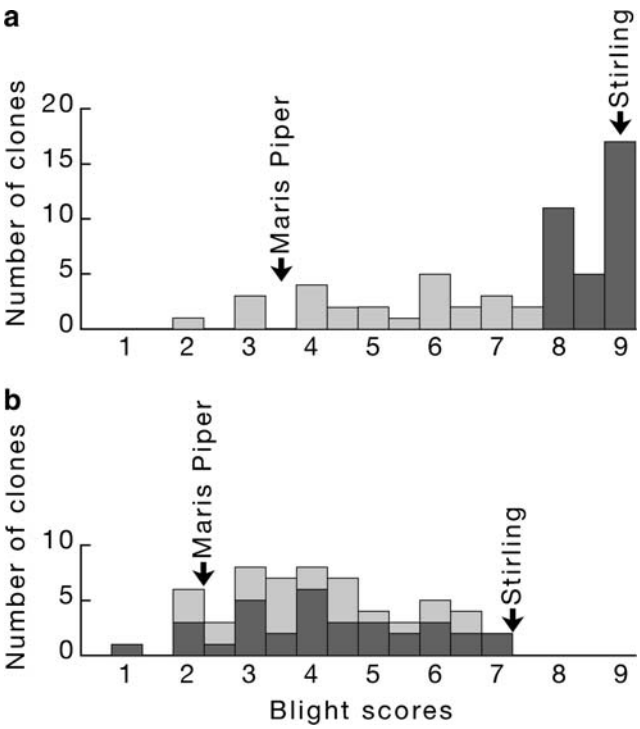

Fig. 1 Blight scores (mean of two replicates) on 1–9 scale of increasing resistance for the 58 F1 clones from the Stirling × Maris Piper cross when assessed with **a** simple race (1,4) and **b** complex race (1,2,3,4,6,7) of *P. infestans*. *Dark bars* resistant to simple race, *light bars* susceptible to simple race

The results for the 20 F1 clones included in all 3 years of blight testing are shown in Table 1 together with the attempt to classify them into those with and without the R-gene on the basis of the 1999 results. Analysis of variance confirmed a large race × clone interaction ($P < 0.001$) as well as a year × clone ($P < 0.001$) and a year × race × clone ($P < 0.01$) interaction. Maris Piper was moderately susceptible (scores less than 5) in all of the assessments. In contrast, Stirling had no visible symptoms (score 9) when inoculated with the simple race and good resistance to the

Table 1 Blight scores (mean of two replicates) on a 1–9 scale of increasing resistance for 20 F1 clones from the cross Stirling × Maris Piper, assessed in 3 years with a complex (1,2,3,4,6,7) and simple (1,4) race of *P. infestans* (average SED for pair of mean values 0.75), and assumed presence (R) and absence (r) of R-gene from 1999 results

	1999			2000		2002	
	Complex	Simple	R-gene	Complex	Simple	Complex	Simple
Stirling	7.25	9.00	R	8.00	9.00	6.50	9.00
94B13A3	6.0	8.5	R	7.0	9.0	7.0	9.0
94B13A17	6.0	9.0	R	7.0	9.0	7.0	9.0
94B13A18	6.5	8.0	R	6.5	8.5	6.5	8.5
94B13A36	6.5	9.0	R	6.5	9.0	7.5	9.0
94B13A10	2.5	8.0	R	3.5	8.0	5.5	9.0
94B13A24	3.0	9.0	R	7.0	9.0	6.0	9.0
94B13A29[a]	2.0	8.0	R	4.0	9.0	3.0	9.0
94B13A49	1.0	9.0	R	4.0	8.0	5.0	9.0
Maris Piper	2.25	3.50	r	4.50	4.50	4.65	4.33
94B13A27	3.0	3.0	r	4.0	6.5	5.5	6.5
94B13A28	2.0	3.0	r	2.5	3.0	–	4.0
94B13A60	2.0	3.0	r	3.0	3.0	4.5	5.5
94B13A64	2.0	2.0	r	2.0	3.0	3.5	5.5
94B13A35	4.5	6.0	r	6.0	8.0	8.0	8.5
94B13A41	5.5	7.5	r	4.0	8.0	7.0	8.0
94B13A43	2.5	6.0	r	3.5	6.0	4.5	7.0
94B13A47	6.5	7.5	r	5.0	7.5	4.5	–
94B13A53	4.5	6.0	r	6.5	8.0	6.0	8.5
94B13A57[a]	6.5	6.5	r	7.0	6.5	6.5	8.0
94B13A59	4.5	7.0	r	6.5	8.5	6.0	8.5
94B13A61[a]	6.0	7.0	r	7.0	6.5	4.0	–

