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Multiple field and glasshouse assessments increase the reliability of linkage mapping of the *Vf* source of scab resistance in apple

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Abstract Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is an important disease in commercial apple production. A mapping population of 155 individuals, derived from a cross between the apple varieties 'Prima' (resistant) × 'Fiesta' (susceptible), was scored for response to the disease in replicated field and glasshouse trials throughout Europe. Twenty data sets were selected and cluster analysis was used to form a consensus score for the population fitting a 1:1 segregation ratio of resistance:susceptibility. The progeny were scored with molecular markers. A detailed map covering 54 cM of the 'Prima' linkage group containing the *Vf* gene for scab resistance was constructed using 24 molecular markers linked to the resistance gene. One isoenzyme marker (*Pgm-1*), six RFLP markers and 17 RAPD markers formed a linkage group with the consensus measure of resistance to scab. Four marker bridges were established with the corresponding 'Fiesta' linkage group with additional markers (one isozyme, one RFLP, three RAPD and one AFLP). A low chi-square value indicated a good fit of the marker ordering, which was in close agreement with previously reported linkage positions for some of the markers and *Vf*. Differences were observed in the ability of different scoring methods to resolve susceptible and resistant classes. The results obtained for the consensus classification of resistance to scab for the population may suggest the presence of virulent inocula at some sites, which could overcome the *Vf* gene for resistance. The consequences of relying on individual scoring occasions for studying *Vf* scab resistance are discussed in the context of linkage analysis, conventional breeding selection, and marker-assisted selection.

Key words *Malus* · Apple · *Venturia* · Scab resistance · Linkage mapping · Cluster analysis

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Introduction

Apple scab is economically one of the most important diseases of apple trees (*Malus pumila* Mill.), especially in regions of cool, moist springs and summers. The causal organism of apple scab is the fungus *Venturia inaequalis* (Cke.) Wint. The disease is spread by dispersal of fungal spores and affects both leaves and fruit, with scab lesions on fruit reducing the marketable quality and value of the crop. Disease control is primarily achieved in commercial production systems by costly and repeated use of fungicide sprays; reduced fungicide inputs are seen to be beneficial to the grower, environment and consumer. Intensive use of fungicides over a prolonged period of time poses the potential threat of fungicide resistance in the pathogen population due

to the selection of fungicide-insensitive strains and a subsequent loss of control.

The introduction of scab resistance into apple cultivars has been the aim of many modern apple-breeding programmes. The most widely used source of resistance has been derived from the small-fruited ornamental species *Malus floribunda* Siebold ex Van Houte, clone 821. This resistance, assigned to a gene called *Vf* (Williams et al. 1966), has been utilised in apple-breeding programmes throughout the world for more than 40 years, and has been incorporated into a substantial number of apple cultivars, although none has been extensively planted (Crosby et al. 1992).

Until recently, *Vf* was considered to be the most effective source of resistance, because *Vf* cultivars had been free from scab for over 50 years in the different countries where they were grown. Parisi et al. (1993) then identified a pathotype (race 6), isolated in Germany, which was virulent against a number of cultivars or selections carrying *Vf*, whereas *M. floribunda* 821 itself was resistant. They concluded that there was an urgent need to diversify the sources of resistance to *V. inaequalis*. In addition, it is likely that there are additional resistance loci in *M. floribunda* 821 which were not introgressed into the cultivars and selections which are susceptible to race 6. Roberts and Crute (1994) have since identified a pathotype of *V. inaequalis* (*FL1*, isolated in England) to which *M. floribunda* 821 and derived cultivars are susceptible.

Several molecular genetic markers have recently been identified which are linked to the introgressed segment of the *M. floribunda* 821 genome which confers *Vf* resistance to *V. inaequalis*. These include the isoenzyme locus *Pgm*-1 (Manganaris et al. 1994) and at least nine DNA-RAPD markers (Durham and Korban 1994; Koller et al. 1994; Yang and Kruger 1994; Tartarini 1996). All RAPD markers were identified following a bulked segregant approach (Michelmore et al. 1991), using bulks composed of either resistant or susceptible cultivars, or individuals from one or more segregating populations. The bulked-segregant approach is relatively efficient in identifying markers linked to a major-gene phenotype, although the subsequent genetic analyses may be limited if these markers are not placed in the context of a linkage map. Gardiner et al. (1996), Gianfranceschi et al. (1996) and Tartarini (1996) have presented partial maps based on these RAPDs, for introgressed sections of the *Vf* linkage group. More detailed linkage information helps in the analysis of interactions with other genes and can be used to minimise linkage drag by selecting resistant plants that carry the shortest segment of alien chromosome.

