Mapping Quantitative Field Resistance Against Apple Scab in a 'Fiesta' × 'Discovery' Progeny

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ABSTRACT

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Breeding of resistant apple cultivars (*Malus × domestica*) as a disease management strategy relies on the knowledge and understanding of the underlying genetics. The availability of molecular markers and genetic linkage maps enables the detection and the analysis of major resistance genes as well as of quantitative trait loci (QTL) contributing to the resistance of a genotype. Such a genetic linkage map was constructed, based on a segregating population of the cross between apple cvs. Fiesta (syn. Red Pippin) and Discovery. The progeny was observed for 3 years at

three different sites in Switzerland and field resistance against apple scab (*Venturia inaequalis*) was assessed. Only a weak correlation was detected between leaf scab and fruit scab. A QTL analysis was performed, based on the genetic linkage map consisting of 804 molecular markers and covering all 17 chromosomes of apple. With the maximum likelihood-based interval mapping method, eight genomic regions were identified, six conferring resistance against leaf scab and two conferring fruit scab resistance. Although cv. Discovery showed a much stronger resistance against scab in the field, most QTL identified were attributed to the more susceptible parent 'Fiesta'. This indicated a high degree of homozygosity at the scab resistance loci in 'Discovery', preventing their detection in the progeny due to the lack of segregation.

Breeding apple cultivars with disease resistances has gained major importance as a response to consumer demands and environmental concerns, which require a reduction in the amount of fungicides applied to crops and introduced in the ecosystem. Approximately 43% of the pesticide expenses for fruit production in Switzerland concern fungicides, amounting to 4 Mio Euro per year (≈600 Euro/ha). A targeted deployment of resistance genes has the potential to drastically reduce this amount.

Apple scab caused by the fungus *Venturia inaequalis* is the most important disease in apple-growing areas with high spring and summer rainfall. The fungus infects leaf and fruit likewise. Leaf infection causes loss of assimilation surface and, in severe cases, complete defoliation of the tree, resulting in low yield. Additionally, early fruit infection causes deformed, unmarketable fruit, and late infection of fruit leads to losses due to 'storage' scab (37). In commercial orchards, up to 15 fungicide treatments per season are applied to control the disease.

Several major scab resistance genes have been known for a long time (55), originating mostly from small fruited Asiatic *Malus* spp. Three of them, *Vf*, *Vm*, and *Vr*, confer vertical resistance against apple scab, as defined by Vanderplank (51), while the other resistance genes (e.g., *Vb*, *Vbj*, and *Va*) (37) have not yet been characterized. *Vr* (from Russian Seedling R12740-7A), *Vbj* (from *Malus baccata jackii*), *Vb* (from *M. baccata*), *Vm* (from *M. micromalus*), and *Vf* (from *M. floribunda* 821) have been introgressed into breeding lines and selections to make them available for breeding purposes. Until now, only the *Vf* resistance has been incorporated into commercially available cultivars, and over 50

scab-resistant cultivars have been released since 1970 (9,20,28), all carrying this same resistance gene.

Such a major resistance gene shows a great effect when the pathogen lacks the matching virulence. However, this effect also enforces a high selection pressure onto the pathogen population, which has to circumvent only one single hindrance to be successful again. Most known major resistance genes are "recognition genes" (i.e., they allow the recognition of the pathogen and trigger a defense reaction) (3,26). The different resistance genes differ in their recognition ability, whereas the defense reactions may very well be the same in all cases.

As MacHardy et al. (38) stated, all major resistances in apple are ephemeral (38); therefore, it is only a question of time until the pathogen with the matching virulence appears and overcomes such a resistance. It was hardly a decade ago that the *Vf* resistance was reported to be overcome by the pathogen in several locations (2,41). *Vm* (10,54) and *Vr* were defeated (22,46,55; A. Patocchi, unpublished data) even before being used in commercial cultivars.

Many more than the described and named major resistance genes are present in apple, but undetectable in the field because they have been rendered ineffective by the pathogen's virulence alleles (15,16,32,38). The interactions between pathogen and host follow a gene-for-gene pattern (13); therefore, such resistances can be detected only with differential, monoconidial isolates representing a defined set of virulences and avirulences. The postulated ephemeral resistance gene (32) from 'Golden Delicious' recently was identified in this manner, named *Vg*, and mapped (12).

To reduce the risk of resistance breakdown and to achieve more durable resistances, it is necessary to broaden the genetic basis of resistance against apple scab. New sources of resistance should be discovered and tools have to be developed to exploit these resistances in breeding programs. A promising reduction of the risk of

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resistance breakdown is the combination of several functionally different resistance genes in a single cultivar, called pyramiding. Due to the unknown mixture of pathogen races in the field, the observed infection severity allows no conclusions with respect to number and type of resistance genes present in a new cross. To identify the resistance genes and determine their presence in an individual by inoculations with a differential set of pathogen races is impractical for breeding purposes because it is cumbersome and only a limited number of differential races is available.