[a] Selected clones

complex race (scores 6.5–8). Some of the F1 clones gave consistent results. Clones 3, 17, 18 and 36 reacted in a similar way to Stirling, with scores greater than or equal to 8 with the simple race and scores of 6–7.5 with the complex race. It was assumed that they combined the R-gene with moderate to high field resistance, but this was not checked as we knew how Stirling behaves in crosses. Clones 27, 28, 60 and 64 reacted in a similar way to Maris Piper with average scores over all assessments of less than 5 and individual scores in the range 2–6.5. It was assumed that they lacked the R-gene and had moderate to high field susceptibility, but this was not checked as we were not interested in susceptibility. Clones 10, 29 and 49 were resistant to the simple race (scores greater than or equal to 8) and susceptible to the complex race (scores less than or equal to 5.5). It was assumed that they had the R-gene but lacked field resistance. Clone 29 was chosen as typical of these for the backcross to check this hypothesis. The scores for clones 35, 57 and 61 were similar for the simple and complex race with clone 57 moderately resistant in all assessments (scores of 6.5–8). It was assumed that they lacked the R-gene but had useful levels of field resistance. However, with clone 35 in the year 2000 there were a few spreading lesions on the youngest leaves with the complex but not the simple race. Hence, clones 57 and 61 were chosen for the backcrosses to check the hypothesis. For the other clones there were race differences in the blight scores in one or more years and these raised doubts about the 1999 classification. Do clones 41, 43, 53 and 59 have the R-gene but it is not always highly expressed, and whilst clone 24 has and clone 47 lacks the R-gene, what is their level of field resistance?

Backcross 94B13A29 × Maris Piper

An analysis of variance of the blight scores for the backcross clones revealed a large race × clone interaction (variance ratio 4.00, $P < 0.001$). With the simple race, 84 clones had a mean score of 7 or more and 22 had a score of 5.5 or less, compared with Stirling at 9, 94B13A29 at 8.75 and Maris Piper at 3.33 (Fig. 2a). For the 22 clones with a score of 5.5 or less, there was good agreement between the scores with the simple and complex race, all clones being clearly susceptible. There were six clones which could not be classified because they scored 6 or 6.5 with the simple race as a result of a disagreement between the two replicates with one resistant and the other susceptible. The ratio of 84 resistant to 22 susceptible clones was not significantly different from the 5–1 ratio (Chi-square = 1.275, $P > 0.05$) expected if the parent was duplex for the R-gene, and was