The European Apple Genome Mapping Project was initiated in 1989 to consolidate the studies on apple genetics carried out by the major European apple breeding institutions (King et al. 1991). Replicated reference populations were established and distributed amongst partners in six countries (King 1994). A wide range of molecular-marker and trait data have now been accumulated (King 1997), including comprehensive assessments for resistance to *V. inaequalis* under both field and glasshouse conditions. The data reported here represent the most detailed assessment of linkage relationships for this major source of apple-scab resistance.

Materials and methods

Plant material

A cross between 'Prima' and 'Fiesta' was carried out at CPRO-DLO, Wageningen in 1988. 'Prima' (Dayton et al. 1970) is a variety selected in the USA Co-op programme (Crosby et al. 1992), where the *Vf* source of scab resistance has been introgressed over four generations. The scab-susceptible variety 'Fiesta' was selected at East Malling from a cross between 'Cox' and 'Idared'. The seedlings were raised in pots, and planted in the nursery at Wageningen in the winter of 1990/1991. Five replicate trees of each genotype were obtained by bud-grafting wood of 155 seedlings onto M27 dwarfing rootstock in early 1992 and grown for another year. The trees were grown for another year and distributed to the six sites in early 1993 (Table 1), with one population being divided between Germany and Italy. Potted trees for glasshouse testing were obtained by grafting wood from the respective field plantations onto M9 (East Malling) and MM106 (Angers) rootstocks.

Fungicides were applied as sprays to the grafted trees at Elst and Cadriano throughout 1993 to establish healthy growth. At Angers and Ahrensburg, fungicides were applied until June 1993. At East Malling and Naoussa the trees were left unsprayed for the duration of the study.

Table 1 Locations of field and glasshouse sites for screening 'Prima' × 'Fiesta' progeny for resistance to apple scab

Site	Location		Country	Propogation
	Grid reference	Altitude (m)		
East Malling	51°17′ N 00°27′ E glasshouse	32	England	M27 cordons M9 in pots
Elst	51°55′ N 05°50′ E	8	The Netherlands	M27 staked
Angers	47°30′ N 00°35′ W glasshouse	57	France	M27 staked MM106 in pots
Ahrensburg	53°40′ N 10°15′ E	46	Germany	M27 staked
Cadriano	44°32′ N 11°23′ E	30	Italy	M27 staked
Naoussa	40°37′ N 22°07′ E	121	Greece	M27 staked

Assessment of scab infection

The segregating population was assessed for disease response on 41 occasions in 3 years over six field sites, and glasshouse trials at two sites. Different methods for scoring symptoms and incidence were used, some of which were based on those currently employed in breeding selection. Twenty two data sets were selected and cluster analysis used to form a 'consensus' score fitting a 1 : 1 segregation ratio over the population.

Glasshouse assessments were carried out at HRI-East Malling and INRA, Angers (see Table 3). At East Malling plants were inoculated with the *E1* isolate of *V. inaequalis* (Kirkham 1957), whilst at Angers the inoculum was prepared from dried scabbed leaves collected from orchards around the INRA station at Angers. Previous characterisations of these scab populations showed that they are generally very aggressive and are primarily represented by race-1 spores. At East Malling a conidial suspension of 2×10^5 spores ml^{-1} was used for inoculation, whilst at Angers the concentration varied between 4×10^5 and 6×10^5 spores ml^{-1}. Inoculations were carried out by spraying and performed only on trees which were actively growing and healthy. To maintain a high humidity, plants were covered with transparent polythene for the first 48 h following inoculation: at East Malling, plants were enclosed individually in polythene bags, whilst at Angers each tray of plants was covered. A humidity of 80–100% and a temperature of 18°C was maintained throughout the experiments. Symptom development was recorded after 2 weeks using the Vi-GH-1 descriptor scale at both sites (Table 2). In addition, at Angers two replicate plants per individual genotype were screened. Trees were inoculated for a second time after 12 days and a second assessment of scab infection was made 15 days following the second inoculation using the Vi-GH-1 descriptor scale.

Field assessments of scab infection were made on several occasions (Table 3) at each site (Table 1). Only one plot had been subject to a fungicide spraying programme (Angers). Scoring was carried out using several different scoring methods or 'descriptors' described in Table 2. The level of field infection at each site was likely to be dependent upon several factors, including the population structure of the pathogen and the environmental conditions. The relative abundance and virulence of the different pathotypes within a local

Table 2 Methods used to assess the incidence and symptoms of scab occurrence in field (Vi-F-1, Vi-F-2, Vi-3, Vi-5) and glasshouse (Vi-GH-1) tests