The only practical way of pyramiding different resistance genes in a single cultivar is the application of molecular markers, closely linked and co-segregating with the desired genes. Such markers are known for *Vf* (14,19,33,48,49,56), *Vr* (22), and *Vm* (7), and more are being developed (C. Gessler, unpublished data).

New sources of resistance are being represented either by so far-unknown ephemeral (major) resistance genes, which might be successful in combination with other major genes (i.e., by pyramiding), or by combining with genes conferring quantitative or partial resistance against scab. Quantitative resistances have been described in apple for mildew (5,24,45) and scab (30,43) and are believed to be responsible for the phenotypic expression of the low scab susceptibility of certain apple cultivars carrying no known major resistances.

In contrast to major genes, partial or quantitative resistance genes as defined by Vanderplank (51), conferring race-independent, horizontal resistance (38,51), generally have smaller effects and therefore result in only low selection pressure on the pathogen. They are believed not to be based on recognition systems, as is the case with most major genes, but the possibility cannot be excluded (51). Also, the assumption that they are truly horizontal (i.e., race independent) may in some cases not be true. The distinction between major and quantitative genes is an artificial, theoretical construct; whereas, in reality, the borderlines blur as investigations on pathogen race-specific quantitative trait loci (QTL) show (42). To achieve a comparable level of resistance as with a major gene, several quantitative resistance loci would have to be combined. This necessarily also leads to a high selection pressure on the pathogen; however, in this case, the pathogen has to circumvent several different, unknown resistance mechanisms. Little is known about the modes of action of quantitative resistance genes and, therefore, on the probability of circumvention.

Due to the different recognition and defense mechanisms involved in pyramided major genes or in combined quantitative resistances (and the resulting high threshold for the pathogen to overcome), this strategy is believed to result in a more durable resistance. However, pyramiding resistance genes does not always lead to the aimed goal of a durable resistance, as work conducted in wheat against mildew showed (6). The durability of a resistance is dependent on the pathogen's evolutionary potential (40), and it might be necessary to selectively combine major and quantitative genes to achieve the desired durability in resistance.

With the availability of apple genetic linkage maps (8,23,36,39,45) and the possibility of calculating the positions of QTL on these maps (21,27,34,53), molecular markers for quantitative disease resistance can be developed.

The aim of this investigation was to determine whether single QTL with effects large enough to be detected despite a potentially large environmental variability could be found in a cross between the two apple cvs. Fiesta and Discovery. This cross was assessed in the field for 3 years and the resistance against apple scab was recorded. Several QTL were found and their observable effects as well as their positions in the genome are presented. The applicability of such resistances in resistance breeding is discussed.

MATERIALS AND METHODS

Plant material and orchard locations. Seeds of a cross between the apple cultivars Fiesta and Discovery, carried out in 1995 at Plant Research International, Wageningen, The Netherlands, were purchased by the Swiss Federal Institute of Technology, Zurich. Discovery, an open-pollinated descendant from 'Worcester Pearmain' and, supposedly, 'Beauty of Bath', was discovered in 1949, and is assigned a high, polygenic resistance to apple scab (29,43,47). Fiesta, introduced in 1985 as a cross of 'Cox's Orange Pippin' × 'Idared' from HRI East Malling, is reported to show no appreciable resistance against scab (1).

Seeds of this cross were sown in 1996 and grown on their own roots for 3 years at the Swiss Federal Research Station for fruit growing, viticulture, and horticulture (FAW), Wädenswil, Switzerland. The entire progeny of approximately 330 plants was triplicated in summer 1998 by bud grafting on M27 rootstocks and planted in the following winter at three different locations in Switzerland: Wädenswil (Zurich) at 47°13'20"N, 8°40'05"E, 455 m altitude; Conthey (Wallis) at 46°12'30"N, 7°18'15"E, 478 m altitude; and Cadenazzo (Ticino) at 46°09'35"N, 8°56'00"E, 203 m altitude. Five trees of each parental cultivar were planted in fall 2000 at each location. The trees were planted in rows 3.5 m apart and with tree-to-tree distances of 50 cm in Wädenswil and Conthey and of 125 cm in Cadenazzo. Orchard and plant maintenance included mulching between the rows, in-row herbicide treatments and sprays against aphids at all sites, and sprinkler watering in Conthey. No fungicide treatments and only a minimum of pruning were applied. Of the total progeny, 30 genotypes did not survive the 3 years of observation or were too small to be scored. Simultaneously with the Fiesta × Discovery progeny, 275 genotypes of an unselected cross of 'Iduna' × 'A679-2', a *Vf* carrier (44), were planted in Wädenswil and in Conthey in the neighboring rows of the same experimental plot.

