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Evaluation of apple genotypes and *Malus* species for resistance to Alternaria blotch caused by *Alternaria alternata* apple pathotype using detached-leaf method

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Abstract

Alternaria blotch, caused by *Alternaria alternata* apple pathotype, is an economically important disease of apple (*Malus* × *domestica* Borkh.). Information on the resistance level of apple cultivars to Alternaria blotch is the key to a strategy for integrated control of the disease, however, such information on new apples recently released or on scab-resistant apples is lacking. The aim of this study was to develop an advanced technique, which allowed one a reliable assessment of apples for resistance to Alternaria blotch, and to evaluate resistance levels of various apple genotypes, especially scab-resistant apples. Detached-leaves, youngest opened leaf (leaf 1), second (leaf 2), third (leaf 3), fourth (leaf 4) and fifth (leaf 5) at shoot tip removed from growing shoots of four cultivars, were inoculated with conidial suspension of *A. alternata* apple pathotype, and the responses were rated at 48-hour postinoculation; a modified six-class disease scale was established for the rating system of Alternaria blotch. Leaf 1–3 were suitable for the evaluation and discrimination of resistance level of apple genotypes to the disease. Reliability of assessment was demonstrated in successive 3-year inoculation tests with detached-leaves of leaf 2 on 16 cultivated apples; incidence of the disease caused by natural infection of *A. alternata* at a field was well correlated with the resistance levels determined by the detached-leaf method among 11 apples. Two-year inoculations using detached-leaf method on 41 apple genotypes revealed that 17 scab-resistant apples, 'Freedom', 'Orlovim', 'Orlovski Pioner, 'Pervinka, 'R12740-7A', 'Regrindis', 'Antonovka', 'Dayton', 'Florina-Querina', 'Galarina', 'Liberty', 'Redfree', 'Reanda', 'Remo', 'Priam', 'Regine' and 'Priscilla', showed no visible symptoms and were regarded as resistant (R) to *A. alternata* apple pathotype. In addition to the above genotypes, five scab-resistant genotypes, 'GoldRush', 'Prima', 'Renora', 'Rewena' and 'Retina', showed pit-type small spots on a few inoculated leaves and were also included in this category of resistance level. Three scab-resistant genotypes, 'Jonafree', 'Reka' and 'Co-op 25,' exhibited extended necrotic lesions in the inoculated leaves and were regarded as susceptible (S) to the disease. Among 38 cultivated apples and selections of Japanese origin, 27 genotypes were R; 'Kitaro', 'Kotaro', 'Santaro', 'Chinatsu', 'Shinano Sweet', 'Shinano Red', 'Shinano Gold', 'Aori 13', 'Akibae', were included in this category, seven genotypes were S; 'Beninomai', 'Natsumidori' and several selections were included in S category, and the remaining four genotypes were moderately resistant. Parentage information clearly indicated that source of the susceptibility in the cultivated apples and selections developed in Japan were 'Delicious' or 'Indo'. In contrast to the genotypic differences for levels of resistance to *A. alternata* apple pathotype in cultivated apples, all *Malus* spp. tested exhibited complete resistance to *A. alternata.*

Key words: *Malus* × *domestica* Borkh. — *Alternaria alternata* apple pathotype — artificial inoculation

Introduction

Alternaria fungi cause two different diseases of apple (*Malus* × domestica Borkh.): Alternaria blotch and moldy core. Initial symptoms of Alternaria blotch, caused by *Alternaria alternata* apple pathotype, appear on the leaves in late spring to early summer as circular brown or blackish spots, gradually enlarging to 2–5 mm in diameter. Some spots exhibit secondary expansion, becoming irregular necrosis and infected leaves are often abscised early in the season, resulting in serious defoliation and decreased fruit quality and marketability. *Alternaria alternata* apple pathotype has a distinct and limited host range characterized by the production of host-selective (or host-specific) toxins (HSTs) essential for pathogenesis (Kohmoto and Otani 1991, Otani et al. 1995). Moldy core of apple, caused by *Alternaria* spp., is a postharvest disease and produces internal decay of commercial apples. The disease is characterized by the growth of fungal mycelium within the locules and may become invasive and lead to a slow, dry rot confined to the flesh immediately around the core (Reuveni et al. 2002); it is not associated with the production of HSTs.

Alternaria blotch of apple is an economically important disease in Japan and other Asian countries (Sawamura 1990). An outbreak of the disease in Japan was noted in 1956 and disease occurrence appeared to correspond to increased cultivation of highly susceptible cultivar 'Starking Delicious', a red-sport of 'Delicious', in Japan (Filajdic and Sutton 1991). *Alternaria alternata* apple pathotype (previously *Alternaria mali* Roberts) was first described in 1924 in the United States by J. W. Roberts and has recently become a problem in the southeastern United States; Alternaria blotch has the potential of becoming an important disease throughout the apple-producing regions where susceptible cultivars, strains of 'Delicious', are grown (Filajdic and Sutton 1991). Although some fungicides are effective to reduce disease incidence and defoliation caused by *A. alternata* apple pathotype (Filajdic and Sutton 1992), the cultivation of apple varieties resistant to Alternaria blotch appears to be the most effective and favourable method of controlling the disease, since the apple industries in most developed countries are currently under pressure to produce high-quality fruit while minimizing the use of agricultural chemicals.

Therefore, information on the resistance and/or susceptibility levels of apple cultivars to Alternaria blotch is needed; such information is the key to a strategy for integrated control of

the disease. There are some reports on the degree of resistance and/or susceptibility of several apple cultivars to *A. alternata* apple pathotype based on artificial inoculation either in the field or in the greenhouse, or the inoculation using leaves on excised growing shoots in the laboratory (Sawamura and Yanase 1963, Saito and Takeda 1984, Filajdic and Sutton 1991). The results of the ranking or grouping of apple cultivars on their relative resistance have presented useful information on some apple cultivars. However, there is still inconsistency in the results on the ratings of resistance levels on several apples: one popular apple, 'Fuji', was evaluated as resistant to *A. alternata* apple pathotype (Saito and Takeda 1984) while it was regarded as moderately resistant (MR) (Saito et al. 2001). Moreover, either information on new apple cultivars recently released has been increasing the importance of apple production worldwide, or materials for apple breeding are lacking in relative resistance and/or susceptibility levels to the virulent fungus. These factors imply that standardized inoculation methods and disease assessment should be determined to obtain reproducible and reliable results on the resistance to the disease and that comprehensive evaluation of the resistance should be required using apple cultivars.

Development of disease-resistant cultivars has a high priority in most apple breeding programmes throughout the world (Kellerhals and Furrer 1994, Lespinasse et al. 2000). Because there are several economically important diseases for apple production, introduction of multiple resistances into one genotype has been currently undertaken (Fischer 1994, Fischer and Richter 1996). In apple breeding in Japan, breeding for resistance to scab caused by *Venturia inaequalis*, as well as resistance to Alternaria blotch, has been concentrated; crossing of current scab-resistant cultivars with major and/or promising new apples with high dessert quality has been conducted to obtain hybrids resistant to both scab and Alternaria blotch. Nevertheless, there is no available information for the scab-resistant apples on their resistance levels to Alternaria blotch.

The main objectives of our study are (i) to develop a suitable inoculation and disease assessment system for Alternaria blotch of apple using detached leaves to obtain reliable results on the evaluation for disease resistance; and (ii) to evaluate resistance levels of various scab-resistant apple cultivars and new cultivars/selections recently developed in Japan that have not been rated for resistance to *A. alternata* apple pathotype. In addition, we evaluated wild *Malus* species for resistance level to the disease, to gain a clue in which *Malus* species, the resistance or susceptibility to *A. alternata* apple pathotype had originated and how the resistance and/or susceptibility in specific *Malus* was involved in the resistance (susceptibility) of modern apple cultivars. This paper describes the influence of leaf age on the evaluation of resistance to *A. alternata* apple pathotype, and genotypic difference for the resistance; most genotypes including scab-resistant cultivars were regarded as resistant or highly resistant to the fungus. All the *Malus* spp. used in the study exhibited highly resistant response to *A. alternata* apple pathotype.