Descriptor	Type	0	1	2	3	4	5
Vi-F-1; Vi-GH-1	Symptom leaf	No symptoms	Pin point pits	Chlorotic lesions, possibly small necrotic spots	Chlorotic and necrotic spots	Chlorotic and necrotic spots, sporulation present	Sporulation
Vi-F-2	Incidence leaf	No leaves attacked	1–10% leaves attacked	11–35% leaves attacked	36–65% leaves attacked	66–90% leaves attacked	91–100% leaves attacked
Vi-3	Incidence leaf	No infection	Very little infection	Clear spots of infection	–	–	–
Vi-5	Symptom leaf	No observation	Symptoms of resistance	Low susceptibility	Middle susceptibility	High susceptibility	Very high susceptibility

Table 3 Assessments of scab development carried out on the replicated progeny of the cross between 'Prima' × 'Fiesta' at different sites. Sets of scores selected to derive the 'consensus' phenotypes which were used in the linkage analysis. Vi-GH-1 is derived from the method described by Chevalier (1991)

Descriptor	Site	Date month/year	Graded as resistant	Ratio (R : S)	χ^2 1 : 1
Vi-GH-1	East Malling	6/94	0–3	83 : 53	6.62
	Angers	4–5/94	0–2	67 : 63	0.12
	Angers	4–5/95	0–2	65 : 63	0.03
Vi-F-1	East Malling	6/93	0–3	69 : 86	1.86
	East Malling	5/94	0–4	66 : 85	2.39
	East Malling	8/94	0–4	68 : 83	1.49
	Elst	6/94	0–4	76 : 72	0.11
	Ahrensburg	7–9/94	0–4	31 : 36	0.37
	Ahrensburg	9/94	0–4	27 : 21	0.75
	Ahrensburg	95	0–4	34 : 37	0.13
Vi-F-2	East Malling	6/93	0	67 : 88	2.85
	East Malling	8/93	0–1	78 : 77	0.01
	East Malling	5/94	1	63 : 88	4.14
	East Malling	8/94	1	69 : 82	1.12
	Elst	9/94	0	73 : 76	0.06
	Elst	95	0	74 : 74	0
	Elst	95	0–1	75 : 73	0.03
	Ahrensburg	95	0–1	25 : 46	6.21
Vi-3	Ahrensburg	8/93	1	17 : 25	1.52
Vi-5	Angers	7/93	0–4	21 : 21	0

population were expected to differ between sites and between years. For example, the virulent race-6 inoculum of *V. inaequalis* was known to be present at Ahrensburg (Parisi et al. 1993). In addition, environmental conditions suitable for scab infection varied from site to site as well as from year to year. For example, at East Malling the number of infection periods predicted by the Ventem™ infection-warning system (Xu et al. 1995) for the critical period between March and June was 22 in 1993, 29 in 1994, and eight in 1995.

Molecular markers

Isoenzymes

Young, actively growing, leaf tissue for isoenzyme analysis was collected from the field sites in early June and weighed samples were stored at − 80°C until extraction and analysis. Protein extraction and starch electrophoresis were carried out according to Chevreau and Laurens (1987). The staining for Phosphoglucomutase (*Pgm* = EC 5.4.2.2) was carried out according to Wendel and Weeden (1989).

DNA markers

DNA was extracted using either a modified mini-prep CTAB-based method (Doyle and Doyle 1990) or a large-scale nuclei-isolation method (Van der Beek et al. 1992; Roche et al. 1997). RAPD primers (Table 4) were obtained either from Operon (Alameda, California), the University of British Columbia, or custom synthesised by a number of suppliers. PCR reaction conditions were based on a standard protocol (King 1994) adopted by participants in the European Apple Genome Mapping Project, or modified for the OPAM19 and OPAL07 assays (Tartarini 1996). RAPD data were scored in different participating laboratories and reproducibility was confirmed by replicating experiments in different laboratories. RFLP analysis followed the methods described in Roche et al. (1997) as modified from Van der Beek et al. (1992). The pB610 clone used as an RFLP probe was kindly supplied by Dr. Gavin S. Ross, HortResearch, Auckland, N.Z.

A total of 208 markers were selected from a larger dataset for linkage analysis, based on population coverage and segregation ratio (Maliepaard et al., in preparation).

Data management and analysis

Scab infection, marker and related data were entered into the AppleStore relational database (Hyne 1995) which was used to generate files for linkage analysis. Linkage analysis was performed with JoinMap version 2.0 (Stam 1993; Stam and Van Ooijen 1995), using a LOD score of 4.0 for grouping markers into linkage groups and the Kosambi mapping function to calculate map distances. The total chi-square value was calculated by carrying out 297 pairwise recombination-frequency estimates for the 25 markers, resulting in 273 degrees of freedom (*df*).