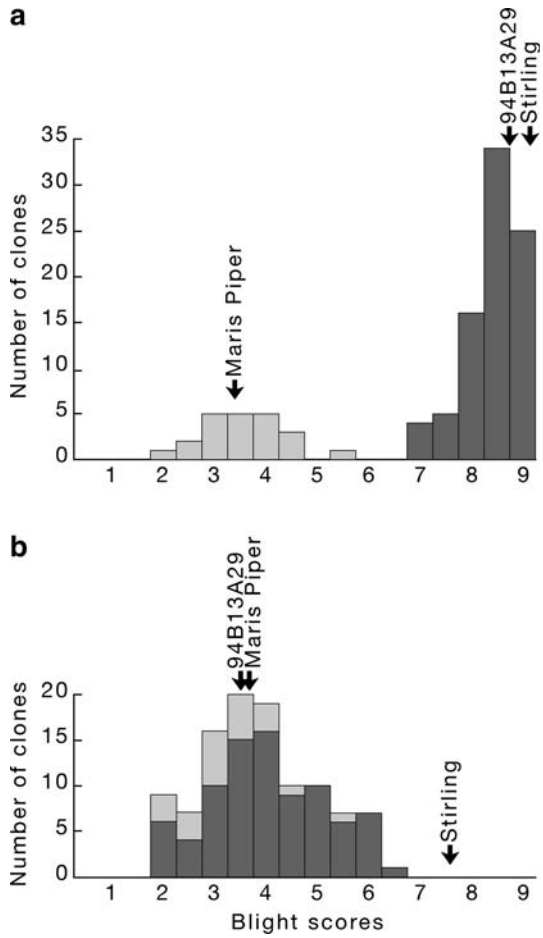

Fig. 2 Blight scores (mean of two replicates) on 1–9 scale of increasing resistance for the 106 clones from the 94B13A29 × Maris Piper backcross when assessed with **a** simple race (1,4) and **b** complex race (1,2,3,4,6,7) of *P. infestans*. *Dark bars* resistant to simple race, *light bars* susceptible to simple race

an even closer fit to a ratio of 82.44–23.55 (Chi-square = 0.132, $P > 0.05$) which would be expected with a coefficient of double reduction of one-sixth. With the complex race, the range of scores was 2–6.5 with a mean of 3.84 compared with Stirling at 7.67, 94B13A29 at 3.5 and Maris Piper at 3.83 (Fig. 2b). The mean values for the 84 and 22 clones with and without the R-gene were 4.04 and 3.23 (SED = 0.256), respectively, a statistically significant difference ($P < 0.01$).

Backcross 94B13A57 × Maris Piper

An analysis of variance of the blight scores for the backcross clones revealed a race × clone interaction (variance ratio 2.53, $P < 0.001$). With the simple race the range of scores was 2–8.5 with a mean of 5.33 compared with 9 for Stirling, 7.5 for 94B13A57 and 3.67 for Maris Piper (Fig. 3a). However, there was a bimodal distribution with 71 clones scoring less than 5 (mean 3.32) and 78 clones more than 5 (mean 7.16), numbers that were not significantly different from a 1–1 ratio (Chi-square = 0.329, $P > 0.05$), nor from the 80.7–68.3 ratio (Chi-square = 2.54, $P > 0.05$) expected with a single copy of an R-gene and a coefficient of double reduction of one sixth. Two clones scoring 4.5 and two scoring 5.5 may have been misclassified as there were large differences between the two replicates, but treating them as missing values and repeating the analyses had a negligible effect on the results. With the complex race the range of scores was 1–8 with a mean of 3.42 compared with 7.5 for Stirling, 5.75 for 94B13A57 and 2.5 for Maris Piper (Fig. 3b). However, this time the distribution was skewed and not obviously bimodal. The outlier with a score of 8 was based on a single plant. The 71 clones which scored less than 5 with the simple race had similar scores of less than or equal to 5 with the complex race, and hence were susceptible to both races. Interestingly, their average score of 2.52 with the complex race was significantly ($P < 0.001$) less than the corresponding average score of 4.23 (SED = 0.181) for the 78 clones which had scored more than 5 with the simple race. The range of scores with the complex race for the 78 clones was 2–8.

