Europ.J.Hort.Sci., **74** (1). S. 1–9, 2009, ISSN 1611-4426. 

# Evaluation of Genus *Rosa* Germplasm for Resistance to Black Spot, Downy Mildew and Powdery Mildew

D. F. Schulz[1)], M. Linde[1)], O. Blechert[2)] and T. Debener[1)]
([1)]Leibniz University of Hannover, Institute of Plant Genetics, Department of Molecular Breeding, Hannover, Germany and [2)]Biotechnology Research Institute, National Research Council, Montreal, Quebec, Canada)

**Summary**

Black spot resistance in the field was visually evaluated for a total of 581 accessions at two locations in central and northern Germany. At Sangerhausen rose garden 289 of 486 accessions have shown a high level of disease resistance. After subsequent resistance tests in the detached leaf assay 33 of these accessions did not show any signs of black spot infections. At Ahrensburg 41 of the 130 wild rose accessions from 65 species were not infected in the field. Leaves of the accessions without apparent black spot infections were sampled and inoculated under laboratory conditions with different single spore isolates and field collected samples of the pathogen. A total of 11 accessions were found to be highly resistant to all black spot isolates. Powdery mildew resistance of 39 accessions at Ahrensburg was identified after field evaluation and artificial inoculation with different samples of *Podosphaera pannosa* from naturally infected leaves. Downy mildew resistance was estimated in detached leaf assays after inoculation of a subset of 85 accessions from Ahrensburg with single spore isolates and field collected samples of *Peronospora sparsa* among which 13 wild rose accessions were found to be resistant. Multiple resistances to two of the pathogens were found in 13 of the investigated accessions of which *R. majalis* 93-09-01 is highly resistant to all three pathogens, except for isolate *Diplocarpon rosae* 'AHE10'. These accessions, primarily those carrying multiple resistances, are a valuable genetic resource for the introduction of resistance genes to black spot, downy mildew and powdery mildew into cultivated garden roses.

**Key words.** black spot – downy mildew – multiple resistance – powdery mildew – wild roses

## Introduction

The genus *Rosa* comprises more than 150 different species with a natural distribution throughout the northern hemisphere (Wissemann 2003) and several thousand cultivars with a ubiquitous distribution. The latter were mainly developed over the last three centuries. Roses are among the economically most important ornamental crops. Worldwide, approximately 8 billion rose stems, 80 million potted plants and 220 million garden roses are sold yearly (Roberts et al. 2003). It is assumed that 10 different species contributed to the gene pool of ancient and modern rose varieties and hence the genome of modern roses comprises a mosaic of different species genomes (Gudin 2000). Despite this genomic diversity, some characters such as resistance to the major pathogens and pests are underrepresented among rose cultivars (De Vries 2000). As the use of pesticides in private and public gardens as well as in commercial production is more and more limited by legal restrictions and public concerns, breeding of disease resistant varieties is one of the most important goals of modern garden rose breeding (Noack 2003). However, as resistant accessions are a prerequisite for every breeding strategy, these have to be searched for among the large number of wild roses. Furthermore, to obtain durable resistance in rose varieties, several resistance genes should simultaneously be introduced to the narrow gene pool of modern roses (Gudin 2000). Rose black spot caused by the facultative hemibiotrophic ascomycete *Diplocarpon rosae* (Wolf) is one of the most devastating diseases of field grown roses (Horst 1982; Drewes-Alvarez 2003a). Whereas black spot is considered to be the most severe disease of field-grown roses (Horst 1983; Debener et al. 1998; Von Malek and Debener 1998; Yokoya et al. 2000), powdery mildew is the major fungal disease of roses grown in greenhouses (Horst 1983; Linde and Shishkoff 2003). It is caused by the obligate biotrophic ascomycete *Podosphaera pannosa* (Wallr.: Fr.) de Bary, which had long been known as *Sphaerotheca pannosa* var. *rosae* (Wallr.: Fr.) Lév (Braun and Takamatsu 2000). The racial structure of *P. pannosa* populations is highly diverse with a large number of different races (Linde and Debener 2003). Downy mildew is caused by the obligate biotrophic oomycete *Peronospora sparsa* (Berk.). The disease is frequently observed in greenhouse cultivation and is an important problem on container plants in retail nurseries and in the production fields of the breeding companies when environmental conditions

favour disease development (Horst 1983; Xu and Pettit 2003). In the present paper we describe for the first time a thorough evaluation of wild rose species for three different important rose pathogens. In detail, we aimed at a combination of field and stringent laboratory methods for disease resistance evaluation and the combination of disease scores for all three pathogens in order to identify accessions with combined resistances.

## Materials and Methods

### *Field observations*

Field observations of black spot resistance were done at two locations in Germany: (1) the former Institute of Ornamental Plant Breeding in Ahrensburg and (2) the Sangerhausen rose garden. In Ahrensburg, most accessions are planted in replicates at two locations about 400–500 m apart whereas in Sangerhausen most accessions were analysed in only one location. Field observations in Ahrensburg (130 accessions) and Sangerhausen (486 accessions) were made in two years. Those plants that remained uninfected in the field were investigated in the lab over the subsequent years. Thirty five of the accessions were both evaluated in Ahrensburg and Sangerhausen. Field observations of powdery mildew with 149 accessions representing 97 species were also made in Ahrensburg within two years and subsequently inoculated in lab experiments.

### *Plant material*

The rose accessions investigated in the present study are part of the germplasm collection of the former Institute of Ornamental Plant Breeding of the Federal Centre for Breeding Research on Cultivated Plants in Ahrensburg and the Sangerhausen rose garden. In addition, a subset of accessions was cultivated in the greenhouse under partially controlled conditions as described in Debener et al. (1998).

### *Fungal isolates for inoculation experiments*

***Diplocarpon rosae.*** Test with plants from Sangerhausen were done with field collected samples*) from experimental sites in Sangerhausen and Ahrensburg. Tests with plants from Ahrensburg were made with the following single spore isolates 'Dort E4' (Dortmund, Germany), 'AHE10' (Ahrensburg, Germany), '121' and '124' (Poona, India), and defined combinations of the isolates '120' (Antonio Prado, Brasil), '121' and '123' (Zimbabwe); '105' (Inowroclaw, Poland) and '116' (Lódz, Poland); and '108' (Gütersloh, Germany), '113' (Portugal), '128' (Hungaria), and '152' (Narre Warren North, Australia) and field collected samples from England (St. Albans), France, Portugal and Germany (Ahrensburg, Dortmund, Lüneburg, Gütersloh, München, Rethmar, Sangerhausen, Siebeldingen, Sparrieshoop, Weinberg) (Debener et al. 1998; Von Malek-Podjaski 1999; Blechert 2005).

*)The term "field collected sample" refers to a crude isolation of spores from an infected site or plant without subsequent preparation of single spore isolates. Therefore, several different races or pathotypes can form a "field collected sample."

