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Developing Resistance to Powdery Mildew (*Podosphaera pannosa* (Wallr.: Fr.) de Bary): A Challenge for Rose Breeders

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ABSTRACT

Powdery mildew is the major fungal pathogen of roses in greenhouses and also an important disease on field-grown roses. In the past decade different tools have been developed allowing breeders to develop resistant roses in a more efficient way. Different pathotypes of the fungus, important for resistance testing, were detected. Resistance mechanisms in rose leaves were found and characterized. Screening techniques to evaluate powdery mildew resistance are available. These methods allow pathotype specific inoculation on detached leaves or can be used for the selection of resistant genotypes within a population of thousands of seedlings. New information on the genetic background of powdery mildew resistance became available. Genetic maps providing information on resistance markers are currently being developed and integrated. Marker-assisted selection is expected to be ready soon for use in rose breeding programs for powdery mildew resistance among other traits. This review aims to provide an overview on fundamental information and methodology available and necessary to make progress in breeding for powdery mildew resistance in roses.

Keywords: breeding, disease resistance, *Rosa*, seedling selection, *Sphaerotheca pannosa*

Abbreviations: **ADR**, Allgemeine Deutsche Rosenneuheitenprüfung; **AFLP**, amplified fragment length polymorphism **Ap**, appressorium; **BTH**, benzo (1,2,3)thiadiazole-7-carbothioic acid S-methyl ester; **CC**, cell collapse; **Co**, conidium; **CP**, conidiophore; **FM**, fungal mycelium; **INA**, 2,6-dichloro-isonicotinic acid; **ISR**, induced systemic resistance; **ITS**, internal transcribed spacer; **LRR**, leucine rich repeat; **MC**, multi cell reaction; **NBS**, nucleotide binding site; **PGPR**, plant growth-promoting rhizobacteria; **PK**, protein kinase; **QTL**, quantitative trait loci; **RAPD**, random amplified polymorphic DNA; **RFLP**, restriction fragment length polymorphism; **RGA**, resistance gene analogue; **SAR**, systemic acquired resistance; **SCAR**, sequence characterized amplified region; **SSR**, simple sequence repeat; **TIR**, toll and interleukin-1 receptor

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INTRODUCTION

Powdery mildew *Podosphaera pannosa* (Wallr.: Fr.) de Bary (syn: *Sphaerotheca pannosa* var. *rosae* (Wallr.: Fr.) Lév.), is one of the main diseases on both cut and garden roses in most parts of the world. Consumer demand for carefree garden roses and an increased concern for environmental issues during recent decades led to disease resistance becoming a prerequisite for new cultivars. Today, new garden rose cultivars with significantly better resistance to most common rose diseases, like powdery mildew, are entering the market. In cut rose production disease resistance is still less important as pathogen control in the controlled environment of a greenhouse is routine. Nevertheless developing more resistant roses for all market classes is necessary.

The availability of unexploited, valuable germplasm in the genus *Rosa* (i.e. species, early generation species hybrids, older cultivars, etc.) as well as new knowledge generated in different research groups (i.e. reproductive biology, taxonomy, phytopathology, etc.) during the last decade offer breeders tools to increase their effort to select for better roses. In this article an overview is given on practical information regarding powdery mildew, including the fungal pathogen, resistance mechanisms in the rose plant and how to apply this knowledge to enhance disease resistance.

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Invited Review

POWDERY MILDEW ON ROSES

The pathogen

Podosphaera pannosa, formerly known as *Sphaerotheca pannosa*, was studied among other *Podosphaera* spp. by Cook *et al*. (1997). Based on conidial surface observations, they could not distinguish the anamorphs of *Podosphaera* and *Sphaerotheca*, which made them conclude that difference between both is only due to the host plant. Internal transcribed spacer (ITS) sequencing (Takamatsu *et al*. 1998) and the combination of morphological and ITS-data (Saenz and Taylor 1999) supported the theory of both species being monophyletic. It was finally decided that all *Sphaerotheca* species belong to the genus *Podosphaera* and therefore have to be renamed after it (Braun and Takamatsu 2000; Braun *et al*. 2002). Recently, Leus *et al*. (2006) found the host plant specificity for powdery mildew is not limited to roses but also applies to *Prunus avium* L. The specificity of the fungal strains to one or both plant species is reflected in the ITS-sequence. Also on sour cherry, *Prunus cerasus* L., infection by *Podosphaera pannosa* was reported (Vajna and Rozsnyay 2006). A broader host range does not necessarily mean the pathogen is not specialized as pathotypes of powdery mildew on rose exist. Pathotypes of rose powdery mildew have been described by Mence and Hildebrandt (1966), Bender and Coyier (1984) and by Linde and Debener (2003). Bender and Coyier (1984) were the first to use monoconidial isolates on detached rose leaves. They tested nine isolates and identified five pathotypes in a host isolate differential using four rose genotypes. Based on differential reactions on ten rose genotypes, Linde and Debener (2003) detected eight different races in eight monoconidial isolates tested. It was proposed that powdery mildew on rose has a very high racial diversity.