Scab-trait data

Inspection of many of the data sets showed them to have a unimodal distribution of scoring grades, with no strong relationship between pairs of data sets (Fig. 1b, d). A subset of data sets did show a clear

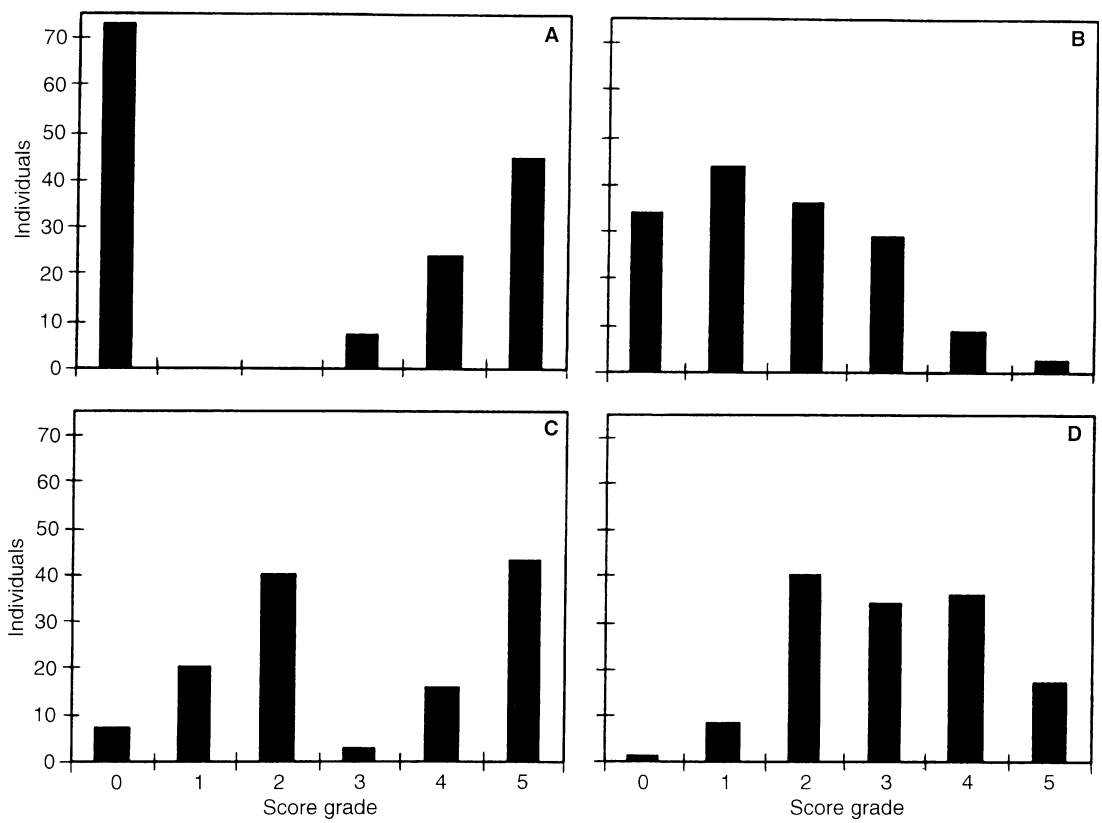

Fig. 1A–D Comparison of distribution of score grades in the segregating population 'Prima' × 'Fiesta' in field and glasshouse tests at different sites. Vi-F-2 incidence scores in the field: (**A**) at Elst in 1994; (**B**) at East Malling in 1993; Vi-GH-1 symptomatic scores in glasshouse tests; (**C**) at Angers and (**D**) at East Malling, both in 1994

bimodality (Fig. 1a, c). Within this subset, there was strong agreement of the classification of trees into each of the two modes. Since these observations fitted the expected model of a single dominant resistance gene with a 1 : 1 segregation ratio (Williams et al. 1966), this model was adopted in the subsequent analysis.

Forty one sets of measurements relating to scab infection were examined. Each data set was inspected to determine the cut point which best divided the plants according to a 1 : 1 ratio. Twenty data sets which allowed a division into resistant and susceptible classes with a segregation ratio between 1 : 2 and 2 : 1 were selected, to form a 'consensus' classification by further analysis. These included 17 field and three glasshouse data sets. Of the data sets discarded, only two had segregation ratios between 1 : 3 and 3 : 1, and many revealed no infection (e.g. at Naoussa). All but three of the sets retained had ratios between 2 : 3 and 3 : 2. Of the data sets retained, the glasshouse assessments were scored using the symptomatic descriptor method Vi-GH-1 (Table 2) in two countries over 2 years, and the majority of the field assessments were scored using the descriptor methods Vi-F-1 (symptomatic) and Vi-F-2 (incidence) in three countries over 2 years. In total, field measurements included data sets from four countries using four scoring systems over 3 years.