Materials and Methods

Plant materials: Four cultivated apples, 'Fuji', 'Orin', 'Gala' and 'Starking Delicious', were used for the test to determine the leaf position suitable for the inoculation with isolate AKI-3 of *A. alternata* apple pathotype. The reported resistant cultivar 'Gala' was used as a resistant control, and the 'Starking Delicious' apple was included as a susceptible control to check the pathogenicity of the inocula. To examine yearly fluctuation in the response of detached apple leaves to *A. alternata* apple pathotype and to confirm the correlated response between inoculation tests and disease incidence at a field, 16 cultivated apple, 'Fuji', 'Tsugaru', 'Orin', 'Starking Delicious', 'Jonathan', 'Hokuto', 'Mutsu', 'Ralls Janet', 'Indo', 'Sansa', 'Jonagold', 'Golden Delicious', 'Kinsei', 'Sekaiichi', 'Redgold' and 'Gala', were used for the inoculation tests and/or for survey of disease incidence caused by natural infection. Forty-one cultivated apples and 33 *Malus* species and interspecific hybrids, originating from different geographic locations (Table 3) in a field of the National Institute of Fruit Tree Science (NIFTS), Morioka, Japan, were subjected to the inoculation test to evaluate their resistance levels against *A. alternata* apple pathotype. Additional 75 genotypes including 38 cultivars and selections developed in Japan (Table 4) in a field of NIFTS were also tested for the resistance levels.

Preparation of inoculum: The monoconidial isolate AKI-3 of *A. alternata* apple pathotype (previously *Alternaria mali* Roberts), originating from an infected apple tree in Akita Prefecture, Japan, was used. The isolate was multiplied on potato dextrose agar medium for 7–10 days at 25°C under continuous fluorescent light. After a 7- to 10-day incubation, conidia formed by the isolate were collected in the sterile distilled water and the number of conidia was adjusted to a concentration of $1–5 \times 10^5$ conidia/ml. The prepared conidial suspensions were preserved at −80°C until use.

Inoculation tests using detached-leaf of different leaf position: Leaves of four apple cultivars, 'Fuji', 'Orin', 'Starking Delicious' and 'Gala', were collected from field-grown trees in NIFTS from June to July. To examine the relationship between leaf position (leaf age) and resistance levels to *A. alternata* apple pathotype and to determine suitable leaf position for the inoculation with detached-leaf, youngest opened leaf at shoot tip (leaf 1), second youngest leaf (leaf 2), third youngest leaf (leaf 3), forth youngest leaf (leaf 4) and fifth youngest leaf (leaf 5) removed from growing shoots in each cultivar were used for the inoculation tests. After mild rinsing of leaf surface with sterilized water, the leaves were dried with a laboratory towel and placed in plastic chambers. Each chamber had a wet paper towel on the bottom for maintaining high relative humidity. Five leaves of respective leaf-position were subjected to the inoculation in each cultivar.

Conidial suspensions that were preserved by freezing were thawed at room temperature and the concentration was re-adjusted to 5×10^4 conidia/ml for the inoculation test. The leaves were then inoculated with a conidial suspension applied with a mist-sprayer to the whole surface of the leaves. Inoculated leaves in the chamber were incubated at 20°C for 48 h in the dark; thereafter, the leaves were visually assessed for resistance levels. The inoculation tests were repeated twice in two separate years.

The non-inoculated controls of different leaf position (three leaves per respective leaf position) in the four apples, which received sterilized water instead of a conidial suspension, exhibited no visible symptoms in all leaves irrespective of leaf position or genotype.

To investigate yearly fluctuation for the response of apple leaves to *A. alternata* apple pathotype, 16 cultivated apples were subjected to the inoculation tests for three successive years from June to July. In the inoculation tests with the 16 cultivars, young unfolded leaves removed from second position of growing shoot in each genotype were used; five leaves of each genotype were subjected to the inoculations. Inoculum, inoculation method and incubation condition were the same as above.

In the first year, three young leaves of each cultivar received sterilized water instead of a conidial suspension as non-inoculated controls except for 'Redgold' and 'Gala'; the non-inoculated controls of the 14 genotypes exhibited no visible symptoms in all leaves irrespective of genotype.

Evaluation of cultivated apples and *Malus* species for resistance levels: Five leaves (leaf 2 and/or leaf 3) collected from cultivated apples and *Malus* species presented in Tables 3 and 4 were inoculated

with the conidial suspensions as described above. After 48-hour incubation at 20°C in the dark, each inoculated leaf was rated for resistance levels. For the cultivars and *Malus* spp. listed in Table 3, the inoculation tests were repeated twice in 2007 and once in 2008; the results obtained for each genotype in 2007 were the same. Inoculation tests were carried out in 2005 and/or 2007 for the cultivars listed in Table 4.

Disease assessment: Disease assessment was performed 48-h postinoculation. The type of response of inoculated leaves observed for the genotypes was categorized using the scores of Saito and Takeda (1984) with modification. The score in our study contains six categories of response: score 0 = no visible symptoms; score 1 = pit-type small spots, < 1 mm in diameter, scattered on leaf surface; score 2 = one or two small necrotic lesions, < 10% of leaf area, with pit-type spots; score 3 = 10–50% of necrosis of inoculated leaf; score 4 = > 50% of necrosis of inoculated leaf and score 5 = almost complete necrosis of whole leaf. According to the criteria of Saito and Takeda (1984), leaves of score 3–5 were considered to be susceptible while leaves of either score 0 or 1 to be resistant. Reaction type of score 2 in our study (Fig. 1) was not described in their report; the response of this type was determined to be intermediate between resistant and susceptible response. Evaluation of each genotype (accession) for resistance levels to *A. alternata* apple pathotype was made by the values of mean scores that were the average of scores of each inoculated leaf in the genotype. According to the modified scoring system, we regarded the resistance levels for each genotype as follows; resistant (R): mean score ≤1; moderately resistant (MR): 1 < mean score ≤2 and susceptible (S): 2 < mean score.

To confirm the reliability of assessment by detached-leaf method, survey for the incidence of the disease development caused by natural infection in 11 cultivars ('Fuji', 'Tsugaru', 'Orin', 'Starking Delicious', 'Jonathan', 'Hokuto', 'Mutsu', 'Sansa', 'Jonagold', 'Golden Delicious', 'Gala') at an orchard in NIFTS was carried out at middle July in 2007. Five to 10 succulent growing shoots per genotype were randomly selected, then youngest 10 leaves of each shoot were assessed for the disease development according to the scoring system described above.

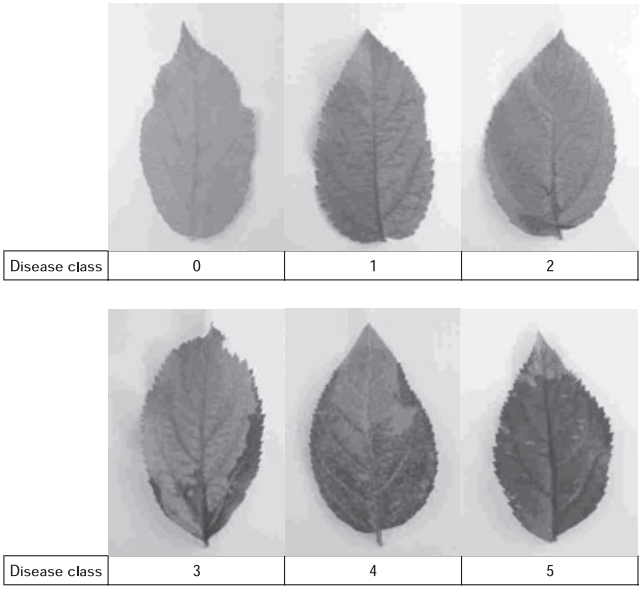

Fig. 1: Disease scale with six classes for scoring Alternaria blotch symptoms. Score 0 = no visible symptoms, leaves apparently healthy; score 1 = pit-type small spots, < 1 mm in diameter, scattered on leaf surface; score 2 = a small necrotic lesion, < 10% of leaf area, with pit-type spots; score 3 = 10–50% necrosis of inoculated leaf; score 4 = > 50% necrosis of inoculated leaf; score 5 = almost complete necrosis of whole leaf

Statistical analysis was carried out using statistical computer software package EXCEL STATISTICS VERSION 6.0 (ESUMI CO., Ltd, Tokyo, Japan). Analysis of variance for the categorical data, on the inoculation test using detached leaves of different leaf position, was performed using a non-parametric test according to Kruskal–Wallis followed by a multiple comparison of mean scores according to Steel–Dwass. For the inoculation test on the yearly fluctuation of the response using 16 apple cultivars, analysis of variance for the data on each cultivar was carried out using only a non-parametric test according to Kruskal–Wallis.