***Peronospora sparsa.*** single spore isolate '3-16' (Sparrieshoop, Germany) and field collected samples 'M2002' from Sparrieshoop and 'DVP' from Melle (Belgium).

***Podosphaera pannosa.*** different field collected samples from Ahrensburg (Germany).

### *Evaluation of black spot resistance*

All accessions were evaluated visually for black spot resistance in the field at Ahrensburg and Sangerhausen. Those that showed typical signs of infection by black spot were referred to as susceptible and not considered any further. From most of the remaining accessions with ambiguous or no black spot infection, leaves were sampled and inoculated under controlled conditions in the laboratory. The artificial inoculation of excised leaves was performed as described earlier (Debener et al. 1998) with $10^5$ viable conidia $ml^{-1}$ sterile tap water in three replications each. The inoculation was done by placing three to six 10 µl droplets of conidial suspension on each leaflet of the leaf. Only young, fully expanded leaves were used for the experiments. For inoculation, the viability of the conidia was tested by staining an aliquot with a 0.05 % solution of phenosafranine (3,7-diamino-5-phenylphenazinium chloride, Sigma-Aldrich) in water (Widholm 1972); nonviable conidia absorb the dye and are stained red, whereas viable conidia remain unstained. This viability test was necessary as the fraction of viable inoculum varied significantly in earlier experiments (Wiegand 2001). The boxes were then incubated for 2 days at 20 °C to promote conidial germination. Afterwards, the inoculation droplets were carefully blotted and the leaves were incubated for 12 more days at room temperature. Inoculations were repeated in three independent experiments. Accessions were considered to be susceptible when the fungal mycelium grew significantly beyond the site of inoculation and/or it was able to sporulate. In those cases where no fungal development could be observed, or where only limited mycelial growth without formation of acervuli occurred, the plant was considered to be resistant. All leaf samples were inspected under a dissecting microscope.

### *Evaluation of powdery mildew resistance*

All accessions at the location Ahrensburg were evaluated visually for powdery mildew resistance in the field during two consecutive years. Additionally, leaves from the 149 accessions comprising 97 species were sampled and inoculated with different field samples of *P. pannosa* under controlled conditions in the lab. Leaflets from three rose accessions were placed in glass petri dishes on water-agar (0.5 % agar) containing 0.03 % benzimidazole to prevent fungal contamination of the agar surface. From each accession six to nine leaflets (third to fifth unfolded leaves from the shoot tip) were used. The leaflets were inoculated with approximately two conidia per square millimeter of leaf surface as described by Linde and Debener (2003). The inoculations were repeated five to seven times. The petri dishes were incubated in a plant growth chamber for 10 days (16 h light, 22 °C). At 10-days post-inoculation, percentages of leaf area covered with conidiophores were estimated in 10 % steps (from zero to 100 %) with a stereomicroscope (8- to 50-fold magnification). Accessions with only single conidiophores on single leaflets in

Table 1. Number of investigated and resistant wild rose accessions after field and detached leaf assay for black spot, powdery and downy mildew at the location Sangerhausen and Ahrensburg.

| Section/ Subgenus | Black spot[1)] | | | | | Powdery mildew[2)] | | Downy mildew[3)] | |
|---|---|---|---|---|---|---|---|---|---|
| | Sangerhausen | | | Ahrensburg | | Ahrensburg | | Ahrensburg | |
| | Evaluated in field | Resistant in field | Resistant in field and detached leaf assay | Evaluated in field | Resistant in field and detached leaf assay | Evaluated in field and detached leaf assay | Resistant in field and detached leaf assay | Evaluated in detached leaf assay | Resistant in detached leaf assay |
| *Bracteatae* | 1 | 1 | – | – | – | – | – | 1 | 0 |
| *Caninae* | 195 | 90 | 4 | 45 | 1 | 49 | 12 | 19 | 7 |
| *Carolinae* | 14 | 8 | 3 | 8 | – | 9 | 2 | 4 | 2 |
| *Cinnamomeae* | 120 | 80 | 13 | 36 | 7 | 43 | 13 | 26 | 3 |
| *Gallicanae* | 2 | 2 | – | 6 | – | 8 | 1 | 6 | 1 |
| *Hesperhodos* | – | – | – | 1 | – | 1 | 1 | 1 | 0 |
| Hybrid | 76 | 48 | 7 | 2 | – | 3 | 1 | 5 | 0 |
| *Indicae* | 2 | 1 | 0 | – | – | 1 | 0 | 1 | 0 |
| *Pimpinellifoliae* | 32 | 32 | 3 | 15 | – | 15 | 5 | 6 | 0 |
| *Platyrhodon* | 1 | 1 | – | 2 | 1 | 2 | 0 | 1 | 0 |
| *Synstylae* | 27 | 22 | 3 | 15 | 2 | 18 | 4 | 16 | 0 |
| Unknown | 16 | 4 | – | – | – | – | – | – | – |
| Total accessions | 486 | 289 | 33 | 130 | 11 | 149 | 39 | 86 | 13 |

1)Those accessions in the field that showed typical signs of infection by black spot were referred to as susceptible and not considered any further. Resistance to *D. rosae* was tested in detached leaf assay using a sub-sample of 178 field resistant accessions. The artificial inoculation of excised leaves was performed with $10^5$ viable conidia $mL^{-1}$ sterile tap water in three replications each. Inoculations were repeated in three independent experiments. Accessions were considered to be susceptible when the fungal mycelium grew significantly beyond the site of inoculation and/or it was able to sporulate. In those cases where no fungal development could be observed, or where only limited mycelial growth without formation of acervuli occurred, the plant was considered to be resistant. The leaves were evaluated at 14 dpi. Thirty five of the accessions from Sangerhausen were also tested in Ahrensburg. Therefore, the total number of tested accessions in both locations is 581.

2)Field observations of powdery mildew were done in Ahrensburg within two years. Different field collected samples of the powdery mildew fungus from Ahrensburg were used in the detached leaf assay. Leaflets from three rose accessions were placed in glass petri dishes on water-agar. From each accession six to nine leaflets were used. The leaflets were inoculated with approximately two conidia per $mm^2$ of leaf surface. Percentages of leaf area covered with conidiophores were estimated at 10 dpi in 10 % steps (from zero to 100 %). Mean DI values of lower than 5 % and with no single estimation over 5 % were treated as resistant. Rose accessions showing a DI of 10 % or more were considered to be susceptible. The inoculations were repeated five to seven times. The experiment was repeated twice.