Climatic conditions at the three locations are characterized with the average temperature (°C), rainfall in millimeters, and hours of sunshine in the growing period between March and October of the years 1999 to 2001 (Table 1). Orchard-specific weather data for

TABLE 1. Climatic characterization of the three orchard locations^a

Location, year	Average temperature (°C) March–October	Deviation from standard (°C)	Total rainfall (mm) March–October	Rainfall % standard	Hours of sunshine, March–October	Sunshine % standard
Wädenswil						
1999	13.6	1.2	1,186	118.6	1,314	103.4
2000	13.9	1.5	1,045	105.6	1,414	108.5
2001	13.6	1.2	1,296	140.4	1,384	107.9
Conthey						
1999	14.7	1.4	475	131.8	1,580	95.7
2000	14.9	1.6	371	100.2	1,686	101.9
2001	14.6	1.3	505	136.5	1,649	98.9
Cadenazzo						
1999	16.2	1.5	2,179	155.7	1,347	88.0
2000	16.3	1.6	1,789	128.3	1,473	95.7
2001	15.8	1.1	1,493	113.7	1,585	103.7

^a Standard values are based on climatic data of the period 1960 to 1990.

the calculation of infection periods and reports on ascospore release were recorded in Wädenswil and Conthey with Luft HP-100 weather recording and disease prediction devices.

Scab inoculations. Ascospore infection pressure was artificially increased by distributing heavily scabbed apple leaves in the orchard. The leaves were collected in orchards within the neighboring 10 km of the experimental sites, carrying a local inoculum mixture. Leaves were collected mainly from 'Golden Delicious' (90%) and supplemented with infected leaves from 'Gala', 'Elstar', and individuals of the cross Iduna × A679-2 (45). To prevent the collected leaves from being blown away by wind or pulled below surface and eaten by insects and worms, 5 to 10 leaves were packed into a flat 15-by-20-cm glass fiber/nylon-mesh bag. The lower side mesh was a fine 1-mm net to protect the leaves from soil creatures, while the upper side consisted of a coarse 9-mm net to allow leaves to be weathered and ascospores to mature under natural conditions. The bags were fixed with tent pegs under the trees with a density of approximately one bag per eight trees. Such bags were installed at all three locations in late fall 1999 and 2000 and were removed again in June of the following spring after ascospore discharge. No herbicide treatment was applied while the inoculation bags were in the field.

Artificial inoculations with spore suspension were performed in spring 2000 in Wädenswil and Conthey. Scabbed leaves were collected in June from the same cultivars as described above at each site and conidia were washed off with water. Spore suspension (20 liters) was applied to the 330 trees with a motorized Birchmeier M-125 backpack sprayer. Spore concentrations at both sites were between 90,000 and 100,000 spores per ml, yielding between 5 and 6 Mio spores per tree. The inoculation in Conthey was carried out at night after 3 h of watering with an overhead sprinkler and at a temperature of 25°C. Trees in Wädenswil were inoculated in the morning after a rainy night and at a temperature of slightly below 20°C.

Detached leaves assay. The same local conidial inoculum as used for the artificial spray inoculations in Wädenswil was used for a detached leaf assay, as described in Yepes and Aldwinckle (57). Conidia concentration was adjusted to 300,000 spores per ml before droplet infection, and leaf discs were prepared for light microscopy as described in Gessler and Stumm (18).

Data collection. Scab assessments were made three to four times per season at each location at 4- to 6-week intervals, starting in May with the first primary infections and ending in August. A quantitative nine-step evaluation scale was used to determine the susceptibility of the investigated trees as follows: 1 = no visible symptoms; 2 = few small scab spots detectable on close scrutiny; 3 = scab immediately apparent; 5 = infection widespread over tree; 7 = multiple lesions per leaf or large surfaces covered by

scab on most leaves; and 9 = nearly all leaves black with scab, eventually scabbed leaf fall. Steps 4, 6, and 8 were intermediate classes (scale developed by L. Parisi, *personal communication*).

In 2001, scab also was evaluated on the harvested fruit on a 0-to-3 scale as follows. 0 = no scab; 1 = fruit not deformed, normal sized, no depressions, only few spots visible and fruit has to be turned and inspected carefully to find spots of <3 mm; 2 = fruit is not deformed or only with light depression at the site of the large flecks (>1 cm), which can be seen on superficial inspection; and 3 = fruit show severe deformations due to early scab infection, are often undersized and cracked (scale developed by G. King, *personal communication*). Fruit were assigned to one of the four infection classes and average fruit scab values were calculated for each tree.

Data analysis. Plants which had not been genotyped previously (31 random plants) (36) or outcrossed individuals (15 plants) were excluded from the analyses or analyzed separately. A set of 251 phenotyped and genotyped individuals was available for trait analyses. Scab scores were averaged over all evaluations per year for each location and individual. Additionally, the maximum score per season was determined for each plant.

The statistical analysis of yearly scab evaluation averages per individual at each location, yearly scab evaluation maxima per individual at each location, and calculated fruit scab values was performed with S-Plus 6.0 (Insightful Corp., Seattle, WA). The phenotypic effect was estimated for each genotype by means of a linear model analysis of variance (ANOVA) with genotype, location, and year as explanatory factors. The amount of total variability explained by those factors was estimated from the type III sum of squares. We did not take into account the somewhat skewed distribution of the averaged disease scores. In the first step of the data analysis, for each genotype we estimated only an effect corrected for location and year. The scale is limited and the genotypes have similar distributions; therefore the effect of distortion can be neglected. No test of significance was performed.