Results

Determination of leaf position suitable for inoculation test

The mean score of diseased leaves inoculated with *A. alternata* apple pathotype differed among different leaf positions in several apple genotypes (Table 1). In 'Fuji', 'Orin' and susceptible control 'Starking Delicious', the values of mean score and severest score (SS) of disease class in younger leaves, leaf 1, 2 and 3 from shoot tip, were higher than those of leaf 4 and 5 in the two successive years. Analyses of variance on categorical scores showed that there was a significant difference for the mean scores of inoculated leaves among leaf positions irrespective of inoculation years in 'Fuji' ($P = 0.01$), 'Orin' ($P = 0.01$) and 'Starking Delicious' ($P = 0.05$), while no significant difference was observed for the mean scores between years regardless of leaf position in the genotypes. In resistant control 'Gala', the reaction of inoculated leaves was completely the same, and no visible symptoms were observed regardless of either leaf position or year of inoculation.

The overall mean scores of the two successive years also differed among different leaf position in 'Fuji', 'Orin' and 'Starking Delicious.' The statistical test of multiple comparison showed that mean scores of leaf 1–3 were higher than those of leaf 4 and 5 in 'Fuji': mean score of leaf 1 tended to be the highest; mean scores of leaf 2 and 3 followed and leaf 4 and 5 were lowest in 'Orin'. In 'Starking Delicious', mean score of leaf 1 was higher than those of leaf 2–5. The resistance level of some apple genotypes to *A. alternata* apple pathotype was therefore influenced by the difference in leaf position. The resistance level of 'Fuji' and 'Orin' was rated as MR and S using leaf 1–3, while the resistance level was rated as R in 'Fuji' and 'Orin' using leaf 4 and 5. In 'Starking Delicious' and 'Gala', the resistance level was not affected by leaf position. The results in Table 1 suggest that young leaves of leaf 1–3 from growing shoots are suitable for the evaluation and discrimination of resistance level of apple genotypes to *A. alternata* apple pathotype.

The resistance level was seldom influenced by the difference in year of inoculation test when the resistance level of the 16 cultivated apples to *A. alternata* apple pathotype was tested by the application of a detached-leaf method using young leaves of leaf 2 and/or leaf 3 from growing shoots in three successive years. In 'Fuji', 'Orin', 'Starking Delicious', 'Mutsu', 'Ralls Janet', 'Indo', 'Jonagold', 'Golden Delicious', 'Kinsei', 'Sekaiichi' and 'Redgold', the difference in mean scores was not statistically significant among the three successive years and thus the resistance levels were the same regardless of different test years in the cultivars (Table 2). Although the mean scores were different among the years in 'Hokuto' ($P = 0.01$) and 'Mutsu' ($P = 0.05$), the resistance levels in 'Hokuto' and 'Mutsu' were determined to be S and were the same irrespective of differing test year. In 'Tsugaru', 'Jonathan', 'Sansa' and

Table 1: Influence of leaf position on the evaluation for resistance levels of detached leaves of apple genotypes to *Alternaria alternata* apple pathotype

		2006		2007		Pooled	
Genotype	Leaf position	Mean score	S S	Mean score	S S	Mean score[1]	Resistance level
Fuji	First	1.6	2	1.4	2	1.5a	MR
	Second	1.2	2	1.6	2	1.4a	MR
	Third	1.0	2	1.2	2	1.1ab	MR
	Fourth	0.4	1	0.2	1	0.3bc	R
	Fifth	0.2	1	0	0	0.1c	R
				Significance			
	Year	ns					
	Leaf position	**					
Orin	First	3.2	4	2.7	4	3.0a	S
	Second	2.2	3	2.4	3	2.3ab	S
	Third	2.2	3	2.0	3	2.1b	S
	Fourth	1.0	2	1.0	2	1.0c	R
	Fifth	1.0	2	1.0	2	1.0c	R
				Significance			
	Year	ns					
	Leaf position	**					
Starking Delicious	First	5	5	5	5	5a	S
	Second	4.6	5	4.6	5	4.6ab	S
	Third	4.2	5	4.6	5	4.4b	S
	Fourth	4.2	5	4.4	5	4.3b	S
	Fifth	4.0	5	4.2	5	4.1b	S
				Significance			
	Year	ns					
	Leaf position	*					
Gala	First	0	0	0	0	0 ns	R
	Second	0	0	0	0	0 ns	R
	Third	0	0	0	0	0 ns	R
	Fourth	0	0	0	0	0 ns	R
	Fifth	0	0	0	0	0 ns	R
				Significance			
	Year	ns					
	Leaf position	ns					

SS represents severest score. Resistance levels were determined as follows; resistant (R): mean score ≤ 1; moderately resistant (MR): 1 < mean score ≤ 2; and susceptible (S): 2 < mean score.
**, * and ns represent significant difference at P = 0.01, difference at P = 0.05 and no statisical difference, respectively, by non-parametric test according to Kruskal–Wallis.
[1]Pooled mean scores with different letters are significantly different among leaf position by non-parametric multiple comparison test at P = 0.05 according to Steel–Dwass.

'Gala', all the inoculated leaves showed complete R reaction regardless of differing inoculation year.

Incidence of the disease caused by natural infection of *A. alternata* in the field was well correlated with the evaluation using the detached-leaf method in 11 cultivated apples (Table 2). No disease symptom was observed in the leaves of R cultivars 'Tsugaru', 'Jonathan', 'Sansa', 'Jonagold', 'Golden Delicious' and 'Gala', while more than 10% of leaves were seriously damaged and exhibited typical susceptible symptoms in the cultivars 'Orin', 'Starking Delicious', 'Hokuto' and 'Mutsu', hence these cultivars were regarded as S at field condition. Disease symptom was observed in several leaves of 'Fuji', but the symptom was restricted and different from those observed in the S cultivars. Hence 'Fuji' was regarded as MR at field condition. The resistance levels of the above cultivars were generally consistent to the results on the disease resistance levels reported previously (Table 2).

Differences in resistance levels among apple cultivars and *Malus* spp.

The results of inoculation with the conidial suspension of *A. alternata* apple pathotype using detached second youngest leaves, collected from growing shoots in respective apples, indicated that there was a marked difference in the responses of the inoculated leaves to the pathogen among cultivars and *Malus* species (Table 3). Nineteen cultivars showed no visible symptoms on all inoculated leaves, and these were regarded as R to *A. alternata* apple pathotype; scab-resistant 'Freedom', 'Orlovim', 'Orlovski Pioner', 'Pervinka', 'R12740-7A', 'Regrindis', 'Antonovka', 'Dayton', 'Florina-Querina', 'Galarina', 'Liberty', 'Redfree', 'Reanda', 'Remo', 'Priam', 'Regine' and 'Priscilla' were included in this category of resistance level. Besides the scab-resistant cultivars, 'Gala' and 'Granny Smith' exhibited no visible symptoms and were evaluated as R. Five scab-resistant cultivars, 'GoldRush', 'Prima', 'Renora', 'Rewena' and 'Retina', showed pit-type small spots in a few inoculated leaves and no symptoms in the other leaves in 2007 and/or 2008, and thus were regarded as R to *A. alternata* apple pathotype. In addition to the five cultivars, 'Cripp's Pink', 'Braeburn', 'Discovery', 'Fiesta', 'Honeycrisp' and 'Jonagored' also showed only pit-type symptoms and were regarded as R. While many cultivars were categorized as R for the resistance level against this pathogen, seven cultivars, 'Redgold', 'Northern Spy', 'Spartan' scab-resistant 'Jonafree', 'Reka', 'Co-op 25' and susceptible control 'Starking Delicious',

Table 2: Yearly fluctuation on the evaluation by detached-leaf method for resistance levels to *Alternaria alternata* apple pathotype and relationship of the evaluations between detached-leaf method and observation of the disease incidence at a field among 16 cultivated apples

	Evaluation by detached-leaf method								
	Mean score			Significance between years	Resistance level (RL)[2]			Incidence of Alternaria blotch at orchard[1]	RL reported in the literatures[4]
Genotype	2005	2006	2007		2005	2006	2007		
Fuji	1.1	1.1	1.4	ns	MR	MR	MR	MR	MR[4], R[6,8]
Tsugaru	0	0	0	–	R	R	R	R	R[4]
Orin	2.1	2.5	2.4	ns	S	S	S	S	S[8]
Starking Delicious	4.4	3.9	4.2	ns	S	S	S	S	HS[3,6], S[7–8]
Jonathan	0	0	0	–	R	R	R	R	R[3–8], MR[9]
Hokuto	2.9	3.1	4.0	**	S	S	S	S	S[8]
Mutsu	2.2	2.6	3.4	*	S	S	S	S	R[4], S[8]
Ralls Janet	0.5	0.4	0.8	ns	R	R	R	–	MS[3], MR[4–7]
Indo	3.8	4.1	4.4	ns	S	S	S	–	HS[3,4], S[5–9]
Sansa	0	0	0	–	R	R	R	R	
Jonagold	0.3	0.4	0.2	ns	R	R	R	R	
Golden Delicious	0.6	0.9	0.8	ns	R	R	R	R	MR[3,7], R[4,5]
Kinsei	2.9	3.3	3.6	ns	S	S	S	–	
Sekaiichi	1.1	1.4	1.6	ns	MR	MR	MR	–	
Redgold	3.0	–	3.2	ns	S	–	S	–	S[1,7]
Gala	0	0	0	–	R	R	R	R	R[9]