3)Resistance to downy mildew was estimated in detached leaf assay with the. single spore isolate '3-16' (Germany) and complex field sample M2002 (Germany) and DVP (Belgium). The lower leaf side of leaflets from 3–4 different rose accessions with four replications was spotted with several 10 µl drops of a spore suspension of 2 x $10^4$ conidia $ml^{-1}$. Leaf disease was estimated microscopically by a five step classification scheme based on the production of conidiophores at 10–14 dpi. The experiment was repeated twice. DI = 0 (resistant); DI = 1 (resistant, minor symptomatic necrotic lesions); DI = 2 (moderate susceptible, 1 to 10 conidiophores per inoculation); DI = 3 (susceptible; 11 to 25 conidiophores); DI = 4 (highly susceptible; profuse sporulation).

only one experiment were scored as having a disease index (DI) of 5 %. Mean DI values of lower than 5 % and with no single estimation over 5 % were treated as resistant. Rose accessions showing a DI of 10 % or more were considered to be susceptible. The minimum and maximum values were excluded, and the mean was calculated and taken as a disease index as described in Linde et al. (2004). The experiment was repeated twice.

### *Evaluation of downy mildew resistance*

Only young, fully expanded leaves were used for the classification of downy mildew resistance in a detached leaf assay. Leaflets from 3–4 different rose accessions with four replications were placed upside down in a damp chamber and the lower leaf side was spotted with several 10 µl drops of a spore suspension in water with approximately 2 x $10^4$ conidia $ml^{-1}$. Leaves were incubated for 2–3 d in the dark at 12 °C in damp chamber. Then the inoculation drops were absorbed with filter paper and the leaves were incubated at 16 °C under light with a photoperiod of 16 h. Leaf disease was estimated microscopically by a five step classification scheme based on the production of conidiophores at 10–14 dpi (Schulz and Debener 2007). Disease index (DI) = 0 (resistant); DI = 1 (resistant, minor symptomatic necrotic lesions); DI = 2 (moderate susceptible, 1 to 10 conidiophores per inoculation); DI = 3 (susceptible; 11 to 25 conidiophores); DI = 4 (highly susceptible; profuse sporulation with more than 25 conidiophores per inoculation spot). All leaf samples were inspected under a dissecting microscope. The experiment was repeated twice.

Table 2. Wild rose accessions at the locations Sangerhausen (S) and Ahrensburg (A) resistant to black spot evaluated in field and detached leaf assay after inoculation with single spore isolates and field collected samples of the pathogen.

| No. | Accession | Code | Location | Section/Subgenus |
|---|---|---|---|---|
| 1 | *R. bella* Rehd. &. Wils. | 00-57-04 | S/A [3)] | *Cinnamomeae* |
| 2 | *R. californica* Chem. et Schlecht. 'Plena' | 00-32-01 | S/A [3)] | *Cinnamomeae* |
| 3 | *R. canina*L. v. *euoxyphylla* (Borb.) Britton & Gl. | Group 134 | S [2)] | *Caninae* |
| 4 | *R. canina* L. v. *hispidula* (Rip.) Christ | Group 134 | S [2)] | *Caninae* |
| 5 | *R. canina* L. v. *nervulosa* (Debeaux) J.B.V.Keller | 00-33-02 | S/A [3)] | *Caninae* |
| 6 | *R. caudata* Baker | 94-103-01 | A [6)] | *Cinnamomeae* |
| 7 | *R. caudata* Baker | 94-103-02 | A [1)] | *Cinnamomeae* |
| 8 | *R. cinnamomea* L. v. *typica* | Group 226 | S [2)] | *Cinnamomeae* |
| 9 | *R. davidii* Crép. v. *elongata* Rehd. & Wils. | Group 146-15 | S [2)] | *Cinnamomeae* |
| 10 | *R. forrestiana* Boulenger | Group 150 | S [2)] | *Cinnamomeae* |
| 11 | *R. giraldii* Crép. v. *venulosa* Rehd. & Wils. | Group 147-1 | S [2)] | *Cinnamomeae* |
| 12 | *R. glomerata* Rehd. & Wils. | Group 127-1 | S [2)] | *Synstylae* |
| 13 | *R. gymnocarpa* Nutt. | Group 150 | S [2)] | *Cinnamomeae* |
| 14 | *R. harisonii* Rivers v. *vorberghii* (Graebn.) Rehd. | Group 146-10 | S [2)] | *Pimpinellifoliae* |
| 15 | *R. harisonii* Rivers v. *vorberghii* (Graebn.) Rehd. (1830) | Group 146-10 | S [2)] | *Pimpinellifoliae* |
| 16 | *R. hemsleyana* Taeckh. | Group 150 | S [2)] | *Cinnamomeae* |
| 17 | *R.* Hi 364 | 00-35-01 | S/A [3)] | Hybrid |
| 18 | *R. x hibernica* Templ. | Group 112 | S [2)] | Hybrid |
| 19 | *R. inodora* Fr. v. *hispida* (M. Schulze) Britton & Gl. | Group 133 | S [2)] | *Caninae* |
| 20 | *R. lunellii* Greene | 00-37-01 | S/A [3)] | *Cinnamomeae* |
| 21 | *R. majalis* Herrm. | 93-09-01 | A [1,5)] | *Cinnamomeae* |
| 22 | *R. majalis* Herrm. | 93-09-02 | A [1)] | *Cinnamomeae* |
| 23 | *R. marginata* Wallr. v. *typica* Christ | Group 134 | S [2)] | *Cinnamomeae* |
| 24 | *R. multiflora* Thunb. Ex Murr. | 93-27-02 | A [1)] | *Synstylae* |
| 25 | *R. multiflora* Thunb. f. *watsoniana* (Crép.) Matsum. | 00-38-01 | S/A [3,4)] | *Synstylae* |
| 26 | *R. multiflora x R. wichurana* | 00-38-02 | S/A [3)] | Hybrid |
| 27 | *R. nutkana* C. Preisl. | 93-30-01 | A [1)] | *Cinnamomeae* |
| 28 | *R. palustris* Marshal | Group 112 | S [2)] | *Carolinae* |
| 29 | *R.* 'Patrizia' *(R. helenae x R. multiflora)* | 00-39-01 | S/A [3,4)] | Hybrid |
| 30 | *R. pendulina x R. spinosissima* | 00-40-01 | S/A [3)] | Hybrid |
| 31 | *R. pendulina x R. spinosissima* v. *lageroides* | 00-40-03 | S/A [3)] | Hybrid |
| 32 | *R. roxburghii* Tratt. | 93-35-02 | A [1)] | *Platyrhodon* |
| 33 | *R. rubiginosa* L. | 93-11-06 | A [1)] | *Caninae* |
| 34 | *R. sempervirens x R. centifolia* | Group 126 | S [2)] | Hybrid |
| 35 | *R. setigera*Michx. v. *serena* Palm. & Steyerm. | 00-47-02 | S/A [3)] | *Synstylae* |
| 36 | *R. setipoda* Hemsl.& Wils. | Group 146-4 | S [2)] | *Cinnamomeae* |
| 37 | *R. sweginzowii* Koehne 'Macrocarpa' | 93-45-01 | A [1)] | *Cinnamomeae* |
| 38 | *R. sweginzowii* Koehne 'Macrocarpa' | 93-45-02 | A [6)] | *Cinnamomeae* |
| 39 | *R. sweginzowii* Koehne | Group 146-6 | S [2)] | *Cinnamomeae* |
| 40 | *R. texarcana* Rydberg | 00-53-07 | S/A [3)] | *Carolinae* |
| 41 | *R. virginiana* Mill. | 00-42-02 | S/A [3,4)] | *Carolinae* |
| 42 | *R. wichurana* Crép. | 93-99-01 | A [1)] | *Synstylae* |
| 43 | *R. x longfort* (*R. gallica x R. multiflora*) | Group 129-1 | S [2)] | Hybrid |
| 44 | *R. x wintoniensis* Hillier | Group 146-4 | S [2)] | *Cinnamomeae* |