Resistance breeding

Racial diversity in powdery mildew implies breeding for resistance in roses should not be directed towards specific, monogenic or vertical resistance, but towards quantitative resistance. Quantitative resistance is based on a polygenetic background. In general however this type of resistance (also called polygenic, horizontal or partial resistance) is less studied and depends upon multiple genes in the host plant (Tuzun 2001). Often slow fungal development and reproduction are noticed by a delayed onset of infection, reduced final extent of leaf area with symptoms and reduced spore production. Frequently also the term tolerance is used to indicate this type of resistance.

Since the postulation of resistance genes, much effort in breeding research was directed towards monogenic or so called qualitative or vertical resistance as these resistances are easier to monitor in a breeding program. Often in selection a binary response, resistant or susceptible, can be used and expectations for segregation can be made. In roses a single dominant resistance gene *Rpp1* was shown by Linde and Debener (2003) based upon a 1:1 segregation for one specific powdery mildew pathotype. Segregation of this resistance gene was observed in several diploid rose populations (Linde *et al*. 2004).

GENETIC BACKGROUND: DISEASE RESISTANCE MAPPING

Genetic maps in rose were developed during last decade by several research groups in order to locate genes and to be used for marker-assisted selection for specific phenotypic characters. Mapping in diploid roses was done by Debener and Mattiesch (1996, 1999), Crespel *et al*. (2002), Linde *et al*. (2004), Dugo *et al*. (2005), Yan *et al*. (2005) and Hibrand-Saint Oyant *et al*. (2008). For tetraploid roses maps were constructed by Rajapakse *et al*. (2001), Yan (2005) and Zhang *et al*. (2006). Linde *et al*. (2004), Yan (2005), Yan *et al*. (2005), Dugo *et al*. (2005) and Linde *et al*. (2006) used genetic mapping to locate powdery mildew resistance. Linde *et al*. (2004) isolated amplified fragment length polymorphism (AFLP) markers linked to the resistance gene *Rpp1*. In this study the most closely linked AFLP marker was also converted to a sequence characterized amplified region (SCAR). Dugo *et al*. (2005) used random amplified polymorphic DNA (RAPD) and simple sequence repeat or microsatellite (SSR) markers to construct a genetic map and to locate quantitative trait loci (QTLs) for horticultural traits and powdery mildew resistance in a diploid rose population. Two QTLs for powdery mildew resistance were found. Yan *et al*. (2005) constructed an integrated map for a diploid rose population with AFLP, SSR, protein kinase (PK), resistance gene analogue (RGA), restriction fragment length polymorphism (RFLP), SCAR and morphological markers for a diploid population using a total of 520 molecular markers (Yan *et al*. 2005). SSR markers for roses as those developed by Esselinck *et al*. (2003) are now widely used in the different maps providing anchor points for the alignment of diploid and tetraploid maps and for consolidation of existing maps.

These markers are also very useful for the actual construction of a consensus map for rose. The map presented by Yan *et al*. (2005) is an essential step towards the development of this reference map. Also comparative genetics and synteny studies among Rosaceae are possible by SSR markers as is discussed by Hibrand-Saint Oyant *et al*. (2008). Besides an integrated map based on diploid populations, Yan (2005) also created a map based on a tetraploid population. Yan (2005) used a population resulting from two partly resistant tetraploid cultivars. In general the tetraploid level is more similar to the commercial roses as most are tetraploid. However, for genetic mapping a higher ploidy level is more challenging. Generating a map with sufficient coverage on in total 56 linkage groups (basic chromosome number in roses: x=7; four times seven or 28 chromosomes for each parent), and up to eight possible alleles is tedious. Meiosis in roses can lead to the formation of univalents, bivalents or multivalents and depends on ploidy, homology between genomes of ancestral species and structural changes and chromosome rearrangements as reviewed by Zlesak (2006). In gametogenesis in tetraploids by double reduction, partly homozygous gametes can influence segregation. Therefore genetic work in tetraploids on a quantitative trait is complex (Yan 2005).

Yan (2005) made a comparison between the diploid and tetraploid populations used in his separated studies. The same AFLP and SSR primer combinations were used on both populations. The map positions of allelic SSR markers on both maps are anchor points for the alignment of the two maps. Still, it would be useful for more anchor points to be developed. Especially polygenic resistances, as found, can allow pyramiding resistance genes in rose by marker-assisted selection (Yan 2005).

