New Approaches to Breeding for Late Blight Resistance: Objectives, Sources and Technology

J. E. Bradshaw[1], H.E. Stewart[1] and G.R. Mackay[1]

In 1991, a multitrait genotypic recurrent selection program was set up at SCRI to combine late blight resistance with other disease and pest resistances and the yield and quality required for new potato cultivars to be commercially successful in Britain. The program operates on a three-year cycle and includes seedling progeny tests for foliage blight and tuber blight resistance in year two. Clones are selected from the best tuber progenies in year three for use as parents in the next round of crossing, as well as for evaluation as new cultivars. This includes more blight testing. Resowings of the best progenies are also made to provide more clones for assessment as potential cultivars. As a result of the 1998 progeny tests, it was possible to select 12 progenies with a mean foliage blight score superior to Stirling selfed, the resistant control, and a tuber blight score as good as Stirling selfed. It can be concluded from the full set of progeny tests that good progress has been made in combining blight resistance with other desirable traits, but the critical assessment will come in 2001 when the best clones that can be selected from the 12 progenies are compared with the parents used in the original crosses. The blight resistance in the 12 progenies came from four of the original parents —Cramond, Stirling, Torridon and clone 8204a4 (a parent of Stirling)— and was derived primarily from relatively few accessions of *Solanum demissum*. The Commonwealth Potato Collection is, therefore, being screened for new sources of resistance for future use and a start has been made with resistant accessions of *S. papita* and *S. verrucosum*. Diallel analysis has confirmed that Cramond, Stirling, Torridon and 8204a4 all have high general combining abilities for foliage and tuber resistance and a more detailed genetic analysis of Stirling's resistance is underway using molecular markers. An AFLP marker (p75m48=5) present in double-dose in Stirling accounted for 34 per cent of the variation in foliage blight scores in cross 12601ab1 x Stirling, assessed in a field trial in Ayrshire, Scotland, in 1998. Prospects for molecular marker-assisted introgression, breeding for blight resistance and cloning natural resistance genes are briefly discussed.

Today, in Britain, the most widely grown potato (*Solanum tuberosum* subsp. *tuberosum*) cultivars are susceptible to a range of pests and diseases. These must be controlled by the widespread use of chemicals, such as fungicides for late blight [*Phytophthora infestans* (Mont) de Bary], nematicides for cyst nematodes, and insecticides for aphid-transmitted virus diseases. However, chemical control is expensive, not always effective, and raises environmental and food safety concerns particularly over large-scale insecticide use and pesticide residues in tubers for human consumption. Hence, new cultivars with higher levels of disease and pest resistance are highly desirable but, for commercial success, they must have acceptable marketable yields and meet the quality requirements of processors and supermarkets. Therefore, in 1991, we set up a multitrait genotypic recurrent selection program to combine the blight resistance which was already available in *S. tuberosum* as a result of past breeding and introgression from *S. demissum* with these other desirable traits. We decided to concentrate on quantitative field resistance, since it has proved more durable than dominant R gene resistance (which the fungus can readily overcome). We also decided to select for resistance in the tubers as well as in the foliage because one does not guarantee the other. Susceptible tubers can be infected by spores produced over a relatively long period of time from the slowly spreading sporulating lesions of a leaf-resistant cultivar.

[1] Scottish Crop Research Institute, Scotland, U.K.

The Recurrent Selection Program operates on a three-year cycle. Crosses were made in 1991, 1994 and 1997, and the resulting full-sib families were assessed in seedling progeny tests in the glasshouse in 1992, 1995 and 1998. These included tests for foliage blight resistance and tuber blight resistance. The best families were then evaluated in the field as tuber progenies at our high-grade seed site in 1993 and 1996. The same will happen again in 1999 (tuber progenies are required in order to assess processing quality and other traits, but need not concern us here). Clones were selected from the best tuber progenies for use as parents in the next round of crossing, as well as for evaluation as new cultivars. More plants have been raised from the best crosses of each cycle to provide further clones for evaluation as potential cultivars. This year, for example, 200 true seeds from each of 12 of the best progenies from the 1998 seedling progeny tests will be sown in a glasshouse (resowings) to provide up to 2,400 clones for visual assessment in four plant plots at our seed farm in 2000. The selected clones will then enter a multistage multiplication and selection program involving replicated yield trials along with quality and disease testing. Means of selecting for blight resistance in such a scheme were described by Bradshaw et al. (1995a). We intend to adopt the two-stage screening strategy proposed by Stewart et al. (1994) in which clones are first tested for foliage resistance at our blight site in Ayrshire, on the western seaboard of Scotland, where the weather favours the development of late blight. This will be done before assessing the resistant clones for tuber resistance in a glasshouse trial. Details of the blight tests can be found in Stewart et al. (1994). We do now recommend two replicates of two plant plots at Ayr, rather than three replicates of single plant plots, for ease of seeing the plots, and eight, rather than five, plants of each clone in each replicate for tuber blight in order to ensure 20 tubers for laboratory testing. This strategy exploits the lower environmental component of variation for foliage blight in Ayrshire rather than for tuber blight in the laboratory test on glasshouse-grown tubers, and any correlation that exists between foliage and tuber resistance when one is working with parents with resistance or susceptibility to both (e.g. the phenotypic correlation between foliage resistance and tuber susceptibility was r = -0.58 in 14016a7 x Stirling). The resistance of clones, particularly in the laboratory test for tuber blight, should be confirmed with field-grown plants in more than one year.

