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RFLP and RAPD markers linked to the rosy leaf curling aphid resistance gene (Sd_1) in apple

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Abstract Sd_1 is a dominant gene for resistance to biotypes 1 and 2 of the rosy leaf curling aphid, *Dysaphis devecta* Wlk., which can cause economic damage to apple trees. This report describes the identification of three RFLP and four RAPD markers linked to Sd_1 in a cross between the *D. devecta* susceptible variety 'Prima' (sd_1sd_1) and the resistant variety 'Fiesta' (Sd_1sd_1). Potted trees were artificially infested in the glasshouse, and the ratio of resistant:susceptible plants supported the hypothesis that the resistance was under the control of a single dominant gene. The position of the gene was mapped to a single locus on a 'Fiesta' chromosome, within 2 cM of three tightly linked RFLP markers (MC064a, 2B12a and MC029b); the four RAPD markers were located further away (between 13 and 46 cM). This is the first report of molecular markers for an aphid resistance gene in tree fruit crops. The potential application of these markers in a marker-assisted resistance breeding programme is discussed.

Key words Apple · *Dysaphis devecta* · Insect resistance · Marker-assisted selection · Molecular markers · Plant breeding

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Introduction

In the UK, the rosy leaf curling aphid (*Dysaphis devecta* Wlk.) is a pest of cultivated and ornamental apples. In the absence of control, the aphid typically affects the same trees year after year, causing severe leaf curl with conspicuous red galls (Massee 1954; Gratwick 1992). The occurrence of the aphid has been reported further afield, including Germany, Hungary, Iran and Italy (e.g. Pfeifer 1994; Jenser and Balazs 1991; Rezwani and Radjabi 1987; Baronio and Briolini 1985).

The aphid is capable of causing economic damage to apple crops although, in general, it is well-controlled by routine aphicide applications. However, economic, regulatory and environmental pressures are changing attitudes towards the use of agrochemicals, and alternative control measures are being sought. In the case of *D. devecta*, sources of genetic host-plant resistance are readily available in apple varieties.

Resistance to *D. devecta* was first reported by Dicker (1954) who observed that the aphid did not attack the variety 'Cox's Orange Pippin'. Alston and Briggs (1968) found that resistance in 'Cox' and 'James Grieve' was controlled by a single dominant gene. In a later report, Alston and Briggs (1977) identified three aphid biotypes and three functionally distinct resistance genes (Sd_1, Sd_2 and Sd_3). 'Cox's Orange Pippin' carries the Sd_1 resistance gene and is resistant to biotypes 1 and 2. In the same report, Alston and Briggs suggested that a number of varieties (including 'Cox') were heterozygous for a precursor gene (Sd_{pr}) at an additional locus, without which any one of the three known *Sd* resistance genes is ineffective.

Currently, selection for resistance to *D. devecta* is dependent on insect-plant bioassays in the glasshouse

or field. These techniques are simple and can give well-defined, reproducible results within 1 month. However, there are problems associated with conventional screening, including inoculation failures or contamination with aphid predators, which can result in an extension of the seasonally dependent screening programme. A marker-assisted approach conducted independently of such constraints could eliminate these problems.

Through the European Apple Genome Mapping Project (King et al. 1991; King 1994; 1996), a saturated linkage map is being constructed of DNA and isoenzyme markers in apple using a cross between 'Prima', susceptible to *D. devecta*, and 'Fiesta', which is resistant, carrying Sd_1 from 'Cox'. The results of an investigation into the association of the *D. devecta* resistance gene (Sd_1) with DNA markers in this progeny are described in this paper.

Materials and methods

Mapping population

The population used in this investigation results from a 'Prima' (sd_1sd_1) × 'Fiesta' (Sd_1sd_1) cross made at CPRO-DLO, Wageningen by Dr J. Janse and J. Verhaegh in 1988. A seedling population was established in the field (CPRO-DLO, Elst) and used as a source of propagating material and leaf material for marker-screening. In 1993, 161 bud-grafted trees on M27 rootstocks were sent to East Malling and planted as cordon rows in an unsprayed plot. Potted trees suitable for glasshouse testing were obtained by bench-grafting wood from these trees, and the parental genotypes, onto M9 rootstocks. Replicate sets of trees were distributed to the other sites (HRI-W, IZZ and DCA) where they were used as sources of material for molecular marker analysis.