Of specific value for disease resistance mapping are PK, involved in incompatible plant-pathogen interactions (Vallad *et al*. 2001), and RGAs, both used on roses by Yan *et al*. (2005). RGA detection is based on the Nucleotide Binding-Site (NBS) profiling technique which makes use of conserved domains within functionally important families of NBS-containing resistance gene analogues (Van der Linden *et al*. 2004). RGAs were also obtained in rose by Xu *et al*. (2005) in *R. roxburghii* Tratt. They have cloned 11 toll and interleukin-1 receptor (TIR) type and 23 non-TIR nucleotide binding site-leucine rich repeat (NBS-LRR) RGAs. Hattendorf and Debener (2007) evaluated NBS-LRR-RGAs in the rose genome. They showed that in roses the number and diversity of TIR-RGAs is much higher than those of the non-TIR class. Compared to *R. roxburghii* (section *Microphyllae*), the genomic copy numbers of RGAs found in *R. multiflora* Thunb. ex Murray genotypes and *R. rugosa* Thunb., both belonging to other sections (*Synstylae* and *Cinnamomeae*, respectively), were higher. This indicates that the RGA complexity varies depending on the phylogenetic background. Some of the RGAs were mapped in the

studies of Yan *et al*. (2005) and Linde *et al*. (2006). Genomic regions with RGA clusters or RGAs were found to associate with QTLs for powdery mildew resistance. Mapped PK and RGA markers in the vicinity of genes or QTLs are possibilities for utilizing marker-assisted selection towards disease resistance (Yan *et al*. 2005).

Linde *et al*. (2006) tried to discriminate QTL markers for powdery mildew resistance by studying a segregating rose population under 6 different environmental conditions for multiple years. In total, 28 QTLs were detected. The linkage to RGA markers was low, as only 4 clustered together with the strongly clustered linkage groups. Also the QTLs clustered together, suggesting that other than the tested NBS-LRR class RGAs must be involved. Transgressive segregation was also found by Yan (2005) in a tetraploid population and by Hosseini Moghaddam *et al*. (unpublished) in a diploid rose population. The observed transgressive segregation for resistance in the segregating populations suggests a combination of resistances from both parents. This includes combinations leading to more resistant genotypes in the offspring possibly by heterozygosity or polygenic resistances in the parents rearranged in the offspring. Yan (2005) found isolate-specific markers at a tetraploid level and discussed the assumed polygenic background of powdery mildew resistance. In the study of Linde *et al*. (2006) it was also suggested that pathogen race specific QTLs were present. However, QTLs also clustered over all tested environmental conditions, indicating QTLs were valid for general powdery mildew resistance under different environments.

More information is still needed to use marker-assisted selection as a practical tool in rose breeding. Molecular markers can enhance breeding efforts especially for the efficient introgression of resistances from wild species into the cultivar gene pool. In back crosses markers can help to discriminate better resistant genotypes and to reduce unwanted genetic background of the species rose (Debener *et al*. 2004). Also for black spot resistance, marker-assisted selection is developed in roses (Debener *et al*. 2001). To our knowledge molecular tools are so far only used for research and on an experimental scale and not in commercial rose breeding for better resistance or other characteristics.