The trees were planted in the same order in all three locations; therefore, special attention was paid to neighboring effects. Data sets of each scab evaluation were tested for unexpected high numbers of infected, adjacent plants according to Vanderplank (50): $p = X \times ([X - 1]/n)$, where p is the expected number of infected pairs, n is the number of consecutive plants examined, and X is the number of infected plants observed.

QTL analysis. QTL analyses of the ANOVA estimates as well as of the average and maximum values per year and location were performed with MapQTL 3.0 (53). Both single-parent genetic linkage maps as well as the integrated linkage map of 'Fiesta' × 'Discovery' (36) were used to determine the positions and the effects of the QTL with the Kruskal-Wallis single-locus analysis

TABLE 2. Scab evaluation data of 251 progeny plants and 15 outcrosses at the three orchard locations over 3 years^a

Location ^b	1999			2000			2001		
	Max	Average	OC	Max	Average	OC	Max	Average	OC
Wädenswil									
Mean	1.53	1.22	1.40	1.46	1.19	1.80	1.90	1.59	2.80
Max	6.00	4.33	4.00	5.00	4.33	4.00	6.00	5.00	7.00
SD	1.03	0.51	0.77	0.94	0.42	1.00	1.06	0.77	1.67
Conthey									
Mean	1.28	1.12	1.29	1.18	1.08	1.40	1.53	1.32	1.69
Max	6.00	3.66	3.00	3.00	2.75	3.75	5.00	3.66	4.00
SD	0.75	0.34	0.52	0.44	0.25	0.78	0.75	0.51	0.95
Cadenazzo									
Mean	1.06	1.03	1.10	1.17	1.09	1.53	1.39	1.28	2.63
Max	6.00	4.00	2.50	5.00	3.33	4.00	4.00	4.00	7.00
SD	0.42	0.24	0.39	0.55	0.31	0.86	0.74	0.58	1.97

^a Each individual was evaluated three to four times per year and location. Max: only maximum scab scores for each progeny plant considered; Average: average scab scores for each progeny plant considered, and OC: average scab scores for each outcrossed plant is considered.

^b Mean = mean value calculated from 251 progeny plants and 15 outcrossed genotypes, respectively; Max = maximum value obtained by a single genotype; and SD = standard deviation of scab scores of 251 and 15 genotypes, respectively.

and the maximum likelihood-based interval mapping approach. The integrated map consists of markers with different segregation types; therefore, the 'all marker mapping approach' (31) was applied. This method employs not only the flanking markers but also markers from neighboring intervals to calculate the probabilities of the presence of QTL. For the interval-mapping approach, five neighboring intervals and a step size of 1 cM was applied.

RESULTS

Field scorings. *Leaf scab.* Scab scoring means of the field evaluations decreased slightly, but not significantly, from 1999 to 2000, and increased significantly from 2000 to 2001 in Wädenswil and Conthey; whereas, in Cadenazzo, a steady increase was observed (Table 2). Development and severity of scab epidemics in Wädenswil and Conthey were in good accordance with the recorded local weather conditions and ascospore reports.

The ranking of the three locations with respect to population mean scab incidence was stable over the 3 years. Trees in Cadenazzo always showed the lowest scab incidence in 2000 and 2001 together with plants in Conthey. Highest scab incidence for all 3 years was observed in Wädenswil. This ranking was emphasized when maximum scores were used instead of average values for the genotypes (Table 2). Although all 15 outcrossed individuals were identified (36), they remained planted in the orchard, and it is noteworthy that the outcrosses in 1999 showed a similar and, in 2000 and 2001, a significantly higher scab incidence than the rest of the population (Table 2; Fig. 1).

The parental cultivars were planted only in autumn 2000; therefore, only one season of field scorings is available. The scorings were averaged across all trees and evaluations per locations for both parental cultivars; averages for Fiesta were 2.7, 1.8, and 1.4 in Wädenswil, Conthey, and Cadenazzo, respectively. Discovery scorings averaged 1.0 in all locations, indicating no scab infections at all.

The distribution of plants in the different incidence classes confirmed the increase in scab incidence over time by a reduction in the number of plants in resistance class 1 and an increase in the number of plants in all higher classes (Fig. 2). A comparable distribution was obtained when not genotyped and outcrossed individuals were included, reaching score averages up to 4.8.

Variability of leaf scab incidence contributed by the genotype and by the environment were estimated at 30 and 10% of the total variability, respectively. An astonishingly high residual variability of 60% remained, representing the variability within genotypes. A detailed analysis, taking all interactions into account, revealed a negligible year–location interaction of less than 1% and an equal contribution of interactions between genotype–year, genotype–location, and the triple interaction genotype–year–location of approximately 20%. With yearly average scores and yearly maximum scores, identical variability proportions were obtained.