These 20 data sets, with the plants classified as resistant or susceptible, were then combined using cluster analysis with the complete-link clustering algorithm (Matula 1977) in the package Genstat 5 (1993). The classification was carried out for each data set individually with the cut point based solely on the assumption of a 1 : 1 segregation. Any discrepancies in relation to the actual scoring grade were not taken into account, but were addressed later in the analysis. Indeed, it was found that this approach had no significant effect on the composition of the consensus classification. Two distinct groups were formed and these were taken to represent the resistant and susceptible plants. This consensus classification was then subjectively assessed by comparing it to each of the original 41 data sets, to establish the degree to which there was disagreement.

To assess the impact of the arbitrary selection of the threshold values of 1 : 2 and 2 : 1 as the criterion for inclusion of data sets into the consensus-forming set, the clustering was repeated following either inclusion of additional sets or removal of existing sets, by setting the threshold values at either 3 : 1 and 1 : 3 or 2 : 3 and 3 : 2. The effect of changing the thresholds was assessed by comparing the output for the classification of individuals into each of the resistant and susceptible groups.

Results

Compilation and assessment of the consensus score

There were differences between the threshold grade required to distinguish between resistant and susceptible individuals, in data sets scored using the same descriptor scale both between sites and within sites. In some data sets with a bimodal-score distribution, where very few plants were assigned to the intermediate-score grade, then the choice of threshold to form a 1 : 1 ratio resulted in a misclassification of that grade with respect to the consensus classification. For example, the Vi-F-1 data set from Elst in 1994 gives the optimal segregation ratio (76 : 72) when score-grades 0–4 are considered resistant. All of the plants scoring grade '4' are, however, susceptible according to the consensus score, and indeed were susceptible in the opinion of the scorers of that occasion, and re-classifying in this way gives a segregation ratio of 71 : 77. In other data sets the fit to the consensus classification cannot be improved by adjusting the threshold. The difference in thresholds assigned to different data sets must therefore be attributed either to different levels of attack, interactions with different *V. inaequalis* populations, patchy distribution of different pathotypes in field plots, or discrepancies in classification by different assessors.

The clustering process carried out on the 20 sets of data to produce a consensus classification formed two groups at a similarity level of about 0.2. The resistant group consisted of 77 individuals, and the susceptible group 78. The robustness of the consensus classification to variations in the threshold for inclusion of data sets in the consensus-forming group was tested, as described in the Materials and methods section. The consensus classification was unchanged by varying the datasets included in its formation. It showed a very high level of agreement with the field data sets from Elst, with only four classification discrepancies in individual scores over four scoring occasions. With glasshouse data from Angers, seven discrepancies occurred in two data sets.

The number of classification discrepancies present in the remaining non-consensus forming data sets was greater. In measurements taken in Naoussa where there was low infection pressure, and most trees showed no symptoms, susceptibility was observed in only 4 of the 150 trees present. In data sets from other sites discrepancies in the resistant:susceptible classification were present for up to 45 individuals (about 30% of the total). In some pairs of data sets from different dates in the same year at one site, or at the same time using different scoring methods, there were a large number of discrepancies in resistant:susceptible classification, with different individuals being responsible for the discrepancies in the different data sets. An inspection of the patterns of misclassifications failed to show any evidence of specific genotypes being consistently miss-scored.

Assessment of scab infection

The ability of different assessors to agree on scoring grades was tested at Elst. This experiment is pertinent to the reliability of scoring systems used in breeding selection. Two teams of two people each carried out scoring within 3 days of each other in September 1995, using the Vi-F-2 descriptor scale. There was good agreement between the two teams, with 112 trees having identical scoring grades, 34 differing by one scoring grade, and only two plants differing by two scoring grades. Of the 112 trees which received identical scoring grades, 72 were scored as zero by both assessor teams. Even excluding these easily classified trees, the two assessor teams agreed on the scoring grades for 40 plants. In the cases where there was disagreement, one assessor team scored 14 plants at a lower grade, and 22

plants at a higher grade. For only one plant was the susceptibility assessed differently by the two assessor teams (grade '3' and grade '1').

The effectiveness of the different scoring systems was compared. In field tests with low levels of scab inoculum many of the consensus susceptible individuals showed no symptoms. All of the data sets scored in the glasshouse show some consensus susceptible plants scored as resistant, and two of the three data sets show at least one consensus resistant plant scored as susceptible, possibly as a result of the increased infection pressure. The divergence from the consensus classification is higher in the data from East Malling than that from Angers.

Of the data sets scored in the field, the symptomatic and incidence descriptors Vi-F-1 and Vi-F-2 gave the same information about susceptibility at Elst, where the scores were in strong agreement with the consensus. However, on scoring occasions where there was deviation from the consensus, the two descriptors gave complementary information. At East Malling in 1994, for example, both Vi-F-1 and Vi-F-2 were scored in May and August. On both occasions the consensus susceptible plants all exhibited sporulating scab, with all but four or five plants scoring a grade '5' (sprorulating) on the Vi-F-1 scale. Fewer than one in five of the plants scored less than a grade '2' (up to 10% of leaves attacked) on the Vi-F-2 scale.