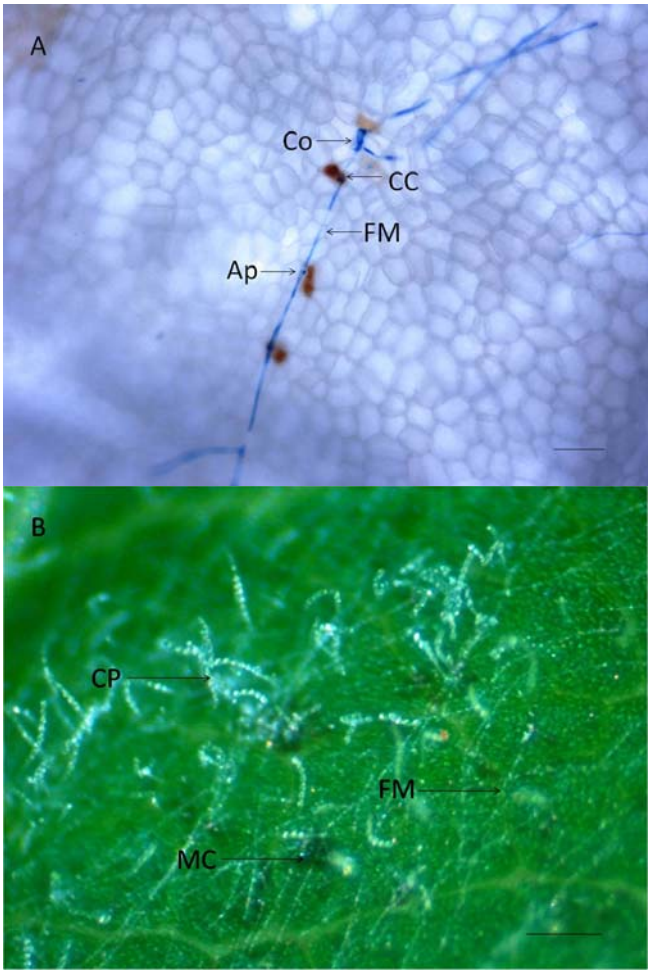

Fig. 1 Examples of resistance reactions in rose leaves towards powdery mildew. (**A**) Single cell collapse in *Rosa wichurana* Crépin after staining of the fungal structures with lactophenolblue (bar = 40 µm). (**B**) Multi cell reactions in 'Excelsa' leaves (bar = 100 µm). Ap = appressorium, CC = cell collapse, Co = conidium, CP = conidiophore, FM = fungal mycelium, MC = multi cell reaction.

RESISTANCE MECHANISMS IN ROSE PLANTS

Characterization of resistance mechanisms in roses

In compatible powdery mildew–host plant interactions, the fungus develops conidia, penetrates the outer epidermal cuticle and cell wall of the host and establishes haustorial complexes within epidermal cells (Koh *et al*. 2005). Resistance in the plant can be caused by different mechanisms that are constitutive or induced at the moment of a pathogen attack. Defence responses towards powdery mildew attack have been studied in model plants like *Arabidopsis thaliana* (L.) Heynh., but also in commercial crops like barley, cucumber and tomato. Resistances in roses influence mycelium formation and sporulation of *P. pannosa*. Hypersensitivity responses in roses have been described by different authors (Mence and Hildebrandt 1966; Conti *et al*. 1985; Hajlaoui *et al*. 1991; Dewitte *et al*. 2007). Because hypersensitivity is a specific reaction of a host genotype to a specific pathogen, the presence of certain resistance genes is involved. Partial resistance in a tetraploid rose population was assessed by Yan *et al*. (2006) for two monospore isolates. Dewitte *et al*. (2007) made a more in depth study on morphological barriers, papillae formation, induced single and multi cell reactions (**Fig. 1**) with or without cell collapse and formation of abnormal haustoria with or without extra-haustorial matrix in different rose genotypes for two powdery mildew isolates. They concluded that resistance reactions were plant genotype and pathotype dependent for the number of resistance reactions, but less for the resistance mechanism. The resistance in the plant was not only controlled by the resistance reaction itself, but also by the speed at which it occurred in the case of induced resistances as shown for 'Excelsa' (syn. 'Red Dorothy Perkins').

Systemic acquired and induced resistances in roses

Plant-pathogen relationships become more complex when also the induction of resistances is addressed. This phenomenon is known as induced systemic resistance (ISR) and systemic acquired resistance (SAR). The classic form of SAR can be triggered by virulent, avirulent, and nonpathogenic microbes, or artificially with chemicals such as salicylic acid, 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Ryals *et al*. 1996). ISR is potentiated by plant growth-promoting rhizobacteria (PGPR), of which the best characterized are strains within several species of *Pseudomonas* (Vallad and Goodman 2004). The constitutive accumulation of specific isozymes of hydrolytic enzymes, or other defense related gene products, is an integral part of both multigenic resistance and the phenomenon of ISR (Tuzun 2001). Phenotypically, ISR is similar to SAR that is triggered by necrotizing pathogens (Bakker *et al*. 2003). Observations made *in situ* showed that ISR and SAR were characterized by hypersensitivity responses, cell wall strengthening, etc. often also in distant (systemic) parts of the plant (Yamaguchi 1998). In roses SAR was demonstrated by the salicylic acid analogues INA for powdery mildew (Hij-

Table 1 Overview of different methods used for powdery mildew resistance screening in roses.

Method	Advantages	Disadvantages	References
Inoculation tower	-Use of specific pathotypes is possible -Standardized conditions -Useful for lab tests	-Repetitions are needed due to a low reproducibility -Labour intensive -Not suited for large screenings	Linde and Debener 2003
Conidia suspension	-Easy quantification of inoculum amount -Useful for greenhouse and lab tests -Use of specific pathotypes is possible	-Temperature control needed -Possible disadvantages on conidia quality	Yan *et al*. 2006
Greenhouse inoculation (inoculation plants or ventilator)	-Suitable for large screenings -Cost and labour effective	-Use of specific pathotypes is difficult -Inoculum quantification not possible	Linde *et al*. 2006; Leus *et al*. 2008
Field observation	-No labour for inoculation needed	-Dependent on natural infection -Pathotypes can differ	Wisniewska-Grzeszkiewicz and Wojdyla 1996; De Vries and Dubois 2001; Ferrero *et al*. 2001; Carlson-Nilsson and Uggla 2005; Linde *et al*. 2006; Leus *et al*. 2007, 2008

wegen *et al*. 1996) and BTH for *Diplocarpon rosae* Wolf (Suo and Leung 2001a, 2001b) and *Podosphaera pannosa* (Leus 2005). Also ISR for different strains of *Pseudomonas aeruginosa* (Schroeter) Migula, a strain of *P. putida* (Trevisan) Migula and *P. fluorescens* Migula and an SAR for an extract of *Reynoutria sachalinensis* (F.W. Schmidt ex Maxim.) Nakai was observed towards powdery mildew in the rose cultivar 'Excelsa' (Leus 2005).