[1)]Tested with single spore isolates of *D. rosae* and field collected samples from England, France, Portugal and Germany (Ahrensburg, Dortmund, Lüneburg, Gütersloh, München, Rethmar, Sangerhausen, Siebeldingen, Sparrieshoop, Weinberg); [2)]Tested with field collected samples from Sangerhausen and Ahrensburg; [3)]Tested with single spore isolates and field collected samples from Ahrensburg, Gütersloh and Sparrieshoop; [4)]These accessions are first tested resistant in Sangerhausen, but were susceptible in detached leaf assay in Ahrensburg; [5)]Only susceptible to isolate 'AHE10' of *D. rosae*, but resistant to all other isolates of black spot, downy and powdery mildew; [6)]Tested with single spore isolate 'Dort E4' and 'AHE10' and field collected samples from England, France, Portugal and Germany (Ahrensburg, Dortmund, Lüneburg, München, Rethmar, Sangerhausen, Siebeldingen, Sparrieshoop, Weinberg).

## Results

### *Evaluations of black spot resistance at Sangerhausen rosary*

In Sangerhausen, 486 accessions were evaluated for black spot resistance in the field of which 289 accessions (59.5 %) were not infected by *D.rosae* (Table 1). The section *Caninae* cover the major part of the selected test plants with 195 accessions followed by the section *Cinnamomeae* with 120 accessions. Interestingly, all 31 evaluated accessions of the section *Pimpinellifoliae* were resistant in the field as were more than 80 % of the accessions in the section *Synstylae*, 66.7 % in the section *Cinnamomeae* and 46.1 % within the *Caninae*. Additionally, resistance to *D. rosae* was tested in detached leaf assay in the lab using a sub-sample of 178 accessions of which only 33 accessions remain uninfected after inoculation with field collected samples of the pathogen (Table 1 and 2). The major fraction of 13 resistant accessions (= 39.4 %) belongs to the *Cinnamomeae*, followed by the section *Caninae* (4 accessions), *Carolinae* and *Synstylae* (each 3 accessions) and *Pimpinellifoliae* (2 accessions). Eight of the resistant accessions were hybrids. From that data, it seems that black spot resistance is more widespread in the section *Cinnamomeae*, where 10.8 % of the accessions are resistant compared to the resistant 2.1 % in the section *Caninae.*

### *Evaluation for black spot resistance at the location Ahrensburg*

At Ahrensburg, 130 accessions belonging to 65 species were screened visually in the field, of which 41 accessions were not infected by black spot. Over the subsequent years, these accessions were inoculated in the lab with the single spore isolate 'Dort E4', which displayed the broadest infection pattern so far (Debener et al. 1998) and with several field collected samples and single spore isolates of the pathogen (Von Malek-Podjaski 1999; Blechert 2005). Resistance to black spot could be confirmed only for 15 of the originally tested 130 accessions from Ahrensburg. Finally, these 15 accessions were tested with the new race *D. rosae* 'AHE10' resulting in 11 highly resistant accessions (Table 1). After all, a total of 44 accessions (Table 2) from the locations of Sangerhausen and Ahrensburg were shown to be resistant to black spot after field and lab investigations of which 20 accessions are in the section *Cinnamomeae*.

### *Evaluation for powdery mildew resistance*

In the field, 149 accessions representing 97 species in nine sections were tested for powdery mildew resistance of which 56 are resistant to the pathogen in the field. In subsequent *in vitro* screenings using field collected samples of the powdery mildew fungus 39 of the 56 field resistant accessions were resistant to *P. pannosa* in the detached leaf assay, as well (Table 1 and 3). Resistant accessions were found in six sections of the subgenus *Rosa* and one in the subgenus *Hesperhodos*. Similar to black spot most of the resistant accessions (13) are in the section of the *Cinnamomeae* followed by *Caninae* (12). Five accessions belong to the *Pimpinellifoliae*, four are from *Synstylae*, two are from *Carolinae*, and one is found in *Hesperhodos* and *Gallicanae*. One of the resistant accessions is the hybrid *R.* x *hibernica*. The highest content of resistant accessions are in the two sections of the *Cinnamomeae* and *Caninae* with 30.2 and 24.5 % resistant accessions, respectively (Table 1).

### *Evaluation for downy mildew resistance*

Downy mildew did not occur in rose fields in Ahrensburg during 2002 to 2005. Therefore, resistance data for downy mildew were collected only in detached leaf assay. Resistance was tested for 86 accessions from 3 subgenera and 8 sections including 26 accessions in the section *Cinnamomeae*, 19 in *Caninae*, 16 in *Synstylae*, six in *Gallicanae* and *Pimpinellifoliae*, four in *Carolinae* and one in *Indicae* and *Banksiae*. One accession each is from the subgenera *Hesperhodos* and *Platyrhodon* and five of the accessions are hybrids (Table 1). Thirteen of the 86 accessions (15.1 %) of the tested wild roses were resistant in detached leaf assay, of which seven accessions were found in the *Caninae.* Three resistant plants are found in the section *Cinnamomeae*, two in *Carolinae* and one in *Gallicanae* (Table 1 and 4).

### *Resistance to more than one pathogen*

Resistance to black spot was found preferentially in the *Cinnamomeae*, whereas resistance to downy mildew was primarily detected in the *Caninae*. Only for powdery mildew we found similar percentage of resistance in both sections. However, there is a group of accessions with overlapping resistance (Table 5). Resistance to *P. sparsa* and *P. pannosa* was tested with 67 accessions, 53 accessions were tested with *P. sparsa* and *D. rosae*, 64 with *D. rosae* and *P. pannosa*, and 44 of all accessions from Ahrensburg and Sangerhausen were tested against isolates of all three pathogens. In these combinations 13 accessions have shown double resistance to two of the pathogens. Most of them are *Cinnamomeae* (8 accessions) followed by *Caninae* (4 accessions) and *Carolinae* (1 accession). Interestingly, six of the eight double resistant *Cinnamomeae* are resistant to black spot and powdery mildew, whereas three of four *Caninae* prefer the combination of downy mildew and powdery mildew resistance (Table 5). The downy and powdery mildew resistant *R. majalis* 93-09-01 was additionally resistant to all tested field collected samples and single conidial isolates of black spot in field and detached leaf assays. So far, this resistance is broken only by the race 'AHE10' of black spot first reported in 1998 in the experimental field of Ahrensburg (Von Malek-Podjaski 1999).