Aphid samples

Aphid populations were scarce at the start of the 1994 season, and aphids obtained from the Royal Horticultural Society's Garden, Wisley (Surrey, England) were supplemented with aphids from Boughton Monchelsea, a fruit-growing region in Kent. The aphid was more abundant in 1995, and all the aphids were collected from the principal 1994 source at Wisley.

The Wisley aphids are thought to be biotype I based on tests with differential hosts ('Fiesta' and 'Northern Spy') in 1995, but the biotype of the Kent aphids is unknown.

Resistance testing

The 1994 and 1995 aphid screening experiments were conducted on single plants of 141 individuals and their parents, 'Prima' and 'Fiesta', in unheated insect-proof glasshouses during May and June. A single adult aphid or two nymphs were placed on a growing point of each potted tree; the reaction was assessed after 1 week using the scoring procedure of Alston and Briggs (1968). Individuals were scored as susceptible (conspicuous galling and reddening of the leaf), intermediate (development of small chlorotic lesions) or resistant (no symptoms). Those plants without aphid colonies were re-tested in weeks 2 and 3.

The presence of *D. devecta* colonies on the trees in the unsprayed East Malling field plot was also recorded in both years.

DNA extraction and restriction fragment length polymorphism (RFLP) analysis

At CPRO-DLO, DNA extraction and RFLP analysis were based upon the method described by Van der Beek et al. (1992) with minor modifications. Frozen leaf tissue (2.5 g) was homogenised for 30 s in 15 ml STE-buffer (0.35 *M* sorbitol, 0.1 *M* TRIS-HCl, 5 m*M* EDTA, pH 7.5 and 20 m*M* Nabisulfite). The homogenate was filtered, rinsed with 20 ml cold STE-buffer, and centrifuged (2000 *g* for 15 min). The green pellet was rinsed with 20 ml cold STE-buffer with 0.4% Triton X-100 and centrifuged again. After a further round of rinsing and centrifugation, the pellet was resuspended in 1.25 ml STE-buffer and then mixed with 1.75 ml nuclear lysis buffer (0.2 *M* TRIS-HCl, 0.05 *M* EDTA, 2 *M* NaCl, 2% CTAB w/v, pH 7.5) and 0.6 ml of 5% Sarkosyl. The mixture was incubated at 65°C for 1 h, with occasional mixing, before being extracted with chloroform: isoamylalcohol (24:1); the DNA was precipitated with an equal volume of cold isopropanol. DNA was hooked out and rinsed with 76% EtOH/10 m*M* NH_4Ac for 30 min, dried and dissolved in TE buffer (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 7.5). The DNA concentration was measured using a fluorometer (TKO 100; Hoefer Instruments).

One hundred and fifteen *Malus* cDNA clones were evaluated in a set of plants which included 'Prima', 'Fiesta', 'Cox's Orange Pippin' and ten of the 'Prima' × 'Fiesta' progeny, using the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII and *Xba*I; 55 of these clones were then used to score the progeny plants. In addition, one genomic DNA clone, 2B12 from IZZ, was screened on these plants after an initial screening had suggested linkage to the resistance allele.

Random amplified polymorphic DNA (RAPD) analysis

Leaf material was harvested from the parental and segregating accessions at the various sites (DCA, IZZ, HRI-W). DNA minipreps were done following the CTAB method of Doyle and Doyle (1990) with minor modifications. Purified DNA was dissolved in water and adjusted to a final concentration of 10 μg ml^{-1}. Random decamer primers were purchased from Operon (Alameda, Calif.) and Genosys (Cambridge, UK). A standard RAPD polymerase chain reaction (PCR) procedure was followed (Williams et al. 1990). Hybaid Omni-Gene thermal cyclers were used in all laboratories.

Amplification products were separated on agarose gels, stained with ethidium bromide, illuminated with UV light and recorded with either a digital camera system or on Polaroid film.

Linkage analysis

Linkage analysis was performed using JOINMAP version 2.0 with the Kosambi mapping function (Stam 1993; Stam and Van Ooijen 1995). JOINMAP 2.0 allows linkage analysis in a segregating progeny from heterozygous parents of an outcrossing species so that markers with different segregation types (segregating 1:1, 3:1, 1:2:1 and 1:1:1:1) can be integrated into a linkage map, and linkage phases can be estimated simultaneously. A LOD score of 4.0 was used for grouping markers into linkage groups.