Observations on cucumber (Hijwegen and Verhaar 1995), red cabbage (Hijwegen and Verhaar 1993) and rose (Hijwegen *et al*. 1996) showed variable reactions in different rose cultivars to powdery mildew as responsiveness to SAR. Hijwegen *et al*. (1996) concluded enhancers like INA facilitated not only the control of the disease, but also discriminated between partially resistant and susceptible genotypes, which are useful for the selection of parents in a breeding program. The cultivar 'Excelsa' used in the study of Leus (2005) showed partial resistance towards powdery mildew (Dewitte *et al*. 2007).

SELECTION FOR RESISTANCE

Although about 25,000 rose cultivars are registered (Roberts *et al*. 2003), their genetic background is rather limited. According to Wylie (1954) only 8 to 15 species contributed to the modern cultivars, while between 100 and 250 botanical rose species exist (Henker 2000). This offers breeders opportunities to broaden the rose cultivar germplasm using rose species. For introgression of disease resistance genes in a cultivar genepool the use of wild related species is common. However, compared to diploid crops, information on the inheritance of characteristics in tetraploids is often hampered. Because of more allelic combinations, pyramiding resistance genes for quantitative resistance breeding is more complex in tetraploids. On the other hand the use of diploid species in interploidy rose hybridization with tetraploids is possible. Obtained triploid offspring from interploidy crosses had some fertility and can be used for further backcrossing with tetraploid cultivars (Van Huylenbroeck *et al*. 2005). In garden roses some recently released cultivars with good disease resistance are triploids. This suggests a broader parental background than tetraploid cultivars (triploids are the result of a cross between a diploid and a tetraploid) and the (re)use of species and species related genotypes in actual crossbreeding. Powdery mildew resistance of these cultivars is among other diseases documented by the Allgemeine Deutsche Rosenneuheitenprüfung (ADR) in Germany and Toproos tests in The Netherlands. In these trials garden rose cultivars are tested at different locations without pesticide treatments for disease control and for other important traits. Roses that meet the quality standards can be designated 'Toproos' or ADR-rose.

A requirement for resistance breeding is the availability of effective selection procedures, either under field or under laboratory conditions. An overview of the different methods described in the literature to screen for rose powdery mildew resistance as well as their (dis)advantages is given in **Table 1**.

Artificial inoculations to screen individual genotypes

Besides the development of resistance screening methods applicable in a rose breeding programme to handle large populations, well characterized information on resistance (mechanisms and degrees) in parent plants and individual genotypes is necessary. Records on disease resistance for different fungal diseases are often made by field evaluations (Wisniewska-Grzeszkiewicz and Wojdyla 1996; De Vries and Dubois 2001; Ferrero *et al*. 2001; Carlson-Nilsson and Uggla 2005; Linde *et al*. 2006; Leus *et al*. 2007, 2008). However, the existence of different pathotypes, e.g. for powdery mildew, may influence disease severity scores. ITS sequencing showed that two different powdery mildew isolates can occur simultaneously in a test field (Leus 2005). Linde and Debener (2003) demonstrated by a screening on a differential plant set, that different pathotypes can occur in a small geographical region. Therefore, controlled artificial inoculations should give more useful information. Artificial inoculations with powdery mildew are difficult compared to most other fungal pathogens, since mildew is an obligate parasite and cannot be cultured on artificial media. *In vitro* plants are useful to maintain specific isolates and to produce inoculum (**Fig. 2**). The quality of the obtained spores as well as germination capacity and infectivity can be influenced by environmental factors as temperature and light

Fig. 2 Conidia production of a monoconidial *Podosphaera pannosa* isolate on a rose plant *in vitro*.