R, resistant; MR, moderately resistant or moderate; MS, moderately susceptible; S, susceptible; HS, highly susceptible.
**, * and ns represent significant difference at P = 0.01, difference at P = 0.05 and no statisical difference, respectively, by non-parametric test according to Kruskal–Wallis.
[1]Resistance levels were determined as follows; resistant (R): mean score < 1; moderately resistant (MR): 1 < mean score ≤ 2; and susceptible (S): 2 < mean score.
[2]Incidence of the disease for each genotype at orchard were determined according to the scoring system and criteria used in detached-leaf method, –: not tested.
[3]Sawamura and Yanase (1963).
[4]Tsuchiya et al. (1967).
[5]Tsuyama et al. (1971).
[6]Saito and Takeda (1984).
[7]Kitajima (1989).
[8]Saito et al. (2001).
[9]Miyashita et al. (2003).

exhibited extended necrotic lesions in the inoculated leaves and/or almost complete necrosis of the leaves and were regarded as S to *A. alternata* apple pathotype. Two cultivars of scab-resistant 'Sir Prize' and 'Rebella' showed one or two necrotic lesion(s), which were rather restricted to <10% of leaf area in a few leaves, and the other leaves of the cultivars showed R response; resistance level of the two cultivars was considered to be intermediate between R and S. Thus, 'Sir Prize' and 'Rebella' were evaluated as MR.

In contrast to the genotypic differences for levels of resistance to *A. alternata* apple pathotype in cultivated apples, all *Malus* spp. tested exhibited complete resistance to *A. alternata* despite the diversity of geographic locations and origin (Table 3).

There was also a discernible difference in the resistance levels to *A. alternata* among modern apple cultivars and selections developed in Japan (Table 4). The result indicated that among 38 apple cultivars and selections of Japanese origin, 27 genotypes were R; 'Kitaro', 'Kotaro', 'Santaro', 'Chinatsu', 'Shinano Sweet', 'Shinano Red', 'Shinano Gold', 'Aori 13', 'Akibae', were included in this category, seven genotypes were S; 'Beninomai', 'Natsumidori' and several selections were included in S category, and the remaining four genotypes such as 'Kio' was MR. Among the 37 old cultivars, 23 cultivars were R; 'Rome Beauty' and 'McIntosh', which were regarded as important founding clones in modern apple breeding worldwide as well as 'Golden Delicious' and 'Jonathan', belonged in this category, 'Cogswell' was MR, whereas the remaining 13 cultivars were S.

Discussion

A detached-leaf inoculation method using leaves of similar age seems to be advantageous in obtaining reproducible results on the rating for resistance to *A. alternata* apple pathotype over traditional inoculations using all leaves varying in leaf position (leaf age), attached to growing apple shoots, in either field or laboratory tests, because difference in leaf position affects the assessment of apple genotypes for the resistance level. In the detached-leaf inoculations, younger leaves tended to show higher susceptibility than older leaves removed from lower positions in the growing shoots of apple genotypes (Table 1). A similar tendency was reported in *Venturia inaequalis* – an apple pathosystem in which old leaves became 'resistant' even in susceptible genotypes; the resistance observed in the older leaves to *V. inaequalis* was considered as ontogenic non-race-specific resistance (Schwabe 1979, Gessler and Stumm 1984). Since resistance and/or susceptibility levels are often known to vary among genetically identical plants and/or organs of different ages (Bell 1981), as is the case of *A. alternata* apple pathotype – apple pathosystem, the detached-leaf method with leaves of similar age offers a reproducible alternative to the traditional inoculation methods for resistance to *A. alternata* apple pathotype. Besides the *A. alternata* apple pathotype – apple pathosystem, inoculation methods using detached leaves have been efficiently applied for the ratings of resistance levels to pathogenic species of *Alternaria* e.g. *A. alternata* Japanese pear pathotype (Kozaki 1973, Sanada et al. 1988),

Table 3: Difference in the resistance levels to *Alternaria alternata* apple pathotype among cultivared apples and diverse *Malus* species

Genotype	Species	Response of inoculated leaves				Resistance level	Remarks[1]
		2007		2008			
		Mean score	S S	Mean score	S S		
Starking Delicious	*Malus* × domestica Borkh.	4.0	5	3.8	5	S	Susceptible control
Gala	*M.* × domestica Borkh.	0	0	0	0	R	Resistant control
Freedom	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Goldrush	*M.* × domestica Borkh.	0.2	1	0	0	R	*Vf*
Orlovim	*M.* × domestica Borkh.	0	0	0	0	R	*Vm*
Orlovski Pioner	*M.* × domestica Borkh.	0	0	0	0	R	*Vm*
Pervinka	*M.* × domestica Borkh.	0	0	0	0	R	*Vm*
Reka	*M.* × domestica Borkh.	2.8	4	4.2	5	S	*Vr*
R12740-7A	*M.* × domestica Borkh.	0	0	0	0	R	*Vr*
Reglindis	*M.* × domestica Borkh.	0	0	0	0	R	*Va*
Antonovka	*M.* × domestica Borkh.	0	0	0	0	R	*Va*
Prima	*M.* × domestica Borkh.	0.2	1	0	0	R	*Vf*
Co-op 25	*M.* × domestica Borkh.	2.6	4	3.6	4	S	*Vf*
Dayton	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Florina-Querina	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Galarina	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Jonafree	*M.* × domestica Borkh.	2.6	4	3.8	5	S	*Vf*
Liberty	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Renora	*M.* × domestica Borkh.	0.4	1	0	0	R	*Vf*
Redfree	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Rebella	*M.* × domestica Borkh.	1.2	2	1.2	2	MR	*Vf*
Reanda	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Rewena	*M.* × domestica Borkh.	0.2	1	0	0	R	*Vf*
Remo	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Retina	*M.* × domestica Borkh.	0.6	1	0.2	1	R	*Vf*
Priam	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Regine	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Priscilla	*M.* × domestica Borkh.	0	0	0	0	R	*Vf*
Sir Prize	*M.* × domestica Borkh.	1.2	2	1.0	2	MR	*Vf*
Redgold	*M.* × domestica Borkh.	3.2	4	3.2	4	S	
Cripp's Pink	*M.* × domestica Borkh.	0.2	1	0	0	R	
Granny Smith	*M.* × domestica Borkh.	0	0	0	0	R	
Northern Spy	*M.* × domestica Borkh.	4.0	5	3.2	4	S	
Spartan	*M.* × domestica Borkh.	3.2	4	4.2	5	S	
Braeburn	*M.* × domestica Borkh.	0.2	1	0	0	R	
Discovery	*M.* × domestica Borkh.	0.2	1	0.2	1	R	
Fiesta	*M.* × domestica Borkh.	0.2	1	0	0	R	
Honeycrisp	*M.* × domestica Borkh.	0.2	1	0	0	R	
Greensleeves	*M.* × domestica Borkh.	0	0	–	–	R	
Idared	*M.* × domestica Borkh.	0	0	–	–	R	
Jonagored	*M.* × domestica Borkh.	0.4	1	–	–	R	
M. sieversii W1-10-49	*M. sieversii* (Lodeb.) Roem.	0	0	0	0	R	
Nagano Borkhousen	*M. prunifolia* Borkh.	0	0	0	0	R	
Marubakaido	*M. prunifolia* Borkh.	0	0	0	0	R	
M. baccata 79091	*M. baccata* Borkh.	0	0	0	0	R	
M. baccata S1-7-15	*M. baccata* Borkh.	0	0	0	0	R	
Nikko Zumi	*M. baccata* Borkh.	0	0	0	0	R	
Mokoto	*M. baccata* Borkh.	0	0	0	0	R	
M. sieboldii Mo 15	*M. sieboldii* Rehd.	0	0	0	0	R	
M. sieboldii Mo 65	*M. sieboldii* Rehd.	0	0	0	0	R	
Sanashi 61	*M. sieboldii* Rehd.	0	0	0	0	R	
Sanashi 63	*M. sieboldii* Rehd.	0	0	0	0	R	
Hayanarisanashi 1	*M. sieboldii* Rehd.	0	0	0	0	R	
M. sylvestris S1-13-17	*M. sylvestris* Mill.	0	0	0	0	R	
M. sylvestris 392390	*M. sylvestris* Mill.	0	0	0	0	R	
M. orientalis W1-11-13	*M. orientalis* Uglitzk. Ex Juz.	0	0	0	0	R	
M. orientalis NA 56041	*M. orientalis* Uglitzk. Ex Juz.	0	0	0	0	R	
M. angustifolia	*M. angustifolia* Michx.	0	0	0	0	R	
Waringo (Kagahan Zairai)	*M. asiatica* Nakai	0	0	0	0	R	
Kanazawa Zairai	*M. asiatica* Nakai	0	0	0	0	R	
M. asiatica Nakai	*M. asiatica* Nakai	0	0	0	0	R	
Jiringo	*M. asiatica* Nakai	0	0	0	0	R	
M. floribunda 821	*M.* × floribunda Sieb.	0	0	0	0	R	
M. florentina	*M. florentina* (Zucc.) Sshneid.	0	0	0	0	R	
Nokaido	*M. halliana* Koehn.	0	0	0	0	R	
Hanakaido	*M. halliana* Koehn.	0	0	0	0	R	
M. honanensis	*M. honanensis* Rehd.	0	0	0	0	R	