There was evidence that the resistance of the consensus "resistant" plants was partially overcome at East Malling. About one-third of the consensus resistant plants displayed sporulating scab in May, and this had increased to about one-half of the consensus resistant plants by August. About one-half of the consensus resistant plants with sporulating scab scored a grade '5' on the Vi-F-1 scale, and only one in six scored more than a grade '1' on the Vi-F-2 scale. Comparing overall performance, on both occasions the Vi-F-1 scale alone misclassified between 15 and 20 plants, while the combined scores misclassified five or six. The Vi-F-2 scores alone misclassified 39 and 24 plants on the two occasions.

It had been observed that the consensus-forming group of data sets included data sets from some sites which consistently contained some individuals which displayed sporulation, whereas in the consensus they were classified as resistant. Removal of these data sets did not affect the consensus classification, but did raise the similarity level of the clustering from 0.2 to 0.3.

Only one false susceptible score was recorded at Angers during the two glasshouse tests, although several susceptible plants were not detected as such. In glasshouse tests at East Malling, with a single isolate, there were a larger number of susceptible plants which were not detected, and several false susceptible scores. The increased accuracy at Angers may be explained by the replication which allowed the rejection of the scores for plants giving inconsistent scores. The screening tests performed on unreplicated seedlings as part of a breeding programme might be expected to have an error rate more similar to that obtained at East Malling.

Additional preliminary scab records of the presence or absence of sporulation from the nursery at Wageningen in 1991 agreed well with the consensus score, with the exception of 8 out of 157 plants. In five cases plants were scored as susceptible while the consensus score was resistant, and three were scored as resistant compared to a consensus of susceptible.

Linkage mapping of markers and consensus scab resistance scores

Twenty four linked markers (six RFLPs, one isoenzyme marker and 17 RAPD markers) were grouped together with the consensus scab resistance (Table 4). The map for this linkage group resulted in a total chi-square value of 74.5 for 273 *df* (mean chi-square of 0.273) indicating a good fit of the marker ordering. Double-recombinant individuals were checked on the original score sheets and on autoradiographs. The most closely linked markers were the RFLP derived from M18-CAPS (Gianfranceschi et al. 1996) and the original RAPD OPM18-0900 marker, with no recombination events between the markers and the resistance locus (from a total of 145 plants for which the marker was scored), and OPAL-07 and OPAM-19 (Tartarini 1996), which showed one recombinant plant each (on totals of 150 and 155 plants respectively). OPU-01 (Koller et al. 1994) showed five recombinants (out of 158 plants) and OPD-20–0600 (Yang and Kruger 1994) showed 15 recombinants (out of 153 plants). Recombination percentages and standard errors of the markers with the consensus score for resistance were calculated (Table 4).

Four RFLP loci provided marker bridges with the corresponding 'Fiesta' linkage group. An additional isozyme, a RFLP, three RAPDs and one AFLP marker were present on the 'Fiesta' linkage group, which spanned 42.4 cM. The isozyme locus *Tpi-5*, which was detected by the activity of triose phosphate isomerase, co-segregates with the RFLP locus pB610a, which derives from a cloned sequence having homology to triose phosphate isomerase (personal communication, G. S. Ross).

The most-likely marker ordering of OPU1, OPM18, *Vf* and OPD20 is in agreement with the ordering of Gianfranceschi et al. (1996), who also located OPM18 and OPU1 on one side of *Vf* and OPD20 on the other side. Our marker ordering (for *Pgm*-1, OPU01, *Vf*, OPM18, OPA15, OPD20) also corresponds with results from Gardiner et al. (1996), in a cross between Granny Smith and the *Vf*-heterozygote A679/2, with results from Hemmat et al. (1995) (for PGM-1, OPU1, OPM18, OPA15 and OPD20) in another 'Prima' cross, and with Tartarini (1996) (for OPAM19, OPAL07, OPC09, OPAB19 and OPC08). However, since only

Table 4 Details of marker loci and the recombination percentages of these loci with the consensus score of scab resistance