Fruit scab. Fruit scab scorings in 2001 confirmed the ranking of the location found with leaf scab, with Wädenswil providing best conditions for scab, followed by Conthey and Cadenazzo (Table 3). A low correlation was found between leaf scab and fruit scab incidence (Fig. 3). The correlation analysis (Pearson) with fruit and leaf scab scores revealed an r value of 0.41. Variability proportions of fruit scab incidence contributed by the genotype and the environment (=location) were estimated as 46 and 9%, respectively, and another relatively high residual-within-genotype variability of 45%.

Neighboring effects. The expected number of pairs of infected plants was calculated according to Vanderplank (50) for each row at each site on each evaluation. In approximately 50% of all evaluations with pairs of infected plants, significantly more pairs were observed than would have been expected. Furthermore, it was noticed that up to 25% of the observed infected pairs occurred also in one or both other locations.

QTL for scab resistance. QTL for scab resistance were found on seven linkage groups (LGs), six QTL associated with leaf scab

Fig. 1. Scab epidemic development in 3 years based on scab class means of 311 to 314 progeny plants and 15 outcrosses at the evaluation dates in Wädenswil, Conthey, and Cadenazzo. Arrows indicate the artificial conidia inoculation sprays in June 2000. Leaf scab: 1 = no visible symptoms, 2 = few small scab spots detectable on close scrutiny, and 3 = scab immediately apparent with lesions thinly scattered in the tree.

resistance and two with fruit scab resistance (Table 4). For both traits, the strongest QTL were mapped on LG 17 of both parents and are located very close to each other on the same parental chromosome. All leaf scab resistance QTL, except one, were detected in all environments over several years. On LG 10, the QTL could be mapped only with data from Wädenswil; no association was found in Conthey or Cadenazzo. Of all eight QTL, six were found to be inherited from Fiesta. Cumulatively, the single parent QTL explain 26 and 21% of the phenotypic variability, attributed to the genotypes in Fiesta and Discovery, respectively. The QTL on the integrated map explained between 4 and 23% of the variability and up to 55% for leaf scab incidence and 16% for fruit scab incidence, respectively.

DISCUSSION

Phenotypic data. Scab incidence on the test plants was found to be low in all three environments and increased only slightly over the 3 years of observation. A first reason for such a low disease incidence seemed to be the low infection pressure given by the fact that, in Conthey and Cadenazzo, no other apple trees were nearby which could have provided the necessary scab inoculum. The orchards were laid out new and there were no apple plantings before, which could have provided primary inoculum. Therefore, the amount of primary inoculum in the orchard was artificially increased at the beginning of the second and third year by distributing scabbed leaves under the plants. A second potential reason for the low incidence was the insufficient adaptation of the (local) pathogen populations to the genotypes of this new cross. This is supported by findings from Wädenswil and Conthey, where the experimental plants were neighbored by rows with 275 contemporaneously planted genotypes of an unselected cross of Iduna × A679-2 (*Vf* carrier) (45). In all 3 years, these plants showed significantly more disease symptoms than the Fiesta × Discovery progeny. Disease score averages of the non-*Vf*-carrier in the neighboring population reached up to 3.5 (data not shown), thus proving the presence of primary inoculum. This argument also was supported by microscopic observations of circular 5-mm leaf punches of the ‘detached leaves assay’. Conidia on the leaves, after 10 and 15 days at 18°C and 100% relative humidity, had formed germination tubes and eventually appressoria, but no further development was observed. In contrast, on identically treated Golden Delicious control leaves, successful infections and sporulation could be seen (data not shown).

With the genotyping of the apple population by means of molecular markers, such as random amplified polymorphic DNAs, amplified fragment length polymorphisms, and closely flanking markers (SSRs) (35,36), several outcrossed individuals were identified in this progeny. These plants, although not belonging to the full-sib family which was analyzed, remained in the field. The outcrosses showed much more disease and had, except in 1999, significantly higher mean scab incidences in all locations than the rest of the progeny (Table 2). The slow increase in scab incidence over the observation period indicates an ongoing adaptation of the pathogen population to the new apple genotypes. The increase observed in the outcrossed plants was much stronger than in the rest of the population; this may indicate that the Fiesta resistance was being eroded. The outcrossed individuals were descendants from Fiesta with an unknown father, lacking the Discovery resistance in their genomes. The observed difference in scab severity between Discovery descendants and outcrosses (non-Discovery descendants) suggests that the strong resistance in Discovery is highly heritable. The heavily affected outcrossed genotypes were regarded as valuable for the experiment because (i) they might, in fact, accelerate the adaptation of the pathogen population by providing an intermediate level of resistance, (ii) they proved that inoculum was present and that favorable infection conditions occurred, and (iii) they provided better adapted inoculum than the

imported Golden Delicious leaves. Therefore, they remained in the orchards. It certainly will be helpful to keep the population in all three locations under further investigation for the next few years in order to confirm the pathogen evolution by appropriate sampling and analyses.