## Discussion

Breeding for resistance is a long-term disease-management strategy and an important alternative to the use of pesticides. The use of genetic resistance is usually the simplest, the environmentally most appropriate and most cost-effective strategy of managing pests and diseases (Russell 1978; Allen 1983; De Vries 2000). When resistance to various biotic and abiotic stresses in cultivated germplasm is low, the range of genetic variability is often also narrow and the selection pressure is leading to virulent biotypes of the diseases, the introgression of additional resistance genes from wild species is the key to maintain crop productivity (Kameswara Rao et al. 2003). In the past, characters other than resistance were of pri-

Table 3. Resistance to powdery mildew in wild rose accessions after field evaluation and detached leaf assay at the location Ahrensburg.

| No. | Wild rose accession | Code | Section/ Subgenus |
|---|---|---|---|
| 1 | *R. agrestis* Savi. | 93/02-01 | *Caninae* |
| 2 | *R. bella* Rehd. &. Wils | 00/57-04 | *Cinnamomeae* |
| 3 | *R. californica* Cham. et Schlecht. 'Plena' | 00/33-01 | *Cinnamomeae* |
| 4 | *R. canina* L. | 93/06-02 | *Caninae* |
| 5 | *R. canina* L. | 93/06-01 | *Caninae* |
| 6 | *R. canina* L. | 93/06-05 | *Caninae* |
| 7 | *R. caudata* Baker | 94/103-02 | *Cinnamomeae* |
| 8 | *R. centifolia* 'Muscosa' | 93/55-03 | *Gallicanae* |
| 9 | *R. corymbifera* Borkh. 'Laxa' | 93/10-01 | *Caninae* |
| 10 | *R. corymbifera* Borkh. 'Laxa' | 93/10-02 | *Caninae* |
| 11 | *R. foetida* Herrm. v. *bicolor* (Jacq.) Willm. | 93/13-03 | *Pimpinellifoliae* |
| 12 | *R. foetida* Herrm. v. *bicolor* (Jacq.) Willm. | 93/13-02 | *Pimpinellifoliae* |
| 13 | *R. foetida* Herrm. v. *persiana* (Lém.) Rehd. | 93/14-02 | *Pimpinellifoliae* |
| 14 | *R. forrestiana* Boulenger | 94/102-01 | *Cinnamomeae* |
| 15 | *R. frobellii* Christ | | *Cinnamomeae* |
| 16 | *R. glauca* Pourr. | 93/17-02 | *Caninae* |
| 17 | *R. glutinosa* Sibth & Sm. | 94/108-01 | *Caninae* |
| 18 | *R. helenae* Rehd. &. Wils. | 93/18-01 | *Synstylae* |
| 19 | *R. x hibernica* Templ. | 00/36-01 | Hybrid |
| 20 | *R. hirtella* Rip. | 00/56-05 | *Caninae* |
| 21 | *R. jundzillii* Besser | 94/114-01 | *Caninae* |
| 22 | *R. lunellii* Greene | 00/37-01 | *Cinnamomeae* |
| 23 | *R. majalis* Herrm. | 93/09-01 | *Cinnamomeae* |
| 24 | *R. majalis* Herrm. | 93/09-03 | *Cinnamomeae* |
| 25 | *R. majalis* Herrm. v. *foecundissima* (Muenchh.) Hyl. | 93/08-01 | *Cinnamomeae* |
| 26 | *R. multflora* Thunb. v. *cathayensis* (Boul.) Rehd. & Wils. | 94/107-01 | *Synstylae* |
| 27 | *R. multiflora* Thunb. Ex Murr. | 93/27-01 | *Synstylae* |
| 28 | *R. multiflora* Thunb. Ex. Murr. | 93/27-04 | *Synstylae* |
| 29 | *R. nanothamnus* Boulenger | 00/56-01 | *Cinnamomeae* |
| 30 | *R. nitida* Willd. | 93/29-04 | *Carolinae* |
| 31 | *R. nitida* Willd. | 93/29-02 | *Carolinae* |
| 32 | *R. nutkana* C. Presl. | 93/30-01 | *Cinnamomeae* |
| 33 | *R. omeiensis* Rolfe f. *pteracantha* Rehd. & Wils. | 93/39-02 | *Pimpinellifoliae* |
| 34 | *R. omeiensis* f. *pteracantha* | 93/39-01 | *Pimpinellifoliae* |
| 35 | *R. rubiginosa* L. | 93/11-03 | *Caninae* |
| 36 | *R. serafinii* Viv. | 00/55-01 | *Caninae* |
| 37 | *R. stellata* Wooton v. *mirifica* (Greene) Cockerell | 93/44-01 | *Hesperhodos* |
| 38 | *R. suffulta* Greene | 00/54-04 | *Cinnamomeae* |
| 39 | *R. webbiana* Royle | 00/53-06 | *Cinnamomeae* |

Field observations of powdery mildew were done within two years. Field collected samples of the powdery mildew fungus were used in detached leaf assays. From each accession six to nine leaflets were used and placed in glass petri dishes on water-agar (0.5 % agar) containing 0.03 % benzimidazole. Leaflets were inoculated with approximately two conidia per $mm^2$. At 10-days post-inoculation (16 h light, 22 °C), percentages of leaf area covered with conidiophores were estimated in 10 % steps. Accessions with only single conidiophores on single leaflets in only one experiment were scored as having a DI of 5 %. Minimum and maximum values were excluded, and the mean was taken as DI. Mean DI values of lower than 5 % and with no single estimation over 5 % were treated as resistant. The inoculations in detached leaf assay were repeated five to seven times. The experiment was repeated twice.

mary interest for the commercial rose breeders. Looking for the flower colour, cold tolerance, thorniness or shoot yield and recurrent flowering breeders ignored the disease problem and tended to use germplasm only from well-established but limited gene pools. Therefore, little attention was paid to internal quality including disease resistance. As a result, resistance to the most important fungal pathogens is underrepresented or even missing in the pools of some rose groups (Debener et al. 2003). Natural rose populations own a high potential for resistance

Table 4. Resistant accessions to downy mildew estimated in detached leaf assay with different *P. sparsa* isolates.