An indication of our progress to date is given by the results of the 1998 progeny tests for blight resistance. The tests were conducted as described by Caligari et al. (1984) for foliage blight and Wastie et al. (1987) for tuber blight. However, a 1:1 mixture of races 1,2,3,4,6,7 and 1,3,4,7,10,11 was used in both tests at concentrations of 5×10^4 and 2.5×10^4 zoospores/ml for foliage and tubers, respectively. In the absence of a fully complex isolate, the use of a mixture to overcome any R genes likely to be present in the material was the best possible compromise and ensured, as far as possible, that any resistance expressed was non-race-specific. There was enough seed for two replicates of 25 seeds of 145 and 122 progenies for the foliage and tuber tests, respectively. In the foliage test, the 25 seedlings were left to grow in a 10-cm pot whereas, in the tuber test, 16 seedlings were pricked out into individual pots, from which 2 tubers were harvested to give a bulk of 32 tubers.

Foliage blight was scored on a 1 (greater than or equal to 50% necrosis) to 4 (less than 10% necrosis) scale. The means of the progenies (the 122 in both tests) ranged from 1.0 to 3.0 with a population mean of 1.76 compared with 1.75 for the resistant control (Stirling selfed) and 1.25 for the susceptible control [G8884(2) selfed]. The broad sense heritability of the means was 0.39 compared with 0.63 and 0.55 in 1995 and 1992. The mean of the 12 selected progenies was 1.98, which was better than Stirling selfed.

Tuber blight was scored as the number of infected tubers in each sample, ignoring any infections that had entered via a wound or the stolon scar. The percentage of infected tubers in each sample was calculated and converted to degrees by the variance-stabilising angular transformation. The progeny means ranged from 0 to 62.07 with a mean of 31.86 compared with 19.36 for Stirling selfed (resistant control) and 66.51 for G8884(2) selfed (susceptible control). The heritability was 0.82 compared with 0.80 and 0.91 in 1995 and 1992. The mean of the 12 selected progenies was 18.94, which was as good as Stirling selfed.

The correlation between resistance to foliage blight and susceptibility to tuber blight was statistically significant ($P<0.1\%$) but small in magnitude ($r = -0.350$) and underlined the need to select for resistance to both in a breeding program.

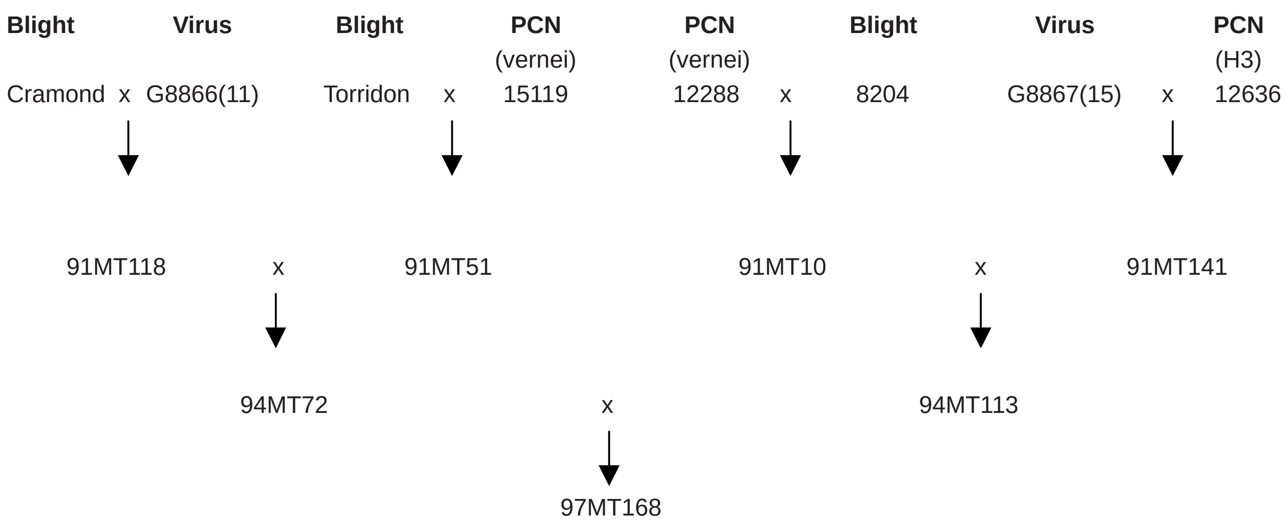
The 12 progenies selected for resowings in 1999 were chosen using the following selection index (SI):