Table 3: Continued

Genotype	Species	Response of inoculated leaves				Resistance level	Remarks[1]
		2007		2008			
		Mean score	S S	Mean score	S S		
M. ioensis	*M. ioensis* Brit.	0	0	0	0	R	
Hong Hai Tang	*M. micromalus* Makino	0	0	0	0	R	
M. platycarpa 73031	*M.* × platycarpa Rehd.	0	0	0	0	R	
M. praecox	*M. praecox* (Pall.) Borkh.	0	0	0	0	R	
M. pratii	*M. pratii* (Hemsl.) Schneid.	0	0	0	0	R	
M. robusta Bailey	*M.* × robusta Rehd.	0	0	0	0	R	
M. tringoides	*M. tringoides* Hughes.	0	0	0	0	R	

[1]Reference from Crosby et al. (1992), Fischer (2000) and Merwin et al. (1994).

A. alternata strawberry pathotype (Maekawa et al. 1984, Takahashi et al. 1990), and *A. alternata* tangerine pathotype (Kohmoto et al. 1991, Solel and Kimchi 1997, Reis et al. 2007).

There was a marked genotypic difference in the resistance level to *A. alternata* apple pathotype as shown in Tables 2, 3 and 4. The resistance levels to *A. alternata* apple pathotype for some major apple cultivars used in both our study and studies by Sawamura and Yanase (1963), Tsuchiya et al. (1967), Saito and Takeda (1984), Kitajima (1989), and Saito et al. (2001) were generally consistent with each other: 'Golden Delicious', 'Jonathan' and 'Tsugaru' were evaluated as R, whereas 'Starking Delicious', 'Indo' and 'Redgold' as S. However, there was inconsistency in the resistance level in 'Fuji'; 'Fuji', regarded as R by Saito and Takeda (1984), was evaluated as MR in our study. Difference in either pathogen virulence and/or race of isolate(s) used in the studies could not explain the inconsistency of the resistance level on the cultivar, as the same isolate of *A. alternata* apple pathotype, AKI-3, was used in both studies. Difference in scales used for scoring Alternaria blotch symptoms is a potential cause of the discrepancy. With the exception of 'Fuji', there were no cultivars essentially having 'MR' response used in the study by Saito and Takeda (1984); this might have resulted in the lack of definition of the 'MR' response in their scoring system, and hence caused a somewhat different determination of the resistance levels for 'Fuji' from its distinctive resistance level. Alternatively, there might be a difference in age of leaves used for the inoculation tests between the two studies, and this could be ascribed to the difference in the resistance levels for Alternaria blotch on 'Fuji;' the resistance level of this genotype was affected by leaf position (leaf age) used for the inoculation test as well as of 'Orin' in our study.

In general, there is no remarkable discrepancy for the resistance levels determined by detached-leaf method and those reported in the literatures among major cultivated apples (Table 2); a virulent isolate AKI-3 was enough aggressive to the leaves of known S cultivars, but could not induce symptoms on the known R ones in the study. The result shows that at present use of the isolate AKI-3 is adequate for the screening of the resistance to *A. alternata* in the apple breeding. In this regards, Saito et al. (1983) investigated the pathogenicity of 56 monoconidial isolates collected from various orchards in northern part of Japan using R and S apples, and reported that no isolate was more virulent than AKI-3. However, they also pointed out that the pathogens may be going to differentiate into races based on the occurrence of large genetic variation on the pathogenicity among the isolates. In the case of apple scab in particular, it is well known that there is a specific interaction between race and genotype, and more importantly, some races have been found capable of overcoming the resistance of some cultivars/selections carrying the *Vf* (Parisi et al. 1993, Roberts and Crute 1994, Parisi and Lespinasse 1996). In this context, continuous study of the behaviour of known R cultivars to various isolates of *A. alternata* collected from many apple production areas will be important to guarantee successful selection for the resistance.

Most scab-resistant apple cultivars used in the study were evaluated as R to *A. alternata* apple pathotype; more than 10 scab-resistant cultivars were included. This may be owing to the resistance of *Malus floribunda* 821 and ancestral cultivars to *A. alternata*. For example, major ancestral founding cultivars of scab-resistant ones developed by the cooperative apple breeding programme (PRI) of Illinois, Indiana and New Jersey, such as 'Prima', 'Liberty', 'Redfree' and 'Freedom', are 'Rome Beauty', 'Golden Delicious', 'McIntosh', and their red sports (Dayton et al. 1970, Lamb et al. 1979, 1985, Williams et al. 1981), and they are R to *A. alternata* (Tables 2–4). According to Saito and Takeda (1984), all progeny are expected to be R to *A. alternata* when crosses are made between R apples. As a result, not only scab-resistance but resistance to *A. alternata* might be transmitted to descendants in the apple breeding for scab resistance. While most scab-resistant cultivars were R to *A. alternata*, a few cultivars e.g. 'Jonafree' and 'Co-op 25' were susceptible. Based on pedigree information on 'Jonafree' (Dayton et al. 1979), source of susceptibility could be 'Red Spy', which was a red sport of 'Northern Spy' (Brooks and Olmo 1997) because the other founding ancestral cultivars were R (Tables 3, 4, Fig. 2), although involvement of 'Galia Beauty' was not ruled out if it was S to *A. alternata*. For 'Co-op 25' source of the susceptibility could be 'Edgewood' and/or 'Melba' on the basis of pedigree information (Janick et al. 2000) and the results on the resistance level of several ancestral cultivars (Tables 2, 3 and 4).

Concerning modern apple cultivars and selections in Japan, not a few of the genotypes tested (7/38) were S (Table 4). This seems to be ascribed to a frequent use of S cultivars, e.g. strains of 'Delicious' and 'Kitakami', as cross parents across generations in apple breeding programmes (Fig. 3). As 'Delicious' and its sports were sources of genes for desirable horticultural traits (Way et al. 1990), they have been frequently used worldwide as parents as well as 'Golden Delicious' and 'Jonathan' in modern apple breeding programmes (Noiton and Alspach 1996), as is the case of apple breeding in Japan. Frequent use of the S parents increased ratio of S progeny in

Table 4: Resistance levels of various apple cultivars and selections to *Alternaria alternata* apple pathotype determined by detached-leaf method