Weather data recorded in Wädenswil and Conthey during the 3 years of investigation can further explain or confirm the epidemiological observations (Fig. 1). Ascospore discharge (primary season) in Switzerland is considered to be completed by mid-June (4,25) and later infections will depend solely on conidia. In Wädenswil, seven and nine infection periods occurred during primary scab season in 1999 and 2000, respectively. The not-well-adapted inoculum had comparable chances to infect the plants in both years. To develop a high scab incidence, conidia of successful pathogen races have to receive favorable conditions after primary season for secondary infections. In contrast to 1999, there were fewer infection periods for secondary infections in 2000, resulting in lower scab incidence. Only five infection periods in the primary season and occasional light infection periods later provided optimal conditions for the adapting scab population in 2001, starting with more primary infections than the years before and developing nicely during the season. The situation reconstructed for Conthey showed much bigger differences between the 2 years. Nine infection periods occurred in primary season 1999 compared with only three in 2000. The slightly better-adapted pathogen population was able to achieve more infections at the beginning, as the first evaluation in 2000 shows, but the adverse conditions during the season did not allow a substantial epidemic development; whereas, in 1999, the few successful pathotypes found optimal conditions for a mass development, which resulted in a higher average incidence. In Conthey, the 2001 epidemic started with nine infection periods in the primary season, providing good conditions for the infection and early development of the adapting pathogen population. The very few infection periods afterward, however, impeded the development in the later season.

The ineffectiveness of the artificial conidia inoculations performed in June 2000 is clearly shown in Figure 1. A much greater impact would have been expected with such a high number of conidia applied per tree. These observations give strong support to the assumption of a nonadapted pathogen population because the inoculation spray with scab inoculum originating mainly from Golden Delicious in Wädenswil remained without significant effect, although coinciding with a heavy infection period. The same probably is also true for the artificial infection with ascospores at the beginning of the second and the third year. However, the arti-

Fig. 2. Distribution of leaf scab scores for the 251 individuals of the ‘Fiesta’ × ‘Discovery’ population, averaged over three locations with three to four evaluations each. The means of the two parental cultivars scored only in 2001 are shown. Leaf scab: 1 = no visible symptoms, 2 = few small scab spots detectable on close scrutiny, and 3 = scab immediately apparent with lesions thinly scattered in the tree.

cial infections were ineffective only against Discovery descendants. The faster development of the disease in the years 2000 and 2001 on the outcrossed individuals (Fig. 1) might, in fact, be a consequence of the artificially increased ascospore density.

For the statistical analysis and QTL mapping, scab score averages over the evaluations in one season as well as maximum scores obtained have been used. Although both approaches delivered comparable results, the two methods are controversial. Assuming that the field scorings were not always impeccable and that some lesions have been overlooked, a number of individuals could have been scored healthy or at least healthier than they really were. This underestimation is mitigated with the consideration of maximum values, because infected plants are unlikely to be overseen in every evaluation. However, if the field scorings are considered accurate, it can be argued that moderate resistance and moderate to high resistance can be distinguished only with the use of average scores over all evaluations in one season. A genotype is regarded to be more susceptible if even a low incidence can be observed several times, indicating a slow disease development, compared with a genotype where low incidence was observed only once and the disease did not develop at all.

An ANOVA of the phenotypic data revealed a rather high ($\approx 60\%$) within-genotype variability (genotype-year, genotype-location, and genotype-year-location interactions) (i.e., larger differences between the scorings of the same genotypes than between the population means). Different infection pressures in different years can, especially with low disease incidence, lead to this kind of variability. Low infection in one year allows all genotypes to stay in lower classes 1 (healthy) or 2 (small spots detectable on close scrutiny). A slightly more severe infection in another year leaves the moderately resistant genotypes in class 2 and eventually 3 (scab immediately apparent), whereas the suscepti-

ble genotypes jump from class 2 to class 5 or even 6 (scab widespread over tree). Only the most susceptible plants were affected; therefore, the majority still scored in the lower classes and the population mean stayed low accordingly. Such changes of single genotypes increase the within-genotype variability fraction, which is expressed as genotype-year interaction. Location-specific pathogen populations are a possible reason for the variability expressed as genotype-location interaction. The scab resistance QTL on LG 10 is possibly a result of such a situation, because this QTL was detected only in Wädenswil, but in all 3 years of observation. Variability proportions accounted for by triple interaction, genotype-year-locations, take into consideration the different local weather conditions in one year causing a higher number of infection periods in one orchard compared with other orchards and other years, as discussed above.

As Gessler et al. (17) reported for downy mildew sampling in vineyards, a proportion of the variability can be due to the sampler. This is most likely true for all diseases of which the incidence or severity is scored rather than measured (e.g., by counting lesions, which are not immediately apparent due to their size and color, or due to a dense, compact foliage). The scoring accuracy not only depends on the education of the scorer, but also on his or her current physical and mental condition. Aware of this difficulty, the same scoring team was commissioned to all evaluations. To ensure the highest possible accuracy of the field data, one orchard was scored twice by different subgroups of the scoring team and the evaluations were compared. A good agreement among the scorers was found; therefore, the variability introduced by the samplers was considered to be low and not considered further.