| No. | Wild rose accession | Code | Section/ Subgenus |
|---|---|---|---|
| 1 | *R. agrestis* Savi. | 93-02-01 | *Caninae* |
| 2 | *R. corymbifera* Borkh. 'Laxa' | 93-10-02 | *Caninae* |
| 3 | *R. foliolosa* Nutt. | 93-15-01 | *Carolinae* |
| 4 | *R. gallica* L. v. *officinalis* | 93-52-01 | *Gallicanae* |
| 5 | *R. glutinosa* Sibth. & Sm. | 94-108-01 | *Caninae* |
| 6 | *R. iberica* Steven | 00-52-02 | *Caninae* |
| 7 | *R. majalis* Herrm. | 93-09-01 | *Cinnamomeae* |
| 8 | *R. majalis* Herrm. | 93-09-03 | *Cinnamomeae* |
| 9 | *R. micrantha* Sm. | 93-23-01 | *Caninae* |
| 10 | *R. nitida* Willd. | 93-29-04 | *Carolinae* |
| 11 | *R. rubiginosa* L. | 93-11-06 | *Caninae* |
| 12 | *R. rubiginosa* L. | 93-11-10 | *Caninae* |
| 13 | *R. rugosa* Thunb v. *rubra* hort. | 00-41-01 | *Cinnamomeae* |

Resistance to downy mildew was estimated in detached leaf assay with the. single spore isolate '3-16' (Germany) and field collected samples M2002 (Germany) and DVP (Belgium). Leaflets from 3–4 different rose accessions with four replications were spotted with several 10 µl drops of a spore suspension of 2 x $10^4$ conidia $ml^{-1}$. Leaf disease was estimated microscopically by a five step classification scheme based on the production of conidiophores at 10–14 dpi. The experiment was repeated twice.

Table 5. Multiple resistance of wild rose accessions to three important rose diseases (+ = resistant; – = susceptible, n. t. = not tested).

| Accession | Code | Section | Ploidy (2n)[1)] | Resistant to | | |
|---|---|---|---|---|---|---|
| | | | | *P. sparsa* | *D. rosae* | *P. pannosa* |
| *R. agrestis* Savi. | 93-02-01 | *Caninae* | 5x, 6x | + | – | + |
| *R. bella* Rehd. & Wils. | 00-57-04 | *Cinnamomae* | 4x | – | + | + |
| *R. californica* Cham. & Schlecht. 'Plena' | 00-33-01 | *Cinnamomae* | 4x | – | + | + |
| *R. caudata* Baker | 94-103-02 | *Cinnamomae* | 2x, 4x | – | + | + |
| *R. corymbifera* Borkh. 'Laxa' | 93-10-02 | *Caninae* | 5x | + | – | + |
| *R. glutinosa* Sibth. & Sm. | 94-108-01 | *Caninae* | 5x | + | – | + |
| *R. lunellii* Greene | 00-37-01 | *Cinnamomae* | 2x | – | + | + |
| *R. majalis* Herrm. | 93-09-01 | *Cinnamomae* | 2x, 4x | + | +[2)] | + |
| *R. majalis* Herrm. | 93-09-03 | *Cinnamomae* | 2x, 4x | + | – | + |
| *R. nanothamnus* Boulenger | 00-56-01 | *Cinnamomae* | 4x | n. t. | + | + |
| *R. nitida* Willd. | 93-29-04 | *Carolinae* | 2x | + | – | + |
| *R. nutkana* C. Presl. | 93-30-01 | *Cinnamomae* | 6x | – | + | + |
| *R. rubiginosa* L. | 93-11-06 | *Caninae* | 5x | + | + | – |

[1)]according to Wissemann (2003)
[2)]*R. majalis* 93-09-01 is only susceptible to the single spore isolate of *D. rosae* 'AHE10'
Sixtyseven accessions were tested with *P. sparsa* and *P. pannosa*, 53 were tested with *P. sparsa* and *D. rosae*, 64 were tested with *D. rosae* and *P. pannosa*, and 44 of all accessions from Ahrensburg and Sangerhausen were tested against all three pathogens.

genes, but up to now only some genotypes of several rose cultivars, breeding lines, and species are investigated for their resistance to fungal diseases (Saunders 1970; Svejda and Bolton 1980; Wenefrida and Spencer 1993; Byrne et al. 1996; Yokoya et al. 2000; Boontiang and Yamaguchi 2002; Colbaugh et al. 2005; Uggla and Carlson-Nilsson 2005; Carlson-Nilsson and Davidson 2006). With their wide natural distribution throughout the temperate regions, rose species constitute a promising source for fungal disease resistance. Therefore, a broad screening with wild rose accessions should discover new resistance sources for the most important rose diseases.Some wild species are known as resistant to black spot, whereas almost no black spot resistance in rose cultivars is found (De Vries 2000). In our own studies, we could confirm the lack of resistance sources in modern rose cultivars. Especially for downy mildew, more than 50 different cultivars were tested for resistance in detached leaf assays, but none was resistant (data not shown). On the other hand, resistant germplasm to all three rose pathogens