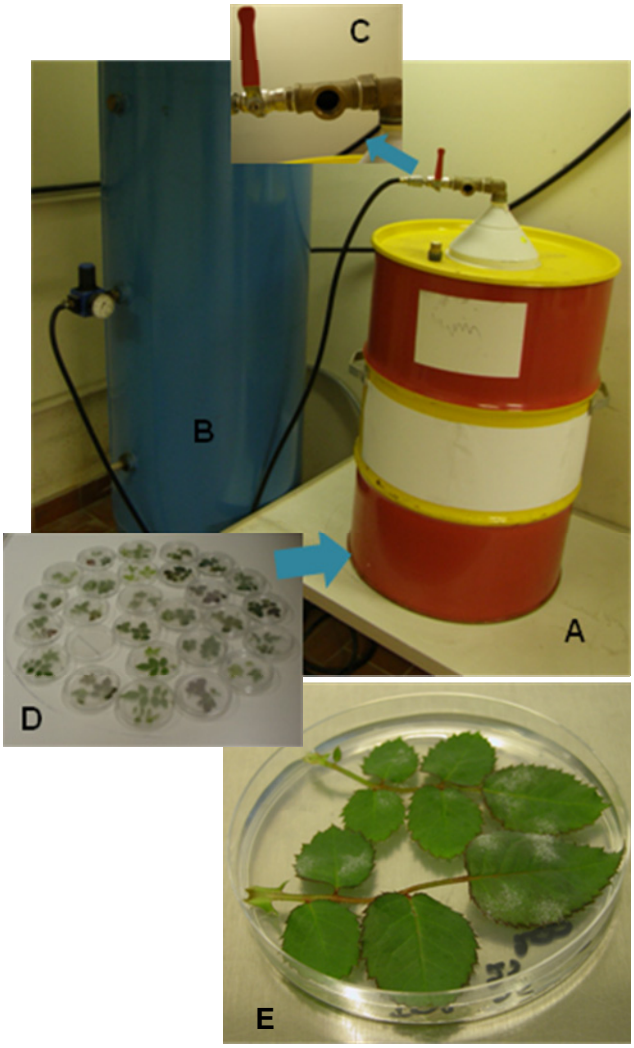

Fig. 3 Inoculation tower for standardized powdery mildew inoculations on detached leaves. (**A**) Barrel used as inoculation tower. (**B**) Pressure vessel to blow compressed air. (**C**) Opening in the funnel for rose leaves infected with powdery mildew. (**D**) Detached leaves on 0.5% microagar with 0.03% benzimidazol in Petri dishes. (**E**) Infection on detached rose leaves 10 days after inoculation with the inoculation tower.

(Butt 1978; Jarvis *et al*. 2002) or by the host plant and media used (Nicot *et al*. 2002; Bardin *et al*. 2007). Also long-term conservation of powdery mildew is difficult. This all makes the use of characterized monoconidial isolates in large quantities difficult.

Standardized inoculation procedures were used by means of an inoculation tower (**Fig. 3**) for lab experiments on detached rose leaves on which conidia are blown with compressed air (Linde and Debener 2003; Leus *et al*. 2007). Another possibility, like a vacuum-operated settling tower, was developed by Reifschneider and Boiteux (1988) and used with *Podosphaera leucotrica* (Ellis & Everh.) E.S. Salmon on apple genotypes by Janse *et al*. (1999). In all tests with the inoculation tower a high variability of scores of individual leaves within one repetition was noticed. Often leaflets of one leaf showed the same high level of infection, whereas other leaves of the same cultivar within the same repetition showed no infection at all. The observed variability between leaves might be due to different leaf ages or physiological conditions, although as much care as possible to select uniform, freshly unfolded leaves were made (Leus 2005). Increased numbers of repetitions are therefore needed to provide proper conclusions on powdery mildew infections established by use of the inoculation tower. Linde *et al*. (2004) suggest five to eight repeated inoculations are needed under highly reproducible conditions. They also found the cut-off point between resistance and susceptible genotypes lies at a disease index of 5%. Plants with a disease index of 5 are considered resistant, while a disease index of 10% or higher means they are susceptible.

The infection pressure can play an important role and can diminish differences in resistances between genotypes (Yan *et al*. 2006). In rose the influence of infection pressure on disease resistance testing is not well studied. Screenings with different inoculation densities with an inoculation tower showed that the infection rate was generally higher when more conidia per cm² were applied (Leus 2005). The use of different conidial densities might be useful to discriminate plants with differences in partial resistance. Publications on powdery mildew on pepper and melon plants correlated the aggressiveness of an isolate by the density of the inoculum used. On melon symptoms were uniform and maximal at spore densities between 200 and 900 conidia per cm², whereas variable at lower densities (Nicot *et al*. 2002). Bushnell (2002) however mentioned that more inoculum did not necessarily result in a higher infection rate. Infection rates decreased as spore density increased because more resistance was induced in the plant by primary germ tubes. Another drawback of these dry inoculation techniques is that calibration of inoculum cannot be done prior to inoculation. Quality and quantity of inoculum deposited onto a plant organ can only be observed after the inoculation process. Moreover, these methods may occasionally result in non uniform dispersal over the host and lead to the deposition of clumps or chains of conidia (Nicot *et al*. 2002). Conti *et al*. (1985) used a conidia-suspension for lab experiments with powdery mildew on roses as well as Yan *et al*. (2005) in their greenhouse infection experiments. The use of microscopically quantified spore suspensions is a routine procedure for many, more amenable, fungal pathogens.