Locus	RAPD sequence or marker type	Map position (cM)	Recombination (%)	SE (%)
'Prima'				
OPAF-12-2000	GACGCAGCTT	0	39.5	3.9
OPAD-12-0510		2	38.8	3.9
MH876a	RFLP genomic	9.1	34	4.9
MC014a	RFLP cDNA	16.5	29.1	4.3
OPC-08-1100	TGGACCGGTG	23	19.7	3.2
OPAB-19-1430	ACACCGATGG	23.7	19.1	3.2
OPO-14-1700		24.5	18.4	3.1
UBC213-2100		28	14.3	3
OPAF-13-2100		28	15.2	2.9
OPA-11-2200	CAATCGCCGT	28	14.3	5.6
OPC-09-0900	CTCACCGTCC	29.1	13.9	2.8
OPD-20-0500	ACCCGGTCAC	35.2	7.5	2.2
OPAG-05-1900	CCCACTAGAC	35.2	8	2.1
MC110a	RFLP cDNA	35.2	8	2.3
pB610a	RFLP: EMBL	35.2	7.8	2.3
MC112a	RFLP cDNA	35.2	7.7	2.2
OPA-15-0900	TTCCGAACCC	38.1	6.2	2
OPAM-19-2200	CCAGGTCTTC	42.7	0.7	0.7
OPAL-07-0580	CCGTCCATCC	42.7	0.7	0.7
Vf	Consensus	43.4		
M18	RFLP	43.4	0	0.7
OPM-18-0900	CACCATCCGT	43.4	0	0.7
OPU-01-0400	ACGGACGTCA	46.8	3.3	1.5
PGM-1	Isoenzyme E.C. 5.4.2.2	49.4	6.4	2.1
OPAG-12-0800	CTCCCAGGGT	54	9.9	2.4
'Fiesta'				
MH876a	RFLP genomic	0		
OPAD-18-1130		11.4		
UBC249-2000		15.5		
MC014a	RFLP cDNA	15.5		
AFLP_F1	AFLP	30.9		
MC110a	RFLP cDNA	34.4		
OPD-07-1600	TTGGCACGGG	34.4		
pB610a		34.4		
TPI-5	Isoenzyme E.C. 5.3.1.1	34.4		
MC112a	RFLP cDNA	42.4		

one recombinant plant was found among the markers OPAM19, OPAL07 and OPM18 and the consensus score of resistance, other orders for this subgroup cannot be excluded from this study, and are most likely to be resolved by physical mapping with large insert genomic clones, as a prelude to map-based cloning. The ordering is also in agreement with an analysis of 50 resistant cultivars (King et al., unpublished).

The recombination frequencies between *Vf* and OPD20, OPC09, OPU01 and OPM18 (Table 4) are within the standard errors of those reported by Gardiner et al. (1996) which were based on field or glasshouse screening. The recombination frequency for *Pgm*-1 is in agreement with the pooled data from four populations reported by Manganaris et al. (1994).

Effect of relying on data from one source to establish linkage positions

The consequences of using only one disease-assessment data set to determine the relative map position of the resistance gene was investigated. Mapping was repeated, using the same parameters, with a set of field data selected from a scoring of Vi-F-1 at East-Malling in May 1994. This data set was chosen as being typical of a reliable scab-screening occasion where more virulent inocula may be present (Roberts and Crute 1994), where the infection rate in the plot was high, and where no fungicide treatment had been applied at the site for several years. Vi-F-1 is a symptomatic scale which may be expected to be more appropriate for distinguishing susceptible from resistant plants on the basis of sporulation.

There were 18 differences from the consensus score. For 14 individuals the consensus score indicated resistance while the East Malling test set indicated susceptibility, and in four cases the consensus score indicated susceptibility while the test set indicated resistance. The ordering of the markers remained identical but the resistance gene could not be inserted between the markers, due to the 'double recombinants' with the most closely linked markers, which now had to be accounted for. Adding the resistance locus based on the

East Malling test set resulted in a map position for *Vf* at the end of the linkage group. However, a large increase in the chi-square value indicated strong discrepancies between this ordering and the original pair-wise recombination-frequency estimates.

Discussion

Compilation of data from widely differing environmental conditions affecting disease development has demonstrated the difficulties involved in the reliable and accurate assessment and classification of resistant and susceptible plants. The use of cluster analysis as a means of forming a consensus classification was successful in identifying the segregation of a major source of resistance. The classification, based on a model which assumed the action of a major gene segregating in a 1 : 1 ratio, was validated by its close agreement with the marker segregation. A poorer fit of the consensus score to the marker segregation would have been observed if the 1 : 1 model had been incorrect. If there had been distortion in the segregation of the resistance score whilst the flanking markers did not show any evidence of distortion this would have caused difficulties in determining the position of the gene. The symptomatic score (Vi-F-1) based on sporulation symptoms was clearly the most reliable in assigning resistance classes, although supplementing this with the incidence of sporulation proved worthwhile at sites where the data suggest a partial breakdown of resistance. The agreement in assessment of the field incidence scale by two teams of assessors at one site indicates that the scale is well defined and robust. In addition, preliminary scab records of the presence or absence of sporulation from the nursery at Wageningen in 1991 agreed well with the consensus score.