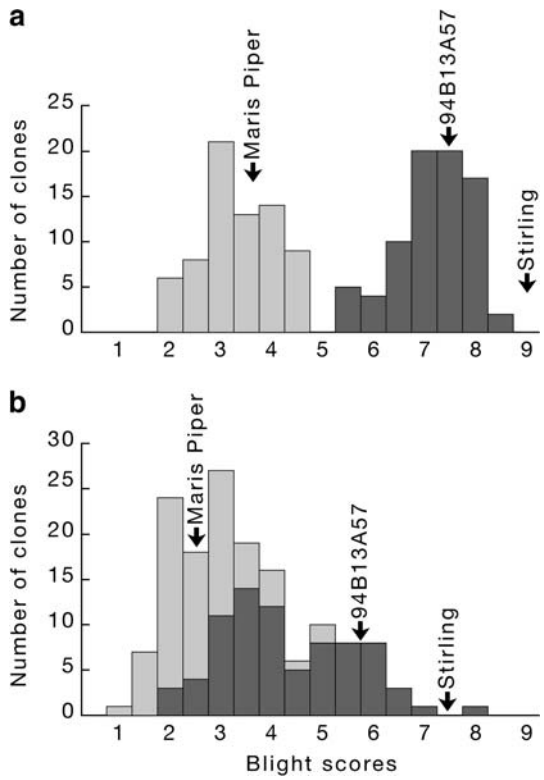

Fig. 3 Blight scores (mean of two replicates) on 1–9 scale of increasing resistance for the 149 clones from the 94B13A57 × Maris Piper backcross when assessed with **a** simple race (1,4) and **b** complex race (1,2,3,4,6,7) of *P. infestans*. *Dark bars* resistant to simple race, *light bars* susceptible to simple race

Backcross 94B13A61 × Maris Piper

An analysis of variance of the disease scores revealed large differences between the clones in this backcross family which were statistically significant ($P < 0.001$) when tested against a smaller but statistically significant race × clone interaction ($P < 0.001$). The components of variance for clones, race × clone interactions and residual error were 1.374, 0.386 and 0.777. With the simple race the range of scores was 2.50–9 with a mean of 6.84, compared with 9 for Stirling, 8 for 94B13A61 and 3.5 for Maris Piper (Fig. 4a). The distribution was skewed but continuous. Clones scoring 7 or more are shaded dark and those scoring 5.5 or less are shaded light, but this is considered an arbitrary division. With the complex race the range was 4.00–8.45 with a mean of 6.51, compared with 8.67 for Stirling, 6.83 for 94B13A61 and 5.0 for Maris Piper (Fig. 4b). However, this time the distribution was approximately normal. The differences for clones between their simple and complex race scores were also approximately normally distributed with a mean of 0.33, and the arbitrary divisions of clones into dark shaded (resistant), unshaded (intermediate) and light shaded (susceptible) for the simple race transfers to their scores with the complex race (Fig. 4).

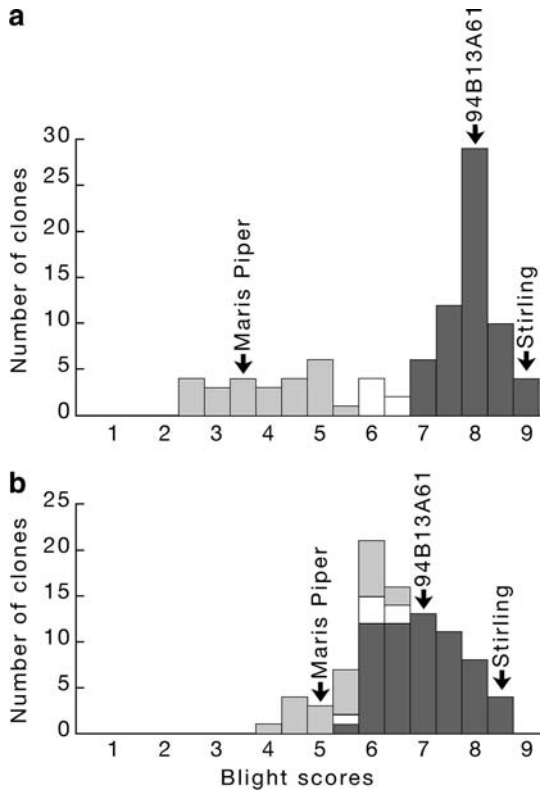

Fig. 4 Blight scores (mean of two replicates) on 1–9 scale of increasing resistance for the 88 clones from the 94B13A61 × Maris Piper backcross when assessed with **a** simple race (1,4) and **b** complex race (1,2,3,4,6,7) of *P. infestans*. *Dark bars* resistant to simple race, *light bars* susceptible to simple race, *white bars* intermediate to simple race