were identified among the investigated rose species. Twenty six percent of the tested 149 accessions are resistant to powdery mildew, about 6 % of 581 accessions are resistant to black spot and 15 % of 86 accessions are resistant to downy mildew. These accessions are a valuable genetic resource to introduce resistance genes into the cultivars and to broaden the genetic background in modern roses. Development of the disease depends on biotic and abiotic conditions, which will vary on the different field plots (Carlson-Nilsson and Davidson 2000). If resistance is estimated only in field tests, it will be difficult to identify the inherent resistance in the different wild rose accessions. The determined field resistance is only related to a distinct area, where a part of the existing races is present. Furthermore, the abiotic conditions vary from year to year and susceptible wild roses may not be infected. For that reason, additional tests are necessary in the lab under constant conditions using broad mixtures of the pathogen and identified races from different locations to assure a high resistance in the evaluated wild rose accessions (Leus 2005). Nevertheless, the occurrence of new races can also overcome that broad resistance, which makes it necessary to continue on resistance screening. In addition to screens for resistance to single pathogens we searched for multiple resistances in wild rose accessions. We used different isolates of the pathogens, complex inocula from different geographic regions, as well as single conidial isolates to increase the probability to identify rose accessions resistant to different races of the pathogen, which probably will comprise different and new resistance genes. The incorporation of several resistance genes into the cultivars may extend the life cycle of each gene by keeping the selection pressure on the pathogen population as low as possible. So far, only three R-genes are known in roses, the black spot resistance genes Rdr1 and Rdr2, which have been described previously and the powdery mildew resistance gene *Rpp1* (Von Malek and Debener 1998; Linde and Debener 2003; Hattendorf et al. 2004; Linde et al. 2004). Our results are also a starting point for basic research on resistance and resistance genes in wild roses. Molecular approaches could involve the detailed characterization of the many genes that confer resistance. Molecular markers are currently developed for disease resistance screening in roses and are used to map additional resistance genes to black spot and powdery mildew (Von Malek et al. 2000; Linde and Debener 2003; Zhang 2003; Linde et al. 2004; Yan 2005). Moreover, quantitative trait loci (QTLs) were mapped for resistance to powdery mildew by Dugo et al. (2005). The introgression of QTLs would provide a more durable resistance than single genes (Linde et al. 2006). The use of accessions resistant to several pathogens would facilitate the incorporation of the different resistance genes into the breeding material and could also reduce the number of steps needed to eliminate the genetic background of wild roses. We found several useful candidates representing valuable resistance to downy mildew, powdery mildew or black spot. None of the different rose species is completely resistant to the pathogens. For example, only two of three *R. majalis* accessions from Ahrensburg are resistant to black spot and two of three *R. nitida* accessions are resistant to powdery mildew. This interaction pattern is to be expected as two of the three pathosystems have been shown to follow the "gene for gene" concept. This is in agreement with the fact that no consistency is found for the modern roses in literature about different degrees of susceptibility between climbing roses, ramblers and hybrid teas, floribunda and polyantha cultivars (Leus 2005). The data from our own experiments show that no taxonomic group within the genus *Rosa* is completely resistant. However, 33.3 % of all accessions resistant to the powdery mildew fungus and 45.5 % resistant to black spot are found in the *Cinnamomeae*. Interestingly, the *Caninae* seem to play no major role in disease resistance to black spot, whereas quite the opposite is the case for downy mildew. Due to ploidy differences and genomic incompatibilities, attempts to introgress resistance from wild species to cultivars are often difficult (Van Huylenbroeck et al. 2005). Modern roses are predominantly tetraploid, while wild species are predominantly diploid, and also tetra-, penta-, hexa- and octaploid species exist (De Vries and Dubois 1996; Byrne and Crane 2003; Wissemann 2003). Crosses between diploid and tetraploid roses often result in sterile triploids. Various techniques can be used to overcome these interploidy barriers by reducing ploidy levels through haploidization or to increase ploidy by use of spindle inhibitors like colchicine and oryzalin (Drewes-Alvarez 1992; Kermani 2003; Leus 2005), through unreduced gametes (Crespel and Gudin 2003), embryo rescue (Drewes-Alvares 2003b) and by protoplast fusion (Squirrell et al. 2005). In our own study, we found that the tetraploid *R. bella* 00-57-04, *R. californica* v. *plena* 00-33-01, *R. majalis* 93-09-03 and *R. nanothamnus* 00-56-01 are highly resistant to black spot and powdery mildew and are primary candidates in breeding programs with the likewise tetraploid garden- and cut roses. To introduce downy mildew resistance *R. majalis* 93-09-01 should be the first choice in a breeding program, because it expressed high resistance against all the tested isolates of the three rose pathogens, with the exception of the black spot race 'AHE10'. In interspecific crosses a large number of unwanted traits are transmitted from wild roses and restrain breeders from using wild species to introgress resistance genes. However, with the use of molecular markers and backcross breeding, the reduction of the genetic background of wild rose species in introgression programs will allow a more efficient utilization of wild rose germplasm (Debener et al. 2003) and could be a solution for the breeders to use wild rose species in future breeding programs. The genotypes we identified in this study could serve as a starting point for such approaches.

## Acknowledgements

This research was funded by AIF (Berlin, Germany) and the German rose breeding companies W. Kordes Soehne, Sparrieshoop, Rosenwelt Tantau, Uetersen and Noack Rosen, Guetersloh. Authors wish to thank Prof. David H. Byrne, Texas A&M University and Prof. Andrew Roberts, University of East London for critical reading of the manuscript.

## References

Allen, D.J. 1983: The Pathology of Tropical Food Legumes: Disease Resistance in Crop Improvement. John Wiley & Sons, Chichester, UK, 428 pp.

Blechert, O. 2005: Molekularbiologische und mikroskopische Untersuchungen am Pathosystem *Diplocarpon rosae* Wolf (Sternrußtau) - Rose. Ph.D. Thesis, Leibniz University of Hannover.

Boontiang, K. and S. Yamaguchi 2002: Breeding for resistance to black spot (*Diplocarpon rosae* Wolf) disease in rose 2: A search for highly resistant rose species in southern Japan. SABRAO J. of Breed. and Genet. **34**, 115–119.

Braun, U. and S. Takamatsu 2000: Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (*Erysiphaceae*) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (*Cystotheceae*) inferred from rDNA ITS sequences – some taxonomic consequences. Schlechtendalia **4**, 1–33.

Byrne, D.H., W. Black, Y. Ma and H.B. Pemberton 1996: The use of amphidiploidy in the development of blackspot resistant rose germplasm. In: Morsot, A. and P. Ricci (eds.): Second international symposium on roses. Acta Hort. **424**, 269–272.

Byrne, D.H. and Y.M. Crane 2003: Meiosis. In: Roberts A, T. Debener and S. Gudin (eds.): Encyclopedia of Rose Science. Elsevier Science, Oxford, pp 273–279.

Carlson-Nilsson, B.U. and C.G. Davidson 2000: Research on blackspot pathogen *Diplocarpon rosae* (*Marssonina rosae*) and resistance in *Rosa*. Acta Hort. **508**, 141–148.

Carlson-Nilsson, B.U. and C.G. Davidson 2006: Variation in resistance to *Marssonina rosae* (Lib.) Died. among different *Rosa* L. cultivars and species including three dogrose species (*Rosa* sect. *Caninae*). Sci. Hortic. **109**, 353–360.

Colbaugh, P.F., W.T. Crow, W.A. Mackay and S.W. George 2005: Rose variety reaction to blackspot disease under minimal input growing conditions. Biological and Cultural Tests for Control of Plant Diseases **20**, O017.

Crespel, L. and S. Gudin 2003: Evidence for the production of unreduced gametes by tetraploid *Rosa* hybrida L.. Euphytica **133**, 65–69.

Debener, T., R. Drewes-Alvarez and K. Rockstroh 1998: Identification of five physiological races of black spot, *Diplocarpon rosae* Wolf on roses. Plant Breed. **117**, 267– 270.

Debener T., B. von Malek, M. Schreiber and R. Drewes-Alvarez 2003: Marker assisted background selection for the introgression of black spot resistance into cultivated roses. Eur. J. Hort. Sc. **68**, 245–252.

De Vries, D.P. and L.A.M. Dubois 1996: Rose Breeding: past, present, prospects. Acta Hort. **424**, 241–248.

De Vries, D.P. 2000: Fungus resistant roses: Fact or fake? Acta Hort. **508**, 149–156.

Drewes-Alvarez, R. 1992: Untersuchungen am Pathosystem Sternrußtau (*Marssonina rosae* (Lib.) Died.)-Rose (*Rosa* L.) zur Klärung der Frage nach pilzlichen Rassen und zur Übertragung der Resistenz aus der diploiden *Rosa multiflora* Thunb. auf tetraploide Gartenrosen. Ph.D. Thesis, University of Hamburg.