The results presented in Table 3 demonstrate that plants carrying the *Vf* resistance gene can exhibit different symptom classes. In glasshouse tests at East Malling it was apparent that plants having chlorotic and necrotic spots possessed *Vf*. The fact that plants displaying the same symptoms in glasshouse tests at Angers did not possess *Vf* may reflect the different origin and level of inocula used. Gardiner et al. (1996) also observed that plants classified into two symptomatic resistant classes (3A and 3B *sensu* Chevalier 1991) possessed the *Vf* gene; these classes correspond to classes 3 and 4 of both Vi-GH-1 and Vi-F-1. The field scores reported in this study appear to indicate that expression of the *Vf* resistance gene is affected by the environment. The resolution of classification in the field may be increased by combining symptomatic and incidence scores. The scanning electron microscope data and histological studies carried out by Chevalier (1991) demonstrated that there was a spectrum of resistant classes (1–4) over which the intensity of the host response decreased, while fungal development increased.

Some care is required in the interpretation of the same scoring scale at different sites. These results suggest that the race-composition of the natural field inoculum and environmental conditions at some sites appear more appropriate for the reliable and accurate selection of plants carrying the *Vf* gene. The evidence from Italy and Greece where scab infection was low highlights the problems associated with a reliance on field infection in breeding selection.

The effect of more virulent races being present in natural inocula at some sites was detected by the interaction between local scores and the consensus set. At Ahrensburg, where the virulent Race 6 has been identified (Parisi et al. 1993), 25 of the 26 plants present in the resistant consensus were scored with sporulating scab on at least one occasion. However, since there were also consensus susceptible plants with no sporulating scab, indicating that the infection rate at this site was sporadic, no quantitative assessment of partial resistance was possible. Even at this site there was a significant relationship between sporulation and the consensus score. At East Malling the results suggested the presence of a more virulent race, or races, which partially overcame the *Vf* resistance. The FL1 race described by Roberts and Crute (1994), which overcomes resistance in *M. floribunda* 821, was found in the vicinity of East Malling.

In some cases repeated symptomatic measurements were taken at one site and inconsistencies found in which trees displayed discrepancies in the scores. This suggests that the discrepancies may not arise from a host genetic factor. Some of the discrepancies, such as those found between a field score early and late in the season, may reflect the turnover of leaf material due to factors such as summer pruning, different timing of bud burst, or growth rate.

Reliance on assessments of resistance or susceptibility solely from one site, or use of only one method, may be misleading when attempting to assign an accurate linkage position to a major source of resistance. This study demonstrates that a different linkage position could have been calculated if the analysis had been based on only one scoring method or one site, in this case probably due to the presence of a more virulent inoculum.

Selection for resistance to scab in apple-breeding programmes currently relies on glasshouse assessment and the culling of young seedlings, followed by more-extensive field assessments against local inocula at a range of trial sites. This process may be inefficient due to practical limitations imposed on replicating young seedlings in glasshouse tests, and the geographical and climatic environmental variation in field screening. The ability to pre-select individual seedlings reliably and accurately based on markers flanking characterised major sources of resistance may contribute to cost and

time savings in such breeding programmes, especially when combined with markers for other important agronomic traits.

Gianfranceschi et al. (1996) have demonstrated the application of *Vf*-linked markers to molecular pre-selection of individuals possessing resistance. Gianfranceschi et al. (1994) had earlier discussed the requirements of a marker-assisted selection (MAS) approach to resistance breeding in apple, where markers are now available flanking sources of scab and mildew resistance, as well as aphid resistances. From the work reported here, it is apparent that there would be advantages to using markers where there are low levels of natural inocula, where virulent races exist, or where environmental conditions or glasshouse facilities are not optimal for scab development. In situations where virulent races (such as Race 6) exist, the advantages of using *Vf* as a component either of developing more durable resistance, or of integrated disease management, have to be evaluated against the tendency to encourage the development of these virulent races. However, there is scope to use the markers to combine *Vf* with other non-allelic or recessive sources of resistance to scab, as well as to use co- dominant markers to identify individuals homozygous for *Vf*. There is evidence that such homozygotes display a stronger resistance response (Gessler et al. 1997).

The data presented not only establish an accurate ordering of markers linked to the resistance from *M. floribunda* 821, but also provide an insight into the efficiency of methods currently used to select for scab resistance in European apple-breeding programmes. The results enable the confident use of flanking markers in order to recover and determine the length of introgressed regions conferring scab resistance in breeding selection. The results also suggest that great care is required when relying upon existing screening methods for breeding selection. Reliance on one test in one environment may lead to loss of valuable material from any one breeding programme. The use of the markers reported here, in combination with suitably segregating material, will enable the accurate assessment of the relationships between pathotypes of *Venturia* and different sources of resistance in *Malus*. The availability of an accurate and saturated linkage map of the region closely flanking a resistance gene is also a pre-requisite for map-based cloning.

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