Genotype	Parentage[1]	Year of inoculation test	Response of inoculated leaves		Resistance level
			Mean score	S S	
Alexander		2007	0	0	R
American Summer Pearmain		2005 + 2007	0	0	R
Baldwin		2007	3.2	4	S
Ben Davis		2007	0	0	R
Benfle		2007	0	0	R
Blue Pearmain		2007	0.2	1	R
Bramley's Seedling		2007	3.2	4	S
Calville Rouge		2007	3.0	4	S
Carolina Red June		2007	0	0	R
Cogswell		2007	1.4	2	MR
Duchess of Oldenburgh		2007	0	0	R
Early Harvest		2007	0.2	1	R
Early Joe		2007	4.2	5	S
Early Strawberry		2007	4.2	5	S
Esopus Spitzenburgh		2007	3.8	5	S
Fameuse		2007	0	0	R
Gravenstein		2007	0	0	R
Jersey Sweet		2007	0.2	1	R
King of Tompkins County		2007	3.2	4	S
McIntosh		2005 + 2007	0.2	1	R
Northern Spy		2005 + 2007	4.0	5	S
Orange Pippin		2007	0.2	1	R
Porter		2007	3.4	4	S
Prinzen Apfel		2007	0	0	R
Rome Beauty		2005 + 2007	0	0	R
Roxbury Russet		2007	0.2	1	R
Sops of Wine		2007	0.2	1	R
Summer Queen		2007	3	4	S
Swarr		2007	3.8	5	S
Tallman's Sweet		2007	0.2	1	R
Twenty Ounce		2007	0.2	1	R
Wagener		2007	2.2	3	S
Wealthy		2007	0	0	R
White Winter Pearmain		2007	4.2	5	S
Winesap		2007	0.4	1	R
Worcester Pearmain		2005 + 2007	0	0	R
York Imperial		2007	0.8	1	R
Akane	Jonathan × Worcester Pearmain	2005 + 2007	0	0	R
Akibae	Sensyu × Tsugaru	2005 + 2007	0.2	1	R
Amanishiki	Golden Delicious × Jonathan	2007	0.6	1	R
Aori 13	Sekaiichi × Akane	2007	0.4	1	R
Beninomai	Fuji × Unknown	2007	2.2	3	S
Chinatsu	Akane × Stark Earliest	2005 + 2007	0.2	1	R
Hacnine	Fuji × Tsugaru	2005 + 2007	0.2	1	R
Haruka	Golden Delicious × Starking Delicious	2005 + 2007	0.2	1	R
Hatsuaki	Jonathan × Golden Delicious	2005 + 2007	0	0	R
Himekami	Fuji × Jonathan	2005 + 2007	0.2	1	R
Hozuri	Fuji × Unknown	2007	0	0	R
Iwakami	Fuji × Jonathan	2005 + 2007	0	0	R
Kio	Orin × Hatsuaki	2007	1.6	2	MR
Kitakami	Tohoku No.2 × Redgold	2005 + 2007	3.4	4	S
Kitaro	Fuji × Hatsuaki	2005 + 2007	0.2	1	R
Kizashi	Gala × Stark Earliest	2005 + 2007	0	0	R
Kotaro	Fuji × Hatsuaki	2005 + 2007	0.2	1	R
Megumi	Ralls Janet × Jonathan	2005 + 2007	0	0	R
Morioka No. 58	Hatsuaki × Fuji	2005 + 2007	0	0	R
Morioka No. 59	Kitakami × Hatsuaki	2005 + 2007	3.8	4	S
Morioka No. 60	Hatsuaki × Starking Delicious	2005 + 2007	4.5	5	S
Morioka No. 61	Tsugaru × Kitakami	2005 + 2007	3.8	4	S
Morioka No. 62	Akane × Maigold	2005 + 2007	1.2	2	MR
Morioka No. 63	Tsugaru × Gala	2005 + 2007	0	0	R
Morioka No. 64	(Megumi × Jonathan) × Sansa	2005 + 2007	0.6	1	R
Morioka No. 65	Orin × Sansa	2005 + 2007	1.8	2	MR
Natsumidori	Kitakami × Meku 10	2007	2.8	4	S
Northqueen	Fuji × Tsugaru	2007	1.2	2	MR
Orei	Golden Delicious × Delicious	2007	2.4	4	S
Santaro	Hatsuaki × Starking Delicious	2005 + 2007	0.4	1	R
Sensyu	Toko × Fuji	2005 + 2007	0	0	R

Table 4: Continued

Genotype	Parentage[1]	Year of inoculation test	Response of inoculated leaves		Resistance level
			Mean score	S S	
Shinano Gold	Golden Delicious × Sensyu	2005 + 2007	0.2	1	R
Shinano Red	Tsugaru × Vista Bella	2005 + 2007	0	0	R
Shinano Sweet	Fuji × Tsugaru	2005 + 2007	0.2	1	R
Shinsekai	Fuji × Akagi	2007	0	0	R
Slim Red	Fuji × Akagi	2007	0	0	R
Toko	Golden Delicious × Indo	2007	0	0	R
Yoko	Golden Delicious × Unknown	2005 + 2007	0	0	R

[1]Reference from Yoshida (1986). Parentage of Chinatsu, Haruka and Kizashi: S. Moriya, H. Iwanami, T. Yamamoto and K. Abe (unpublished data).

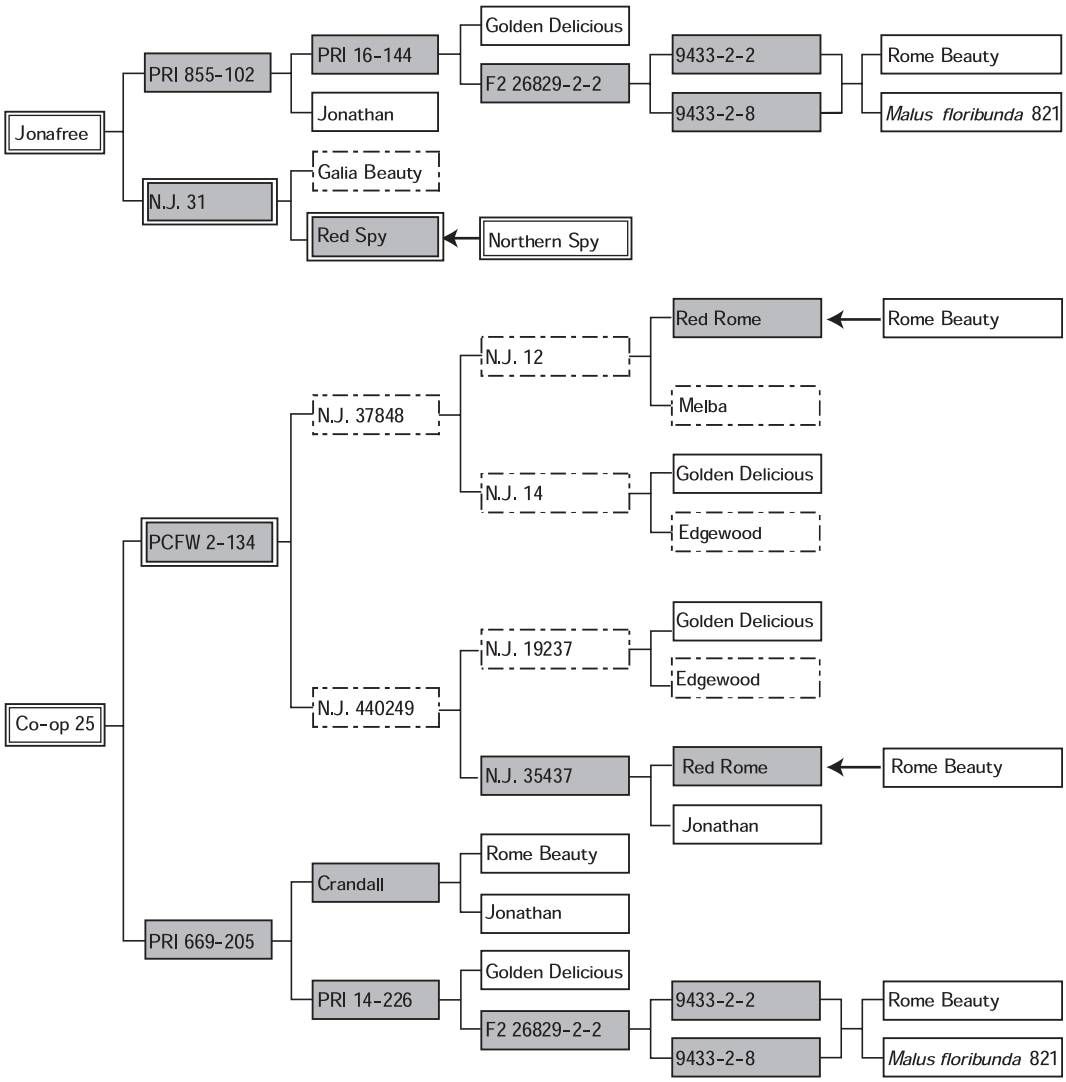

Fig. 2: Predicted source of susceptibility to *Alternaria alternata* apple pathotype for 'Jonafree' and 'Co-op 25.' Pedigree information for 'Jonafree': Dayton et al. (1979), 'Co-op 25': Janick et al. (2000). ▭: susceptible, ▭: resistant, ▭: not determined, ▭: predicted susceptible, ▭: predicted resistant. ← represents bud mutation

breeding populations and it could also increase the chance of selection of S individuals as candidates of new cultivars. Parentage information (Table 4, Fig. 3) by Yoshida (1986) clearly indicate that source of the susceptibility in apple cultivars/selections of Japanese origin were 'Delicious' or 'Indo.' It is interesting to explore the roots of (possible) S cultivars such as 'Northern Spy', 'Edgewood', 'Melba', 'Delicious' and 'Indo.' Pedigree information presented in Fig. 3 also suggests that use of R genotypes as cross parents is important to develop new cultivars resistant to *A. alternata* in future apple breeding. In addition, it is worth noting that most apple breeders in Japan have been working within a population of limited genetic base, which was also the trend in other apple breeding programmes worldwide. As Noiton and