Artificial inoculation demands adapted circumstances for pathogen development. Although many powdery mildews have the ability to develop in very dry conditions, this is not the case for *Podosphaera pannosa* conidia which require near 100% relative humidity. The effect of water on the conidia is not always clear but for many powdery mildews it diminished germination (Sivapalan 1993, 1994; Nicot *et al*. 2002). Sivapalan (1993) found that *Podosphaera* conidia lose their germination ability in water. Other studies postulated that a fine water spray applied after inoculation prevents contact of the conidia with the leaf surface (Butt 1978). Yan *et al*. (2006) used a spore suspension for the inoculation of rose plants with powdery mildew as is the case with other studies on cucumber (Zijlstra *et al*. 1995), tomato (Bai *et al*. 2003) and pepper (Lefebre *et al*. 2003) based on the same method. Quick preparation of the spore suspension and a temperature shift in the greenhouse from 22 to 28°C to evaporate the water after inoculation was essential (Yan *et al*. 2006).

Leus *et al*. (2007) compared results obtained with the inoculation tower to scores given in a field test on 20 rose cultivars. In general, infection rates on both the field test and the detached leaves inoculated with the tower were high, which made it difficult to evaluate the use of the tower to find incremental differences in (partial) resistance. Also, young rose leaves, as used for inoculation with the tower, are frequently more susceptible to powdery mildew. For genotypes in which resistance promptly rises with leaf age, this led to unbalanced scores between the tower and field test. In genotypes with an intermediate reaction (partial resistance) reproducibility was lower. Sufficient repetitions are however needed to have a correct validation.

Artificial inoculations to screen progenies

The setup of rose breeding programs is mainly oriented towards the selection of ornamental traits. Earlier selection for disease resistance is indispensable to identify and move a higher proportion of resistant genotypes forward in the selection process. To change the selection priority in favour

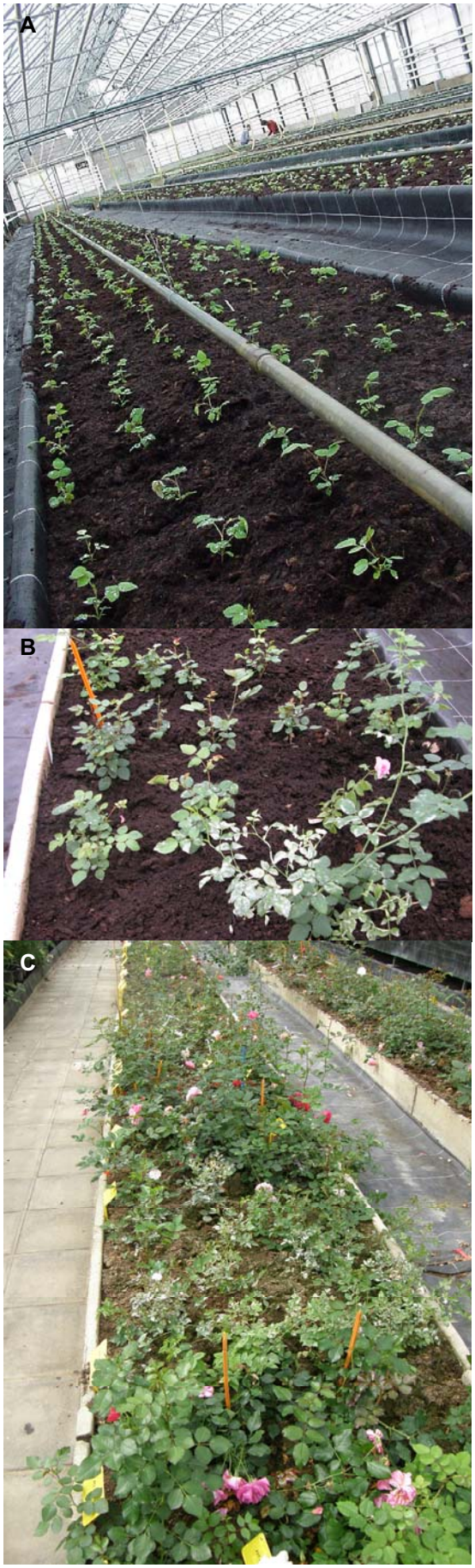

Fig. 4 Greenhouse inoculation for powdery mildew resistance in seedlings. (**A**) Transplanting of garden rose seedlings for greenhouse evaluation during the first selection year. (**B**) Infected inoculation or spreader plant in the foreground for inoculation of seedlings. (**C**) Example of resistant and susceptible seedling families in greenhouse selection after inoculation with inoculation plants.

of disease resistance, auxiliary methodology is needed. The earlier in the selection process disease resistance is evaluated, the more resistant genotypes will proceed in further selection and the sooner susceptible genotypes can be rouged. The difficulty in an early screening of seedlings implies an unreplicated evaluation stage likely to be different in environmental and growth conditions than normal production.