Discussion

Maris Piper and Stirling

Maris Piper is the most widely grown cultivar in Britain and is known to be susceptible to late blight and to lack *S. demissum* derived R-genes. As expected, it was susceptible to late blight in all tests although there was some variation in the degree of susceptibility with scores ranging from 2.25 to 5. Stirling was bred for a high level of field resistance, but its pedigree traces back to the introgression of R-genes from *S. demissum* and includes the R7 differential of Black (Malcolmson and Black 1966). As expected, it was disease free (score 9) in all tests with the simple (incompatible) race 1,4 and highly resistant in those with the complex (compatible) race 1,2,3,4,6,7, with scores ranging from 6.5 to 8.5. The variation in the degrees of susceptibility and resistance seen with Maris Piper and Stirling were no doubt due to environmental differences between tests and possibly differences in the aggressiveness of the isolates used in the tests.

The F1 generation

Previous genetic analysis of Stirling's blight resistance in whole plant glasshouse tests (Bradshaw et al. 2004) had revealed a single copy of an R-gene for qualitative resistance which mapped to the end of chromosome 11 at the same position as *R7* (El-Kharbotly et al. 1996), a result which was consistent with, but not proof for, the gene being *R7*. This needs to be confirmed once races of *P. infestans* are available which can distinguish R7 from the other ten *S. demissum* derived R-genes. The F1 segregation results with the simple race confirmed the presence of a single copy of the R-gene in Stirling. The results with the complex race were consistent with Stirling having a high level of quantitatively inherited field resistance. The blight scores formed a continuous, approximately normal distribution with a mean (4.30) that was similar to the mid-parent value (4.75). The distribution was similar to that obtained in the previous genetic analysis which had used the same complex race and had revealed two copies of an allele for quantitative resistance at a QTL on chromosome 4 (Bradshaw et al. 2004). This locus had explained 29% of the variation in blight scores with a model in which the two copies acted additively. An earlier analysis of crosses including some with Stirling as a parent had revealed a strong relationship between offspring mean and mid-parent values (Bradshaw et al. 2000). Comparisons of the results with the simple and complex race apparently enabled 14 out of 20 F1 clones to

be classified for the presence or absence of the R-gene and to be assessed for their level of quantitative field resistance. The results were less clear for the other six clones.

Backcross 94B13A29 × Maris Piper

It can be inferred from the segregation ratio of resistant to susceptible clones that genotype 94B13A29 has two copies of the R-gene and hence arose by double reduction, a not uncommon event for a locus near the end of a chromosome. The segregation ratios found for the R-gene were not significantly different from those expected for a coefficient of double reduction of one sixth, the commonly but incorrectly given maximum in papers and books. It is easy to see how crossovers in quadrivalents can give a coefficient of one-sixth and hence the reason for using this value, but the correct maximum is in fact one quarter (Luo et al. 2006). There was no evidence for one copy of the R-gene conferring less resistance than two copies. The 22 clones lacking the R-gene were as susceptible to the simple race as Maris Piper, with a mean score of 3.57 compared with 3.33. The complex race overcame the resistance conferred by the R-gene and 94B13A29 and the backcross clones on average were as susceptible as Maris Piper. Whilst a single copy of the R-gene and severe distorted segregation is an alternative explanation of the results, this seems unlikely as there was no distorted segregation in the F1 generation.