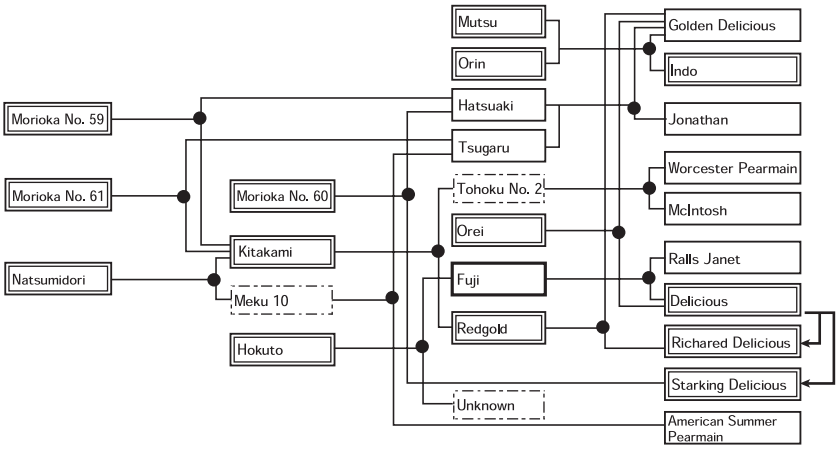

Fig. 3: Pedigrees and source of susceptibility to *Alternaria alternata* apple pathotype for modern apple cultivars and selections developed in Japan. Reference from Yoshida (1986). Parentage for 'Tsugaru': Kitahara et al. (2005). ▭: susceptible, ▭: moderately resistant, ▭: resistant, ▭: not determined. ⟵: represents bud mutation

Alspach (1996) pointed out, this might be handicap future genetic improvement of apple. Since excessive use of particular genotypes as cross parents results in the loss of genetic diversity, which may sometimes cause genetic vulnerability to diseases and pests (e.g. Jordan et al. 1998), it seems to be important to diversify genetic base in a breeding population in future apple breeding programme.

Scab-resistant cultivars exhibiting resistance to Alternaria blotch are useful cross-parents for the acquisition of hybrid double-resistance to the diseases in apple breeding. Some of the scab-resistant cultivars, e.g. 'Reanda' and 'Regine', are reported to be resistant not only to apple scab but to fire blight and/or bacterial canker (Fischer 2000). Since trends in modern apple production have been toward an integrated control of diseases and pests with minimum use of fungicides and pesticides, these multiple-resistant cultivars should be valuable sources for the development of new apples resistant to economically important diseases.

In contrast to the genotypic differences for levels of resistance to *A. alternata* apple pathotype in cultivated apples, all *Malus* spp. tested exhibited complete resistance to *A. alternata* despite the diversity of geographic locations and origin (Table 3). Accessions belonging to both *M. sieversii*, thought to be a putatively maternal ancestor of the cultivated apple (Lamboy et al. 1996, Zhou and Li 2000, Robinson et al. 2001, Forte et al. 2002, Harris et al. 2002) and *M. sylvestris*, the primary progenitor of the apple (Dunemann et al. 1994, Coart et al. 2006), produced no symptoms on all inoculated leaves. This was unexpected because no susceptible offspring was considered to originate from resistant parents; susceptible hybrids to *A. alternata* apple pathotype were observed only in the descendants of susceptible parent(s) (Saito and Takeda 1984). Although our results provided no evidence for the possible origin of the susceptible cultivated apples, this does not always rule out the assumption for the existence of the susceptible *Malus* germplasm that had been the origin of the susceptibility. Since there is great genetic diversity in *M. sieversii* (Volk et al. 2005) and *M. sylvestris* (Larsen et al. 2006), further study will be necessary using as many accessions belonging to *M. sieversii* and *M. sylvestris* as possible to obtain information on the origin of the susceptibility.

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References

Bell, A. A., 1981: Biochemical mechanisms of disease resistance. Ann. Rev. Plant Physiol. **32,** 21—81.

Brooks, R. M., and H. P. Olmo, 1997: Register of New Fruit and Nut Varieties, 3rd edn. ASHS Press, VA, USA.

Coart, E., S. Glabeke, M. de Loose, A. S. Larsen, and I. Roldan-Ruiz, 2006: Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). Mol. Ecol. **15,** 2171—2182.

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goffreda, and A. Voordeckers, 1992: Breeding apples for scab resistance: 1945–1990. Fruit Var. J. **46,** 145—166.

Dayton, D. F., J. B. Mowry, L. F. Hough, C. H. Bailey, E. B. Williams, J. Janick, and F. H. Emerson, 1970: Prima – an early fall red apple with resistance to apple scab. Fruit Var. Hort. Dig. **24,** 20—22.

Dayton, D. F., J. B. Mowry, E. B. Williams, J. Janick, F. H. Emerson, L. F. Hough, and C. Bailey, 1979: 'Jonafree'apple. HortScience **14,** 551—552.

Dunemann, F., R. Kahnau, and H. Schmidt, 1994: Genetic relationship in *Malus* evaluated by RAPD 'fingerprinting' of cultivars and wild species. Plant Breeding **113,** 150—159.

Filajdic, N., and T. B. Sutton, 1991: Identification and distribution of *Alternaria mali* on apples in North Carolina and susceptibility of different varieties of apples to Alternaria blotch. Plant Dis. **75,** 1045—1048.

Filajdic, N., and T. B. Sutton, 1992: Chemical control of Alternaria blotch of apples caused by *Alternaria mali*. Plant Dis. **76,** 126—130.

Fischer, C., 1994: Breeding apple cultivars with multiple resistance. In: H. Schmidt, and M. Kellerhals (eds), Progress in Temperate Fruit Breeding, 43—48. Kluwer Academic Publishers, Dordrecht.

Fischer, C., 2000: Multiple resistant apple cultivars and consequences for apple breeding in the future. Acta Hortic. **538,** 229—234.

Fischer, C., and K. Richter, 1996: Breeding for fire blight resistance within a multiple resistance breeding programme in apples. Acta Hortic. **411,** 375—381.

Forte, A. V., A. N. Ignatov, V. V. Ponomarenko, D. B. Dorokhov, and N. I. Savelyev, 2002: Phylogeny of the *Malus* (Apple tree) species, inferred from the morphological traits and molecular DNA analysis. Russian J. Genet. **38,** 1150—1160.

Gessler, C., and D. Stumm, 1984: Infection and stroma formation by *Venturia inaequalis* on apple leaves with different degree of susceptibility to scab. Phytopath. Z. **110,** 119—126.

Harris, S. A., J. P. Robinson, and B. E. Juniper, 2002: Genetic clues to the origin of the apple. Trends Genet. **18,** 426—430.

Janick, J., J. C. Goffreda, and S. S. Korban, 2000: 'Co-op 25' (Scarlet O'Hara™) apple. HortScience **35,** 150—151.

Jordan, D. R., Y. Z. Tao, I. D. Godwin, R. G. Henzell, M. Cooper, and C. L. McIntyre, 1998: Loss of genetic diversity associated with selection for resistance to sorghum midge in Australian sorghum. Euphytica **102,** 1—7.

Kellerhals, M., and B. Furrer, 1994: Approaches for breeding apples with durable disease resistance. In: H. Schmidt, and M. Kellerhals (eds), Progress in Temperate Fruit Breeding, 93—97. Kluwer Academic Publishers, Dordrecht.

Kitahara, K., S. Matsumoto, T. Yamamoto, J. Soejima, T. Kimura, H. Komatsu, and K. Abe, 2005: Parent identification of eight apple cultivars by S-RNase analysis and simple sequence repeat markers. HortScience **40,** 314—317.

Kitajima, H., 1989: Alternaria blotch, Alternaria leaf spot. (In Japanese). In: H. Kitajima (ed.), Kaju Byogai Kakuron (Diseases of Fruit Trees), 141—151. Yokendo, Tokyo.