Seedlings, in breeding programs for garden roses, are often grown protected in greenhouses during the first year of selection. Traditionally, disease resistance is recorded afterwards when plants are growing outside. Moreover, fungicides were used in the past to protect candidate rose varieties in the breeding process to allow for expression and selection of other, primarily ornamental, traits (De Vries 2000). However, in field trials and large populations undergoing selection, it is very difficult to interpret results on observed resistances as other pathotypes may interfere. Therefore artificial inoculations in the greenhouse of young seedling progenies might be very useful. Linde *et al*. (2006) used a ventilator for artificial inoculation of rose plants in a greenhouse. Leus *et al*. (2008) described a system of inoculation or spreader plants (very susceptible genotypes) that were planted in between rose seedlings (**Fig. 4**). They demonstrated that by using inoculation plants it was possible to introduce a homogenous spread of powdery mildew in a greenhouse. Besides also the first infection occurred much earlier compared to natural infection, which made it possible to have an early resistance screening and selection in young seedlings. The earlier in the selection process resistance screening can occur, the more cost effective because resources won't be invested in raising susceptible clones to maturity.

On greenhouse and outdoor grown roses Frinking and Verweij (1989) observed the highest percentages of sporulating leaf areas on leaves at 4 to 10 days after unfolding; these leaves were probably infected while still at a very young stage. On older leaves sporulation was at a very low level and on leaves from 14 days and older, sporulation was not observed any longer. Frinking *et al*. (1987) discussed the spread of fungal spores in a greenhouse where roses were grown. Test spores from the sporophyte, *Lycopodium* sp., remain suspended for quite a long time in a closed glasshouse. According to Pieters *et al*. (1994) powdery mildew on cut roses needs only 1 week to spread the infection all over a greenhouse.

Linde *et al*. (2006) evaluated powdery mildew incidence in different years and compared scores from roses grown in greenhouses where conidia were dispersed by a ventilator with field scores of the cloned population, scored at the same time. Correlations were found between field and greenhouse scores for powdery mildew and over different years. Similar results were observed by Leus *et al*. (2008) who tested different seedling families in a breeding program both as seedlings in the greenhouse (**Fig. 4**) and in the field one year later. In the first year of selection only one seedling plant is available, while on the field small groups of the vegetatively multiplied seedling are evaluated. A higher correlation was found when scores of progeny groups of crosses, instead of scores for individual seedlings in the greenhouse, were compared to the field scores. A scoring bias on individual plants by differences in disease development due to environmental conditions or occurrence of various pathotypes might cause the lower correlation for individual seedlings. Therefore, the disadvantage of the unreplicated seedling stage could be overcome by family selection. Family selection can avoid the misinterpretation of resistance for individual seedlings in progenies with a good overall level of resistance, but will not be able to select individual resistant plants in segregating populations. The evaluation of seedlings on a family scale could also be used to evaluate valuable parental combinations (Leus *et al*. 2008). The with-in progeny selection seems less imperative for resistance selection in an early stage but is necessary in later field selection on cloned plants to find the best resis-

tance towards powdery mildew in combination with other required traits. The discussed seedling screening is performed in practice in the rose breeding program at the Institute for Agricultural and Fisheries Research (Belgium).

CONCLUSION

A high racial diversity in *Podosphaera pannosa* and different resistance mechanisms towards powdery mildew in rose plants are found. Both qualitative and quantitative resistance are described and genetic mapping studies proved QTLs for powdery mildew resistance can be assigned. However, the tetraploid nature of most rose cultivars makes mapping and breeding more complex. More information on the genetic side is on its way to make markers a valuable tool in powdery mildew resistance selection. On the other hand, also practical screening methods are indispensable. Screening methods are developed not only for seedling populations, but also for the identification of more resistant rose genotypes for use as parents. Characterizing parents in an in depth study with characterized isolates is possible, while this type of resistance screening on large seedling populations may be prohibitive.

Both resistance screens information and developed molecular technology allow breeders to direct limited resources towards the development of roses with enhanced powdery mildew resistance. Some of the techniques discussed can easily find their way into practical garden rose breeding by putting disease resistance selection more in the foreground. An easy tool that does not require much additional effort is screening seedlings for powdery mildew resistance while still in the greenhouse; this technique can become a standard used in breeding programs. Also, marker-assisted selection for powdery mildew resistance and/or other important traits is a tool that may soon be routine in rose breeding programs.

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