The poor correlation between leaf and fruit scab incidence is surprising because fruit on trees with a high leaf scab incidence were subjected to a much higher infection pressure and, therefore, would have been expected to show higher fruit scab incidence as well. Fruit scab was recorded at the time of harvest; therefore, it is possible that the latest infections did not have sufficient time to develop visible lesions. A longer observation of harvested fruit for storage scab would be necessary. The low correlation could be confirmed by not finding QTL for the two traits on the same loci, thus leading to the conclusion that leaf and fruit scab resistance were independent and, in fact, implying a negative correlation between the two traits. This could be verified in a leaf scab independent fruit scab investigation.

Neighboring effects. In many cases, series of consecutive, infected plants involved outcrossed or other not genotyped individuals. Excluding these individuals from the very beginning would have reduced the number of pairs of diseased plants drasti-

TABLE 3. Fruit scab evaluation for all three orchard locations in 2001^a

Variables	Wädenswil	Conthey	Cadenazzo
No. of fruit-bearing trees	110	141	156
Scab score mean	0.40	0.15	0.12
Scab score maximum	2.57	2.00	2.00
Standard deviation	0.55	0.33	0.25

^a Harvested fruit of each tree were assigned to one of the four fruit scab classes: 0, healthy fruit; 1, fruit not deformed, only a few, small (<3 mm) spots visible; 2, fruit with light depression at the site of the large flecks (>1 cm); and 3, fruit shows severe deformations due to early scab infection. Fruit scab values for genotypes were calculated as follows: (number of fruit) $\times$ (fruit scab class)/(number of harvested fruit per tree).

Fig. 3. Unweighted averages of leaf scab versus fruit scab values of 208 fruit-bearing genotypes recorded in 2001, in Wädenswil, Conthey, and Cadenazzo. Leaf scab: 1 = no visible symptoms, 2 = few small scab spots detectable on close scrutiny, and 3 = scab immediately apparent with lesions thinly scattered in the tree. Fruit scab: 0 = healthy fruit; 1 = fruit not deformed, only few, small (<3 mm) spots visible; 2 = fruit with light depressions at the site of the large flecks (>1 cm); 3 = fruit shows severe deformations due to early scab infection. Fruit scab values for genotypes were calculated as follows: number of fruit $\times$ fruit scab class/number harvested per tree.

cally so that no unexpectedly high numbers of adjacent infected plants were present. However, the clearly existing neighboring effects always imposed a higher disease pressure on the same plants. Such plants might have been scored as more resistant when placed near other neighbors. The consequences of this underestimation of possibly moderately resistant genotypes are that the overall observed disease level was higher and the effect of resistance QTL was calculated too low. This would imply that detected QTL might, in fact, be stronger than reported here, and weak QTL probably were not even detected.

QTL. A QTL analysis always investigates the differences between parental alleles at a certain locus. These differences can range from nil to extreme and, accordingly, some QTL alleles are not detected or considered valuable for breeding and for improving future cultivars.

Low LOD QTL. Several QTL for leaf and fruit scab resistance in apple were found with the analysis of field data in combination with a molecular marker map. Although three of the QTL did not reach a LOD score of 2.5 or 3.0, representing a significance level of 95% in the single parent map or the integrated map, respectively (52), they still are presented in Table 4. The calculated low LOD scores can be due to the reported neighboring effects diluting the effect of the superior allele or alleles, thus reducing the phenotypic difference between the carriers of the alternative alleles. Or it can be the result of the actual small difference between the effects of the involved alleles. This is the case when both alleles from one parent or all four possible combinations of alleles from both parents contribute almost equally to the trait. A third possibility for a low LOD score is the genetic background. When several loci contribute to the same trait, the difference between the alleles at one locus can be masked by the effects contributed by the other loci from the same or the alternative parent (i.e., only a small proportion of the total, large variability can be attributed to one locus). Additionally, interactions between loci or between alleles of the same locus, or epistatic effects, can be responsible for a weak or undetectable effect of a QTL.

It has to be clear that such an observed weakness of the QTL is only relevant for the cross or parent under investigation, because the effect measured is the difference between the involved alleles and not necessarily the intrinsic quality of the QTL. Although statistically not significant, these QTL are believed to be mapped accurately and are not random artifacts. The repeated appearance of the QTL in the analysis of field data from different years and locations indicated a reproducible, albeit weak, effect.