Results

The genotypes tested in 1994 were classified as either resistant, intermediate or susceptible. Those genotypes classified as intermediate in 1994 were re-classified as

either resistant or susceptible after re-testing in 1995, when only resistant or susceptible symptoms were observed. Eleven susceptible genotypes were identified in the unsprayed field plot during 1994 and 1995, despite the low level of natural aphid colonisation in both years. These records are in full agreement with the glasshouse screening tests, where the same 11 genotypes were also found to be susceptible.

The field and glasshouse scores for 1994 and 1995 were combined. Of the 141 plants that were tested, there were 75 resistant and 62 susceptible plants while 4 plants remained unclassified since they appeared susceptible in 1994 and resistant in 1995. There was no evidence to suggest any statistically significant departure from a simple 1 : 1 segregation (chi-squared = 1.64, $P = 0.27$). When the data were prepared for linkage analysis, the 4 unclassified individuals were excluded. The analysis was repeated, assigning either resistant or susceptible scores to all the unclassified plants in order to evaluate the influence of these genotypes on the map position of the gene.

Three RFLP markers and four RAPD markers were linked to the resistance gene, with recombination frequencies ranging from 0.015 to 0.346.

One of the two RFLP markers obtained with MC029/*Hae*III had a ⟨aa × ab⟩ segregation type (a = 4.7 kb and b = 2.8 kb). The 2.8-kb fragment, which was present in 'Cox' and 'Fiesta' and absent in 'Prima', was linked with the resistance gene (Table 1). This locus was denoted MC029b, and a sample autoradiograph is shown (Fig. 1). Two RFLP markers were also obtained

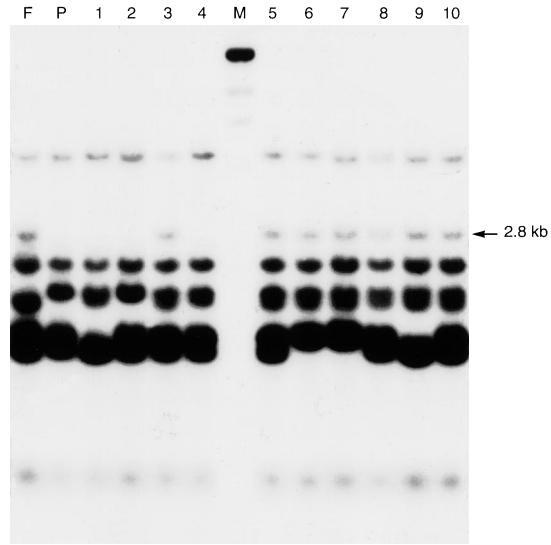

Fig. 1 Southern hybridisation autoradiograph of *Hae*III-digest DNAs probed with MC029. *F* resistant parent 'Fiesta', *P* susceptible parent 'Prima', *lanes 1–10* ten progeny plants, *M* λ/*Hin*dIII molecular weight marker. Of the progeny plants, seven are resistant (*lanes 3* and *5–10*) and three are susceptible (*lanes 1, 2* and *4*). The 2.8-kb fragment linked to the *D. devecta* resistance is shown (←)

Table 1 Segregation analysis of the 'Prima' × 'Fiesta' progeny by molecular marker and reaction to *D. devecta*. The recombination frequency and standard error values are shown

Locus	Segregation type	Banding pattern	Resistant	Susceptible	Number of plants	Recombination frequency	Standard error
MC029b	⟨aa × ab⟩	aa	1	59		0.022	0.013
		ab	73	2			
		Total	74	61	135		
MC064a	⟨ab × ac⟩	aa	0	30		0.015	0.010
		ab	1	30			
		ac	35	1			
		bc	38	0			
		Total	74	61	135		
2B12a	⟨ab × cd⟩	ac	0	30		0.015	0.010
		bc	1	29			
		ad	35	1			
		bd	38	0			
		Total	74	60	134		
OPC-08-1700	⟨aa × ab⟩	aa	8	46		0.147	0.032
		ab	64	11			
		Total	72	57	129		
OPT-09-1200	⟨aa × ab⟩	aa	12	47		0.189	0.035
		ab	56	12			
		Total	68	59	127		
OPA-10-1000	⟨aa × ab⟩	aa	42	18		0.276	0.040
		ab	17	50			
		Total	59	68	127		
GE80-19-0550	⟨aa × ab⟩	aa	26	40		0.346	0.041
		ab	47	20			
		Total	73	60	133		