Kohmoto, K., and H. Otani, 1991: Host recognition by toxigenic plant pathogens. Experimentia **47,** 755—764.

Kohmoto, K., K. Akimitsu, and H. Otani, 1991: Correlation of resistance and susceptibility of citrus to *Alternaria alternata* with sensitivity to host-specific toxins. Phytopathology **81,** 719—722.

Kozaki, I., 1973: Black spot disease resistance in Japanese pear 1. Heredity of the disease resistance. (In Japanese with English summary). Bull. Hort. Res. Stn. **12,** 17—27.

Lamb, R. C., H. S. Aldwinckle, R. D. Way, and D. E. Terry, 1979: 'Liberty' apple. HortScience **14,** 757—758.

Lamb, R. C., H. S. Aldwinckle, and D. E. Terry, 1985: 'Freedom', a disease-resistant apple. HortScience **20,** 774—775.

Lamboy, W. F., J. Yu, P. L. Forsline, and N. F. Weeden, 1996: Partitioning of allozyme diversity in wild populations of *Malus sieversii* L. and implications for germplasm collection. J. Am. Soc. Hortic. Sci. **121,** 982—987.

Larsen, A. S., C. B. Asmussen, E. Coart, D. C. Olrik, and E. D. Kjar, 2006: Hybridization and genetic variation in Danish populations of European crab apple (*Malus sylvestris*). Tree Genet. Genomes **2,** 86—97.

Lespinasse, Y., C. E. Durel, F. Laurens, M. Chevalier, C. Pinet, and L. Parisi, 2000: A European project: D. A. R. E. – Durable Apple Resistance in Europe – durable resistance of apple to scab and powdery mildew: one step more towards an environmental friendly orchard. Acta Hortic. **538,** 197—200.

Maekawa, N., M. Yamamoto, S. Nishimura, K. Kohmoto, M. Kuwada, and Y. Watanabe, 1984: Studies on host-specific AF-toxins produced by *Alternaria alternata* strawberry pathotype causing Alternaria black spot of strawberry (1) Production of host-specific toxins and their biological activities. Ann. Phytopath. Soc. Japan **50,** 600—609.

Merwin, I. A., S. K. Brown, D. A. Rosenberger, D. R. Cooley, and L. P. Berkett, 1994: Scab-resistant apples for the northeastern United States: new prospects and old problems. Plant Dis. **78,** 4—10.

Miyashita, M., T. Nakamori, H. Miyagawa, M. Akamatsu, and T. Ueno, 2003: Inhibitory activity of analogs of AM-Toxin, a host-specific phytotoxin from the *Alternaria alternata* apple pathotype, on photosynthetic O_2 evolution in apple leaves. Biosci. Biotechnol. Biochem. **67,** 635—638.

Noiton, D. A. M., and P. A. Alspach, 1996: Founding clones, inbreeding, coancestry, and status number of modern apple cultivars. J. Am. Soc. Hortic. Sci. **121,** 773—782.

Otani, H., K. Kohmoto, and M. Kodama, 1995: *Alternaria* toxins and their effects on host plants. Can. J. Bot. **73**(Suppl. 1)**,** S453—S458.

Parisi, L., and Y. Lespinasse, 1996: Pathogenicity of *Venturia inaequalis* strains of race 6 on apple clones (*Malus* sp.). Plant Dis. **80,** 1179—1183.

Parisi, L., Y. Lespinasse, J. Guillaumes, and J. Kruger, 1993: A new race of *Venturia inaequalis* virulent to apples with resistance due to the Vf gene. Phytopathology **83,** 533—537.

Reis, R. F., T. F. de Almeida, E. S. Stuchi, and A. de Goes, 2007: Susceptibility of citrus species to *Alternaria alternata*, the causal agent of the Alternaria brown spot. Sci. Hortic. **113,** 336—342.

Reuveni, M., D. Sheglov, N. Sheglov, R. Ben-Arie, and D. Prusky, 2002: Sensitivity of Red Delicious apple fruit at various phonologic stages to infection by *Alternaria alternata* and moldy-core control. Euro. J. Plant Pathol. **108,** 421—427.

Roberts, A. L., and I. R. Crute, 1994: Apple scab resistance from *Malus floribunda* 821 (Vf) is rendered ineffective by isolates of *Venturia inaequalis* from *Malus floribunda*. Norw. J. Agric. Sci. **17,** 403—406.

Robinson, J. P., S. A. Harris, and B. E. Juniper, 2001: Taxonomy of the genus *Malus* Mill. (Rosaceae) with emphasis on the cultivated apple, Malus × domestica Borkh. Plant. Syst. Evol. **226,** 35—58.

Saito, K., and K. Takeda, 1984: Genetic analysis of resistance to Alternaria blotch (*Alternaria mali* Roberts) in apple. (In Japanese with English summary). Japan. J. Breed. **34,** 197—209.

Saito, K., M. Niizeki, M. Wake, and Y. Hidano, 1983: Fundamental studies on breeding of the apple 9. Variation of pathogenicity and chemical tolerance to polyoxin in Alternaria mali Roberts. (In Japanese with English summary). Bull. Fac. Agric. Hirosaki Univ. **40,** 23—30.

Saito, A., N. Nakazawa, and M. Suzuki, 2001: Selection of mutants resistant to Alternaria blotch from in vitro-cultured apple shoots irradiated with X- and γ-rays. J. Plant Physiol. **158,** 391—400.

Sanada, T., T. Nishida, and F. Ikeda, 1988: Resistant mutant to black spot disease of Japanese pear'Nijisseiki'induced by gamma rays. J. Japan. Soc. Hort. Sci. **57,** 159—166.

Sawamura, K., 1990: Alternaria blotch. In: A. L. Jones, and H. S. Aldwinckle (eds), Compendium of Apple and Pear Diseases, 24—25. APS Press, St. Paul, USA.

Sawamura, K., and H. Yanase, 1963: Studies on spotted disease of apples 2. On *Alternaria* sp., causal organism of Alternaria blotch and Japanese name of the disease. (In Japanese with English summary). Bull. Hort. Res. Stn. Series **1,** 77—94.

Schwabe, W. F. S., 1979: Changes in scab susceptibility of apple leaves as influenced by age. Phytophylactica **11,** 53—56.

Solel, Z., and M. Kimchi, 1997: Susceptibility and resistance of citrus genotypes to *Alternaria alternata* pv. *citri*. J. Phytopathology **145,** 389—391.

Takahashi, H., T. Takai, and T. Matsumoto, 1990: Susceptible strawberry cultivars to Alternaria black spot of strawberry (*Alternaria alternata* strawberry pathotype) in Japan. J. Japan. Soc. Hort. Sci. **59,** 539—544.

Tsuchiya, S., Y. Yoshida, and T. Haniuda, 1967: Studies on the disease resistance in apple 1 Investigation on the resistance to Alternaria blotch in apple varieties and hybrids. (In Japanese with English summary). Bull. Hort. Res. Sta. **5,** 9—19.

Tsuyama, H., Y. Kodama, and N. Nakazawa, 1971: Histological studies on the different varietal reaction in Alternaria leaf spot disease of apples caused by *Alternaria mali* Rob. (In Japanese with English summary). Bull. Fac. Agric. Iwate Univ. **10,** 217—231.

Volk, G. M., C. M. Richards, A. A. Reilley, A. D. Henk, P. L. Forsline, and H. S. Aldwinckle, 2005: Ex site conservation of vegetatively propagated species: development of a seed-based core collection for *Malus sieversii*. J. Am. Soc. Hortic. Sci. **130,** 203—210.

Way, R. D., H. S. Aldwinckle, R. C. Lamb, A. Rejman, S. Sansavini, T. Shen, R. Watkins, M. N. Westwood, and Y. Yoshida, 1990: Apples (*Malus*). In: J. N. Moore, and J. R. Ballington Jr (eds), Genetic Resources of Temperate Fruit and Nut Crops, 3—62. Inter. Soc. Hort. Sci, Wageningen, Nertherlands.

Williams, E. B., J. Janick, H. Emerson, D. F. Dayton, L. F. Hough, and C. Bailey, 1981: 'Redfree' apple. HortScience **16,** 798—799.

Yoshida, Y., 1986: Encyclopedia of apple cultivars. (Japanese). In: Y. Yoshida (ed.), 'Amanishiki', 'Megumi', and 'Toko', 20, 605, 1024. Japan Agricultural Cooperatives, Nagano, Japan.

Zhou, Z. Q., and Y. N. Li, 2000: The RAPD evidence for the phylogenetic relationship of the closely related species of cultivated apple. Genet. Resour. Crop Evol. **47,** 353—357.