Undetected QTL. Although cv. Discovery was used as the resistant parent (1), and current field observation confirmed its resis-

tance, the overwhelming majority of all detected resistance QTL was mapped and assigned to cv. Fiesta. As mentioned above, the detected effect of a QTL is based only on the difference between the involved alleles. In the case of Discovery, it must be assumed that other QTL are present and responsible for good resistance and that, except on LG 11 and LG 17, there are no differences between the resistance alleles at the same loci. The two alleles at each locus are similarly effective or even equal (i.e., Discovery is homozygous at the resistance loci involved). The conclusion that Discovery is not valuable for breeding because no or only few resistance QTL could be found would, in fact, be fatal. As the observation of the outcrossed individuals demonstrates, Discovery is a very potent parent for resistance breeding, where all descendants display a high level of field resistance against scab.

Determination of maximal QTL effect and applicability in breeding. Applicability and usefulness of QTL in resistance breeding depends on the “real” effect of the alleles and the ease with which they can be introgressed into commercial cultivars. The percentage of explained variability, delivered as a result of the QTL analysis, concerns only the phenotypic difference between the involved parental alleles. To estimate the real effect of a QTL allele, it has to be compared with an ineffective allele at the same locus. Crosses have to be performed between grand parents, such as Discovery and a susceptible cultivar, known to contribute nothing or very little to the trait under investigation (i.e., donor of the “ineffective allele”). In such a cross, heterozygous carriers of the “effective allele” have to be chosen as parents for the F₂. They must be crossed again, ideally with an ineffective cultivar. The difference between the grand parental alleles (effective versus ineffective) observed in the F₂ represents the real effect of the effective allele. However, the second parent of the F₂ is of minor importance because only the grandparental alleles in the Discovery descendants are of interest.

The difference between the QTL alleles on LG 11 and 17, explaining 11 and 23%, respectively, of the variability observed, represent promising starting points for resistance breeding. Being located in a map region densely populated with molecular markers makes the QTL on F17 the ideal candidate, because SSRs, which are usable in other crosses, exist already. They allow the QTL alleles to be traced in other progenies. To prove their usefulness, they must be tested for stability over a broader range of environments. Most important for their successful applicability in breeding programs will be their performance in different genetic backgrounds and their interactions with other loci, as well as their durability.

Comparison with reported scab resistance QTL or resistance modifiers. Four scab resistance QTL have been reported by Durel

TABLE 4. Quantitative trait loci (QTL) detected for leaf scab resistance and for fruit scab resistance in 251 individuals of a segregating population of ‘Fiesta’ × ‘Discovery’

LG ^a	Leaf scab resistance					Fruit scab resistance				
	Chr ^b	Pos (cM) ^c	LOD ^d	Expl (%) ^e	Integr ^f	Chr	Pos	LOD	Expl (%)	Integr
6	F+	65	3.1	6	2.9 (5%)	...	...	...	...	...
7	F+	65	2.3	4	2.3 (4%)	...	...	...	...	...
10	F+	14	2.2	4	3.1 (6%) ^g	...	...	...	...	...
11	F+	17	...	...	6.0 (11%)	...	...	...	...	...
	D+	16	4.2	8	...	...	...	...	...	...
12	F+	15	2.1	4	2.8 (6%)	...	...	...	...	...
15	...	...	...	...	...	F+	10	2.5	6%	2.8 (7%)
17	F-	15	4.5	8	13.2 (23%)	F-	10	2.8	6%	4.9 (9%)
	D+	32	7.2	13	...	...	...	...	...	...

^a LG = linkage group according to Liebhard et al. (36).

^b Parental chromosome carrying the effective allele, identified by marker phase (36).

^c Position on the chromosome (36).

^d LOD score at this position calculated with MapQTL 3.0.

^e Percentage of explained phenotypic variability attributed to the genotype.

^f LOD score and percentage of explained phenotypic variability attributed to the genotype (in parentheses) calculated on the integrated map of both parental cultivars (36).

^g QTL was detected only with data from Wädenswil.

et al. (11). Two of them are located on the same LGs as the QTL described here (LG 10 and 17), while two others, supposedly located on LG 2 and 3, could not be detected in the current analysis. The already defeated, major resistance gene *Vg*, conferring resistance to race 6 of *V. inaequalis* (12), though located on LG 12, does not coincide with the scab resistance QTL on the same LGs described here. Neither Fiesta nor Discovery carry *Vg*, which would not have been detected here because mainly Golden Delicious inoculum was used for the artificial infections.

Scab resistance QTL and *Vf* scab resistance modifier genes in a cross between Iduna and A679-2 have been described for several LGs (44). One QTL, located on the LG I7/A3, which corresponds to LG 11 in Fiesta × Discovery, is most likely the same as the one reported here. It has a comparable position in relation to the SSR marker CH02d12 and, therefore, is suspected to be the same. A closer investigation of the QTL found on I5/A7, which corresponds to LG 12 in Fiesta × Discovery (35,36) as well as to LG 12 in Prima × Fiesta (39), and a comparison of the SSR positions (E. Van de Weg, unpublished data; 35) suggest that this QTL corresponds to the *Vg* locus (12).

Outlook. To confirm the above speculations, future disease development has to be observed in the orchards and changes in the pathogen population should be monitored. The effectiveness of the detected QTL has to be confirmed at higher disease levels as well as in other genetic backgrounds.

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