for MC064/*Eco*RV; one of these loci (MC064a) gave three allelic fragments (a = 4.1 kb, b = 6.2 kb, c = 2.5 kb) with a ⟨ab × ac⟩ segregation type. The 2.5-kb fragment, present in 'Cox' and 'Fiesta' and absent in 'Prima', was linked with the resistance (Table 1). Clone 2B12 was first analysed at IZZ on a subset of 40 plants with *Hin*dIII, and the 4-kb fragment was found to be linked to the resistance gene. When analysed at CPRO-DLO with *Dra*I, two RFLP markers were obtained. One of these markers (2B12a) displayed a ⟨ab × cd⟩ segregation type (a = 3.2 kb + 2.0 kb, b = 2.5 kb, c = 2.5 kb + 2.0 kb, d = 2.5 kb + 1.5 kb). The 1.5-kb fragment, present in 'Cox' and 'Fiesta' and absent in 'Prima', was linked with the resistance (Table 1). The three other markers described for MC029, MC064 and 2B12 corresponded to unlinked loci.

The four RAPD markers linked to the resistance, listed in order of increasing distance from the Sd_1 gene, were OPC-08-1700, OPT-09-1200, OPA-10-1000 and GE80-19-0550 (Table 1). In all cases, the RAPD fragment was present in 'Fiesta' and absent in 'Prima'.

The unclassified plants were coded as missing values for the resistance locus in the first analysis, and in 2 plants recombination events were identified between the resistance locus and two of the RFLP markers (MC064a and 2B12a, Table 1). These plants were also identified as recombinants for MC029b, together with an additional plant. The marker genotype of the 4 unclassified plants for the three RFLP markers suggests that these plants are probably resistant, as was observed in the 1995 single-biotype trials. Including these 4 plants as resistant instead of missing made no difference in the marker order and minimal changes in the marker distances since no extra recombination events had to be accounted for. However, when these 4 plants were included as susceptible, the resistance gene was placed at the end of the linkage group but with a higher mean chi-square value for the resulting map, indicating more conflicts between the estimated map distances and the original pairwise estimates.

Allelic bridges to the 'Prima' homologue of this linkage group were provided by the RFLP markers MC064a and 2B12a, for which both parents were heterozygous. Markers linked to MC064a and 2B12a on the 'Prima' homologue included RAPD markers OPAB-13-2600, OPA-09-0700 and RFLP marker MC014b. Figure 2 shows the 'Fiesta' linkage group with Sd_1 and the 'Prima' homologue; the 4 unclassified plants were coded as missing values for the resistance locus in this analysis.

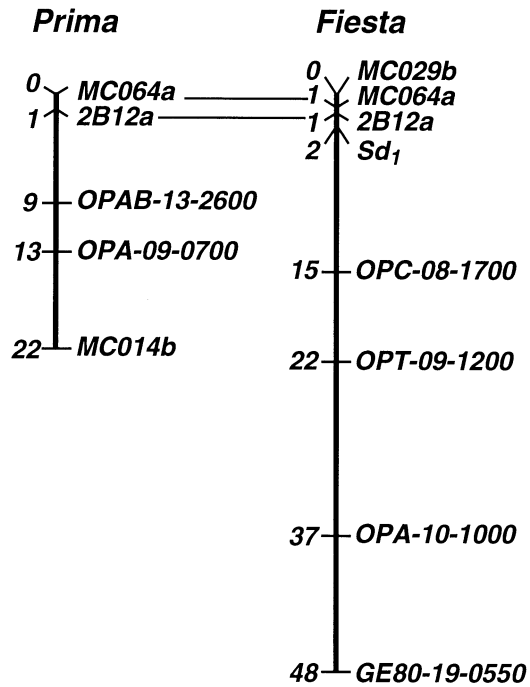

Fig. 2 Homologous 'Prima' and 'Fiesta' linkage groups with markers linked to the *Dysaphis devecta* resistance locus Sd_1. Distances in centiMorgans

Discussion

We have located the position of the Sd_1 gene for resistance to biotypes 1 and 2 of the rosy leaf curling aphid in the context of seven molecular markers (three RFLPs and four RAPDs); this is the first report of molecular markers for an aphid resistance gene in tree fruit crops. Three RFLP markers have been mapped at less than 2 cM from Sd_1, each marker displaying a fragment which was linked in coupling phase to the resistance. In the best fitting marker order, the resistance gene was flanked by the RFLP markers on one side and the RAPD marker OPC-08-1700 on the other side.