Backcross 94B13A57 × Maris Piper

The results for this backcross were surprising as it had been inferred from the reactions of 94B13A57 to the simple and complex race over 3 years that this clone lacked the R-gene but had a moderately high level of quantitative field resistance. In the backcross test with the simple race, however, there was a clear segregation into resistant and susceptible clones and a ratio consistent with 94B13A57 possessing a single copy of an R-gene, but expression of the gene giving an average score of 7.16 rather than immunity. Previous results with other R-genes which do not give complete immunity have been summarised and discussed by Bradshaw et al. (2006). The complex race did not completely overcome the R-gene in the sense that 94B13A57 was more resistant than Maris Piper (5.75 versus 2.5) and the clones presumed to possess the R-gene were more resistant than those without the gene (4.23 versus 2.52). Stewart et al. (2003) reported a similar sized effect for the average of genes *R1*, *R10* and *R11* in a field trial in 2001. The magnitude of the effect depended on the R-gene and the year of the trial. Stewart et al. (2003) also found a similar but smaller effect in the Stirling progeny used for genetic analysis by Bradshaw et al. (2004). The effect was also statistically significant ($P < 0.01$) in the backcross 94B13A29 × Maris Piper but not in the F1 ($P > 0.05$). It is not known if the increased resistance is conferred by the defeated R-gene or linked genes for field resistance. More complex genetical explanations can not be ruled out, but would require a considerable amount of genetic analysis involving molecular markers to confirm or refute. For example, one could hypothesise that the population is segregating for a single copy of Stirling's QTL on chromosome 4 (Bradshaw et al. 2004), but one would then have to explain a large QTL × race interaction which has not been observed previously. With the simple race, the difference between the mean values of the clones with and without the resistant allele was large and the same as the difference between the resistant and susceptible parent, whereas with the complex race the difference between the two groups of clones was much smaller (Fig. 3).

Backcross 94B13A61 × Maris Piper

The low blight score of 4.0 for 94B13A61 with the complex race in 2002 is considered an exception as its scores in the other six assessments ranged from 6 to 8. The conclusion from the backcross is that genotype 94B13A61 lacks the R-gene but has moderate field resistance despite a statistically significant ($P > 0.001$) race × clone interaction and a markedly skewed distribution with the simple but not the complex race. The differences in scores between the simple and complex race were small and approximately normally distributed with a mean close to 0 (0.33). Furthermore, the distributions of scores with both races were continuous with no obvious segregation into resistant and susceptible categories. The cut-off point for shading in Fig. 4a is arbitrary, unlike that in Fig. 1a where the clones scoring 8 or more were clearly phenotypically resistant, and unlike that in Fig. 2a and Fig. 3a where there was obvious segregation into two groups. Differences in the skewness of distributions were seen in the previous genetic analysis of Stirling's blight resistance (Bradshaw et al. 2004). The scores for the glasshouse test with the complex race gave an approximately normal distribution whereas the field assessments had a skewed distribution similar to the one observed with the simple race in the current experiment. Whilst these differences could result primarily from the scale of measurement used to record blight, they do change the observed degree of dominance from none to partial for the QTL with large effect for field resistance, i.e. the scores for genotype Qqqq move closer to QQqq from midway between QQqq and qqqq.

Implications for breeding for blight resistance

In a breeding programme the breeder may have only a race of *P. infestans* that is incompatible with a new source of

resistance. It is therefore instructive to look at the conclusions from the results with the simple race which is incompatible (avirulent) with the R-gene present in Stirling. First, an effective R-gene masks the level of quantitative field resistance which a clone possesses so that one does not know from screening with the simple race that Stirling has good field resistance and 94B13A29 poor resistance like Maris piper. However, crossing the resistant with a susceptible clone does provide such information, as well as the copy number of the resistance gene, provided that the population size is sufficient for the segregation of a reasonable number of clones which lack the R-gene. Thus the F1 generation revealed that Stirling has one copy of an R-gene and good field resistance as the mean score of the clones without the R-gene was 5.34 with a range of 2–7.5 compared with 3.5 for the susceptible parent Maris Piper. In contrast, the backcross of 94B13A29 to Maris Piper revealed that it has two copies of the R-gene and poor field resistance, as the clones without the R-gene were susceptible with a mean score (3.57) similar to Maris Piper (3.33). Likewise, Bradshaw et al. (2006) found that the R11 differential of Black has a single copy of *R11* and poor underlying field resistance. It was possible to confirm these conclusions with a complex compatible race and to demonstrate the known disadvantage of introducing major dominant R-genes from *S. demissum* into an otherwise susceptible potato. As the resistance is rapidly overcome the cultivar becomes susceptible for most of its commercial life, as happened with Pentland Dell (Malcolmson 1969). We have mentioned the strategy of combining R-genes with high levels of field resistance so that the near immunity conferred by R-genes is utilised until they are defeated, when the high levels of field resistance come into operation together with any residual resistance contributed by the defeated R-genes. Useful residual resistance was found for 94B13A57, as previously reported for Stirling's R-gene by Stewart et al. (2003), but this clone would have been rejected as having poor field resistance on the basis of the screening of its backcross progeny with the simple race. Where a resistance gene has been cloned, it should be possible to develop resistant-allele-specific markers for direct recognition of the desired gene, as has recently been achieved for the *RB* gene from *S. bulbocastanum* (Colton et al. 2006). This will allow the incorporation of the resistance gene into clones with high levels of field resistance, either by introgression or genetic transformation.