$$SI = 1.776\ (pref - 4.00) - 0.346\ (pcn - 7.92) + 0.352\ (fb - 1.76) - 0.044\ (tb - 31.86)$$

where "pref" is breeders' visual assessment of tubers on a 1-9 scale of increasing preference (Brown *et al.*, 1988), pcn is the square root of cyst counts of *Globodera pallida* Pa 2/3 (Lindley) (Phillips and Dale, 1982) and fb and tb are the foliage and tuber blight scores. The weights were calculated using Smith's (1936) discriminant function for plant selection with relative economic weights in phenotypic standard deviation units of

1:1:½:½ for the four traits, so that fb and tb together were given a similar weight to "pref" and pcn.

It can be concluded from the full set of progeny tests that good progress has been made in combining blight resistance with other desirable traits, but the critical assessment will come in 2001 when the best clones that can be selected from the 12 progenies are compared with the parents used for the original crosses in 1991. Typical of the 12 progenies is 97MT168, which ranked 3rd on the selection index:



Although seed was secured from eight of the ten blight-resistant parents chosen to start the multitrait program in 1991, just four of them have contributed to the 12 progenies selected for further evaluation this year. They are Cramond, Stirling and Torridon, and clone 8204a4, which is one of the parent of Stirling. All four parents were derived from seedling selections six or more generations removed from *S. demissum* (CPC 2127), and over which selection was mainly for R gene resistance, as well as blight resistant derivatives of *S. demissum*, selected for field resistance in Mexico from material of European and North American origin (M109-3 in Cramond, 8204a4 and Stirling; M136-6 in Stirling and Torridon) and Russian origin (MRu18 in Cramond). Hence, the resistance being used is derived primarily from relatively few accessions of *S. demissum*, although the complex pedigrees of the three cultivars mean that contributions of resistance genes from other species cannot be ruled out. Nor, indeed, can *S. tuberosum*, when one considers the success in improving the resistance of Andigena potatoes (Simmonds and Malcolmson, 1967).

The value of the blight resistance of Stirling and Torridon (and Cramond to a lesser extent) has been demonstrated under organic farming conditions and in experiments on a farm in Ayrshire (Wastie *et al.*, 1993a). The GILB GXE experiment has shown that their resistance is effective in a wider range of environments. It is also hoped that it will prove long lasting, despite its probable narrow genetic base. Nevertheless, we thought that it was essential to seek additional sources of resistance for future use. Helen Stewart and Gavin Ramsay are now in the process of screening the Commonwealth Potato Collection for new sources of resistance. Their results, to date, are presented in a poster at this meeting (Stewart and Ramsay, 1999). Briefly, they have found resistance in 24 of the 47 species tested so far, and in seven taxonomic series. A higher proportion of accessions from Central America (Mexico) were resistant than from South America, although resistance was found in accessions from Bolivia and Argentina. We have started to study resistant accessions of *S. papita* and *S. verrucosum* with a view to marker assisted introgression. We chose the former because a high proportion of accessions were resistant and the latter because we have true breeding inbred lines (it is a self-compatible diploid EBN2 species).

The diallel sets of crosses that we assessed in the 1990s (Stewart *et al.*, 1992; Wastie *et al.*, 1993b; Bradshaw *et al.*, 1995b) showed that Stirling, 7683a12 (a parent of Cramond), Torridon and 8204a4 have high general combining abilities for resistance to foliage and tuber blight. It is, therefore, not surprising that these sources of resistance have survived in the multitrait program, unlike others such as Shelagh and Teena. Specific combining ability was also found for foliage and tuber blight, but not in every diallel. Any desirable variation in progeny means due to specific combining ability is exploited when selecting the best progenies for cultivar production.