This resistance gene has several characteristics which make it suitable for use in breeding programmes. Certain high quality varieties, including 'Cox' and some of its derivatives (e.g. 'Fiesta'), carry the Sd_1 gene. They are readily available to the breeder and have been widely used as parents in many breeding programmes. In addition, the resistance appears to be readily transferred to the progeny in a distinct, simply inherited fashion. In this particular study, the resistance gene segregated in a simple 1 : 1 fashion as would be expected if one of the parents was homozygous for the precursor gene Sd_{pr}. Nevertheless, many varieties are heterozygous for the precursor gene, without which the Sd_1 gene is ineffective (Alston and Briggs 1977). In a cross between two such heterozygous parents ($Sd_{pr}\ sd_{pr}$), only 75% of the plants carrying the Sd_1 gene will be resistant since the remaining quarter will lack the precursor gene (i.e. $sd_{pr}\ sd_{pr}$ genotypes). This hypothesis may now be tested by using appropriate RFLPs to analyse a progeny where the precursor gene is segregating; it may then be possible to map the precursor gene.

Now that the Sd_1 gene has been mapped with closely linked markers, a marker-assisted selection (MAS)

approach becomes a possibility. It has the potential to enhance the process of breeding for resistance to *D. devecta* by reducing the need for insect-plant bioassays; the magnitude of this benefit is dependent upon the precursor gene status of the parents at present. However, the identification of an appropriate molecular marker for the precursor gene could overcome this problem.

A molecular breeding approach is most appropriate with a range of markers that are cost-effective, reliable, robust, easy to score and suitable for screening large numbers of individuals. These factors may explain why marker-assisted selection techniques have been discussed widely in the literature but there are comparatively few practical examples. When compared to genes for resistance to diseases, there have been relatively few reports describing the mapping of genes for resistance to insects in plants. DNA markers associated with resistance to insects have been reported for several crops either as single genes, e.g. mungbean (Young et al. 1992) and rice (Mohan et al. 1994), or as quantitative traits, e.g. maize (Schön et al. 1993), potato (Bonierbale et al. 1994), barley (Nieto-Lopez and Blake 1994) and tomato (Nienhuis et al. 1987; Maliepaard et al. 1995). MAS can be restricted to a small part of a linkage group for a single dominant gene like Sd_1, where the expression of resistance is largely unaffected by environmental variation. Linkage drag can be controlled more easily under these circumstances, and the testing of extra progeny is often unnecessary.

In this study, linkage has been demonstrated in only one progeny but, if the most tightly linked RFLP markers prove to be reliable, for screening progenies they also need to be easy to use and cost-effective. RFLP markers are not readily suited to routine screening programmes. However, the application of MAS techniques could be facilitated by converting candidate RFLPs into allele-specific PCR-based assays (e.g. Penner et al. 1995) which may be amenable to the automated processing of samples. These markers could then be used for the routine screening of progenies where the Sd_1 gene has been introgressed. Nevertheless, the economics of a marker-assisted screening strategy need to be evaluated and compared with the conventional glasshouse screening approach.

There are additional non-economic factors to consider; MAS has the potential to eliminate the problems associated with conventional screening experiments, which are complicated by the occurrence of different aphid biotypes, inoculation failures and the activities of predators of aphids. Indeed, differences in the aphid biotypes may explain the presence of the unclassified and "intermediate" plants, since a supplementary source of aphids of unknown biotype was used in the 1994 experiments.

As the genetic distance between a marker and an agronomic trait increases, the breeder will become more inclined to confirm the MAS result with a plant-based test. However, the gene for resistance to *D. devecta* is associated with three tightly linked markers, and any individual which contains these markers could be selected with a reasonable degree of confidence. For routine screening purposes, it may be possible to rely on one marker, MC064a or 2B12a, for example, both of which correctly classified more than 98% of the 'Prima' × 'Fiesta' population. The MAS result may have to be confirmed by an insect-based test at a later stage of the breeding programme: bioassays would be vital for progenies where the Sd_{pr} precursor gene may be absent in some individuals. The benefits of MAS become even more attractive when selection for more than one locus, for example, Sd_1 aphid resistance, *Vf* scab resistance (Gianfranceschi et al. 1996) and Pl_1 mildew resistance (Markussen et al. 1995) in apple, can be achieved by multiplexing of PCR reactions and gel separation.