Selection for high levels of field resistance

It is therefore useful to discuss the best strategy for selecting high levels of quantitative field resistance. Two selection strategies have been tried, firstly using races of *P. infestans* compatible with the R-genes present in the germplasm and secondly creating R-gene free germplasm so that screening can be done with an isolate of any race (Toxopeus 1964; Black 1970; Wastie 1991; Ortiz 2001). The first strategy requires a compatible race and raises the question of the definition and recognition of a compatible reaction. A high level of quantitative resistance could result in restricted lesions similar to those which are sometimes seen with R-genes. The International Potato Center (CIP) found this strategy difficult to operate in practice and the blight resistance of the cultivars distributed to developing countries from their Population A germplasm was often disappointing (Landeo 2002). In contrast, it proved possible at SCRI to select cultivars such as Stirling with good quantitative field resistance in the presence of R-genes. However, it is clear from this present study that one cannot predict the inheritance of resistance from observations on the extent of restricted or slowly spreading lesions in the field or glasshouse, and the use of more than one race does not necessarily help. Thus clones 94B13A57 and 94B13A61 were both thought to lack the R-gene for qualitative resistance but to have good quantitative resistance. The genetic analysis proved that this assumption was correct for 94B13A61 but incorrect for 94B13A57 where the segregation of the R-gene was observed with the simple race. Use of the complex race would have selected both clones, one with some resistance from a defeated R-gene and the other with quantitative field resistance, but rejected susceptible clones in which the R-gene was overcome, an acceptable outcome.

The second strategy of creating R-gene free germplasm was developed from the experience of the failure of *S. demissum* derived R-genes to provide durable resistance and has been found useful by CIP in developing their Populations B1–B3. Race-specific resistance was eliminated from these populations where necessary by progeny testing with crosses to a tester, like Maris Piper, that does not possess *S. demissum* derived R-genes for resistance (Trognitz et al. 2001). However, the distinction between resistant alleles of large effect at QTLs and R-genes is not always clear cut in terms of recognition and desirability in a breeding programme. The *RB* gene from *S. bulbocastanum* has been cloned and introduced into the susceptible cultivar Katahdin and shown to confer broad spectrum resistance (Song et al. 2003). Because it provides a high level of field resistance (14% foliage infection compared with 65% in susceptible controls) rather than complete immunity, there is cautious optimism about its potential durability, as well as much debate (Colton et al. 2006). In contrast, Bradshaw et al. (2006) could only map the *R10* gene from *S. demissum* as a QTL because of the continuous distribution of blight scores in the cross between the R10 differential of Black and Maris Piper. Nevertheless, *R10* is of little value in breeding for durable resistance as isolates of *P. infestans* capable of overcoming it are common throughout the world

(Swiezynski et al. 2000). For the immediate future, the best strategy to select for high levels of field resistance is probably selection for high levels of resistance with the currently available complex races of *P. infestans*, but also taking into account the blight scores of a clone's parents and sibs. The segregation of a large proportion of susceptible offspring would be indicative of an R-gene masking a high degree of susceptibility and reason to reject the resistant clone. The breeding material should also be screened for maturity to avoid a correlated response for lateness, as found for example in previous research with Stirling (Bradshaw et al. 2004). In the longer term, as more R-genes and QTLs are mapped and diagnostic markers for them developed, it should become easier to combine desirable resistance genes that have qualitative effects with those that have quantitative effects and are not associated with late maturity.

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