Drewes-Alvarez, R. 2003a: Disease/Black spot. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier, Academic Press, Oxford.

Drewes-Alvarez, R. 2003b: Biotechnologies for Breeding / Early embryo rescue. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Academic Press, Oxford.

Dugo, M.L., Z. Satovic, T. Millan, J.I. Cubero, D. Rubiales, A. Cabrera and A.M. Torres 2005: Genetic mapping of QTLs controlling horticultural traits in diploid roses. Theor. Appl. Genet. **111**, 511–520.

Gudin, S. 2000: Rose: Genetics and breeding. Plant Breeding Reviews **17**, 159–189.

Hattendorf A., M. Linde, L. Mattiesch and T. Debener 2004: Genetic analysis of rose resistance genes and their localisation in the rose genome. Acta Hort. **651**, 123–130.

Horst, R.K. 1983: Compendium of rose diseases. Am. Phytopath. Soc., St. Paul.

Kameswara Rao, N., L.J. Reddy and P.J. Bramel 2003: Potential of wild species for genetic enhancement of some semi-arid food crops. Genet. Resourc. and Crop Evol. **50**, 707–721.

Kermani, M.J., V. Sarasan, A.V. Roberts, K. Yokoya, J. Wentworth and V.K. Sieber 2003:Oryzalin-induced chromosome doubling in Rosa and its effect on plant morphology and pollen viability. Theor. Appl. Genet. **107**, 1195–1200.

Leus, L. 2005: Resistance breeding for powdery mildew (*Podosphaera pannosa*) and black spot (*Diplocarpon rosae*) in roses. Ph.D. Thesis, Faculty of Bioscience Engineering, Ghent University.

Linde, M. and T. Debener 2003: Isolation and identification of eight races of powdery mildew or roses (*Podosphaera pannosa* (Wallr.: Fr.) de Bary) and the genetic analysis of the resistance gene Rpp1. Theor. Appl. Genet. **107**, 256–262.

Linde, M. and N. Shishkoff 2003: Disease / Powdery mildews. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier, Academic Press, Oxford.

Linde, M., L. Mattiesch and T. Debener 2004: Rpp1, a dominant gene providing race-specific resistance to rose powdery mildew (*Podosphaera pannosa*): molecular mapping, SCAR development and confirmation of disease resistance data. Theor. Appl. Genet. **109**, 1261–1266.

Linde, M., A. Hattendorf, H. Kaufmann and T. Debener 2006: Powdery mildew resistance in roses: QTL mapping in different environments using selective genotyping. Theor. Appl. Genet. **113**, 1081–1092.

Noack, R. 2003: Breeding / Selection strategies for disease and pest resistance. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier, Academic Press, Oxford.

Roberts, A.V., T. Debener and S. Gudin 2003: Modern Roses XI. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier, Academic Press, Oxford.

Russell, G.E. 1978: Plant Breeding for Pest and Disease Resistance. Butterworth Publ., London-Boston.

Saunders, P.J.W. 1970: The resistance of some cultivars and species of *Rosa* to *Diplocarpon Rosae* Wolf causing black spot disease. The Rose Ann., 118–128.

Schulz, D.F. and T. Debener 2007: Screening for Resistance to Downy Mildew and its Early Detection in Roses. Acta Hort. **751**, 189–198.

Squirrell, J., Z. Mandegaran, K. Yokoya, A.V. Roberts and J. Mottley 2005: Cell lines and plants obtained after protoplast fusions of *Rosa+Rosa*, *Rosa+Prunus* and *Rosa+Rubus*. Euphytica **146**, 223–231.

Svejda, F.J. and A.T. Bolton 1980: Resistance of rose hybrids to three races of *Diplocarpon rosae*. Can. J. Plant Pathol. **2**, 23–25.

Uggla, M. and B.U. Carlson-Nilsson 2005: Screening of fungal diseases in offspring from crosses between *Rosa* sections *Caninae* and *Cinnamomeae*. Sci. Hortic. **104** , 493–504.

Van Huylenbroeck, J., M.J.M. Smulders, T. Debener, H. Nybom, S. Gudin, P. Cox, L. Crespel and J. De Riek 2005: GENEROSE: Genetic evaluation of European rose resources for conservation and Horticultural use. Acta Hort. **690**, 119–123.

Von Malek, B. and T. Debener 1998: Genetic analysis of resistance to black spot (*Diplocarpon rosae*) in tetraploid roses. Theor. Appl. Genet. **96**, 228–231.

Von Malek, B., W.E. Weber and T. Debener 2000: Identification of molecular markers linked to Rdr1, a gene conferring resistance to black spot in roses. Theor. Appl. Genet. **101**, 977–983.

Von Malek-Podjaski, B. 1999: Erschließung von Resistenzquellen gegenüber dem Erreger des Sternrußtau (*Diplocarpon rosae* Wolf) an Rosen unter Verwendung molekularer Marker. Ph.D. Thesis, University of Hanover.

Wenefrida, I. and J.A. Spencer 1993: *Marssonina rosae* variants in Mississippi and their virulence on selected rose cultivars. Plant Disease **77**, 246–248.

Widholm, J.M. 1972: The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. Stain Technol. **47**, 189–94.

Wiegand, J. 2001: Untersuchungen Zum Erreger des Sternrußtaus *Diplocarpon rosae (Lib.)* Wolf and Rosen. Dresden, Germany. Dresden Germany Polytechnic for Technology and Economics, BSc Thesis.

Wissemann, V. 2003: Conventional Taxonomy (Wild Roses). In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier Academic press, Oxford, 111–117.

Xu, X. and T. Pettit 2003: Disease/ Downy Mildew. In: Roberts, A.V., T. Debener and S. Gudin (eds.): Encyclopedia of rose science. Elsevier, Academic Press, Oxford.

Yan, Z. 2005: Towards efficient improvement of greenhouse grown roses: genetic analysis of vigour and powdery mildew resistance. Ph.D. Thesis, Wageningen University.

Yokoya, K., K.I. Kandasamy, S. Walker, Z. Mandegaran and A.V. Roberts 2000: Resistance of roses to pathotypes of *Diplocarpon rosae*. Ann. Appl. Biol. **136**, 15–20.

Zhang, L. 2003: Genetic linkage mapping in tetraploid and diploid rose. Ph.D. Thesis, Clemson University, USA.

Received July 01, 2007 / Accepted September 09, 2008

Addresses of authors: Dietmar Schulz, Thomas Debener, (corresponding authors) and Marcus Linde, University of Hannover, Institute of Plant Genetics, Department of Molecular Breeding, Herrenhäuser Str. 2, D-30419 Hannover, Germany, and Oliver Blechert, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada, e-mail:
schulz@genetik.uni-hannover.de,
debener@genetik.uni-hannover.de.