But what can be said about the variation within pair crosses? The cross between clone 14016a7 (susceptible) and Stirling (resistant) was assessed for foliage blight in a field trial in Ayrshire and for tuber blight in the glasshouse/ laboratory in 1993 (Stewart et al., 1994). It showed continuous variation for both traits with the scores for foliage blight resembling a normal curve, while those for tuber blight had a flatter, more uniform distribution. The heritability was high ($h^2 = 0.93$) for foliage blight, and moderate ($h^2 = 0.76$) for tuber blight, but there was no clue as to the number of genes segregating. Hence, a more detailed genetic analysis of Stirling's resistance was undertaken with Robbie Waugh and his colleagues using molecular markers. An AFLP marker (p75m48 = 5) present in double-dose in Stirling accounted for 32% of the phenotype variation observed in foliage blight scores in cross 12601ab1 x Stirling, assessed in a whole-plant glasshouse test in 1995 (Meyer et al., 1998). In 1998, a further assessment of foliage blight resistance was made on the same 78 clones, together with 69 from the reciprocal cross (Stirling x 12601ab1), but this time at our field site in Ayrshire. The trial had a randomised complete block design with two replicates and two-plant plots, but with three plots of each parent in each replicate. The clones were planted on May 13th in pairs of drills separated by a third 'spreader' drill planted continuously with the susceptible cultivar King Edward. The trial was infected with race 1,2,3,4,6,7 on July 23rd by placing, at metre intervals along the spreader drills, pot plants of King Edward, which had been inoculated three days previously in the laboratory. The trial was scored on a 1 to 9 scale of increasing resistance (Cruickshank *et al.*, 1982) on July 30th and on August 3rd, 7th, 11th and 14th. The fourth score proved the best for discriminating between clones. Stirling had a score of 7.50, 12601ab1 a score of 2.67, and the cross and reciprocal cross means were 4.98 and 4.97, respectively. Both displayed continuous variation with modes at 7.00 (i.e. skewed towards resistance with a long susceptible tail) and heritabilities of 0.84 and 0.91, respectively. The correlation with the glasshouse scores was moderately high (r = 0.723) and, not surprisingly, AFLP marker p75m48=5 accounted for 34% of the phenotypic variation displayed by the 78 clones of the cross. This year, we intend to extend this research by assessing a larger population of 270 clones for tuber blight resistance, and to use microsatellite markers to complement the AFLPs in the hope of finding more of Stirling's blight resistance genes.

Our plans for the immediate future are to complete the multitrait genotypic recurrent selection program, to complete the analysis of Stirling's blight resistance and to explore the possibility of marker-assisted selection in a tetraploid breeding program. We also wish to complete screening the CPC for new sources of resistance and to start their molecular marker assisted introgression into *S. tuberosum*. In the longer term, we may try to clone natural resistance genes.

Acknowledgements

Funding from the Scottish Office Agriculture, Environment and Fisheries Department is gratefully acknowledged, as is funding for the molecular work from the British Potato Council and UNDP/CIP. Thanks are due to all of the people whose work is quoted in the following list of references.

Literature Cited

Bradshaw, J.E., R.L. Wastie, H.E.Stewart and G.R. Mackay. 1995a. *Phytophthora infestans 150*, European Association Potato Research, Pathology Section Conference, Dublin, Ireland, pp 246-54.

Bradshaw, J.E., H.E. Stewart, R.L. Wastie, M.F.B. Dale and M.S. Phillips. 1995b. Theoretical and Applied Genetics 90:899-905.

Brown, J., P.D.S. Caligari, M.F.B. Dale, G.E.L. Swan and G.R. Mackay. 1988. Theoretical and Applied Genetics 76:33-38.

Caligari, P.D.S., G.R. Mackay, H.E. Stewart and R.L. Wastie. 1984. Potato Research 27:43-50.

Cruickshank, G., H.E. Stewart and R.L. Wastie. 1982. Potato Research 25:213-214.

Meyer, R.C., D. Milbourne, C.A. Hackett, J.E. Bradshaw, J.W. McNicol and R. Waugh. 1998. Molecular and General Genetics 259:150-160.

Phillips, M.S. and M.B.F. Dale. 1982. Journal of Agricultural Science, Cambridge 99: 67-70.

Simmonds, N.W. and J.F. Malcolmson. 1967. European Potato Journal 10:161-166.

Smith, H.F. 1936. Annals of Eugenics 7:240-250.

Stewart, H.E. and G. Ramsay. 1999. In: Late Blight: A Threat to Global Food Security, Global Initiative on Late Blight conference , Quito, Ecuador, March 16-19, 1999 (in press).

Stewart, H.E., R.L. Wastie, J.E. Bradshaw and J. Brown. 1992. Potato Research 35:313-319.

Stewart, H.E., J.E. Bradshaw and R.L..Wastie, 1994. Potato Research 37:429-434.

Wastie, R.L., P.D.S. Caligari, H.E. Stewart and G.R. Mackay. 1987. Potato Research 30: 533-538.

Wastie, R.L., H.E. Stewart, G.R. Mackay, J. Bevan and M. Lennartsson. 1993a. Biologi Italiani 23:18-21.

Wastie, R.L., J.E. Bradshaw and H.E. Stewart. 1993b. Potato Research 36: 353-357.