It may be possible to devise a strategy that avoids total reliance on the Sd_1 resistance gene, which can be broken down by the rare biotype 3 (Alston and Briggs 1977). As markers for the Sd_2, Sd_3 and Sd_{pr} genes become available, MAS techniques could be used to combine two or more functionally distinct resistance genes in a single individual. For example, pyramiding the Sd_1 and Sd_3 genes would confer resistance to all three reported aphid biotypes. However, the relative importance of these biotypes is unclear: 'Cox' has been grown widely in the UK for many years, and there have been no reports of the breakdown of resistance in the field.

As the linkage map for the 'Prima' × 'Fiesta' progeny becomes more saturated, further markers flanking the resistance gene may be identified. This could be a useful start for linkage drag studies, map-based gene cloning and an increased understanding of the biochemical basis of the resistance mechanism.

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References

Alston FH, Briggs JB (1968) Resistance to *Sappaphis devecta* (Wlk.) in apple. Euphytica 17:468–472

Alston FH, Briggs JB (1977) Resistance genes in apple and biotypes of *Dysaphis devecta*. Ann Appl Biol 87:75–81

Baronio P, Briolini G (1985) Afidi delle Pomacee. Ital Agric 122:110–121

Bonierbale M, Plaisted RL, Pinedo O, Tanksley SD (1994) QTL analysis of trichome-mediated insect resistance in potato. Theor Appl Genet 87:973–987

Dicker GHL (1954) The apple, pear, and quince aphids. Rep E Malling Res Stn for 1953, East Malling, pp 213–217

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Gianfranceschi L, Koller B, Seglias N, Kellerhals M, Gessler C (1996) Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theor Appl Genet 93 : 199–204

Gratwick M (1992) Crop pests in the UK. Collected edition of MAFF leaflets, 1st edn. Chapman and Hall, London

Jenser G, Balázs K (1991) The possibilities and difficulties in introducing integrated pest management in apple orchards. Növényvédelem 27 : 97–102

King GJ (1994) Progress in mapping agronomic genes in apple (The European Apple Genome Mapping Project). Euphytica 77 : 65–69

King GJ (1996) Progress of apple genetic mapping in Europe. HortScience 31 : 1108–1111

King GJ, Alston FH, Battle I, Chevreau E, Gessler C, Janse J, Lindhout P, Manganaris AG, Sansavini S, Schmidt H, Tobutt K (1991) The 'European Apple Genome Mapping Project' – developing a strategy for mapping genes coding for agronomic characters in tree species. Euphytica 56 : 89–94

Maliepaard C, Bas N, Van Heusden S, Kos J, Pet G, Verkerk R, Vrielink R, Zabel P, Lindhout P (1995) Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*. Heredity 75 : 425–433

Markussen T, Krüger J, Schmidt H, Dunemann F (1995) Identification of PCR-based markers linked to the powdery-mildew-resistance gene Pl_1 from *Malus robusta* in cultivated apple. Plant Breed 114 : 530–534

Massee AM (1954) The pests of fruits and hops, 3rd edn. Crosby Lockwood, London

Mohan M, Nair S, Bentur JS, Rao UP, Bennett J (1994) RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor Appl Genet 87 : 782–788

Nienhuis J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci 27 : 797–803

Nieto-Lopez RM, Blake TK (1994) Russian wheat aphid resistance in barley: inheritance and linked molecular markers. Crop Sci 34 : 655–659

Penner GA, Stebbing J-A, Legge B (1995) Conversion of an RFLP marker for the barley stem rust resistance gene *Rpg1* to a specific PCR-amplifiable polymorphism. Mol Breed 1 : 349–354

Pfeifer B (1994) Erste Eindrücke von Schorf-Mehltauresistenten Apfelsorten an der SLVA in Ahrweiler. Obstbau 19 : 236–240

Rezwani A, Radjabi G (1987) The species of Aphididae family injuring rosaceous fruit trees in Iran. Entomol Phytopathol Appl 54 : 67–68, 165–178

Schön CC, Lee M, Melchinger AE, Guthrie WD, Woodman WL (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70 : 648–659

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J 3 : 739–744

Stam P, Van Ooijen JW (1995) JOINMAP™ version 2.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Van der Beek JG, Verkerk R, Zabel P, Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84 : 106–112

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18 : 6531–6535

Young ND, Kumar L, Menancio-Hautea D, Danesh D, Talekar NS, Shanmugasundarum S, Kim D-H (1992) RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata*, L. Wilczek). Theor Appl Genet 84 : 839–844