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Early Selection of Garden Rose Seedlings for Powdery Mildew Resistance

L. Leus[1)], J. Van Huylenbroeck[1)], E. Van Bockstaele[1,2)] and M. Höfte[3)]
([1)]Plant Unit, ILVO, Belgium, [2)]Dept. Plant Production, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium and [3)]Dept. Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium)

Summary

A selection protocol to screen for powdery mildew resistance at an early stage in rose progenies was evaluated. Seedlings were grown in the first year in two separate greenhouse compartments: one with inoculation plants (C1) and one without (C2; control compartment). In the second year, the same genotypes were tested in the field under natural infection conditions.

In the compartment with inoculation plants a high and uniform infection pressure was established. Selection for disease resistance could start more than one month earlier in the greenhouse compartment with inoculation plants compared to the control compartment. Significant correlations between greenhouse evaluation and field scores were found, especially for the families (F_1) of different crosses. In commercial breeding, the proposed method can significantly reduce selection efforts and allows for a more economical and efficient manner to screen on a larger number of seedlings for resistance.

Key words. rose breeding – disease resistance – *Podosphaera pannosa* – *Rosa* – seedling selection – *Sphaerotheca pannosa*

Introduction

Traditionally, commercial garden rose breeders have given priority to aesthetical flower and plant characteristics. This means that, in contrast to breeders of vegetables or agricultural crops, little attention has been paid to internal quality such as disease resistance. In addition, fungicides were used in the past to protect candidate rose varieties in the breeding process (DE VRIES 2000). Due to changing plant protection legislation and the ecological awareness of consumers, the need to breed rose types with at least a better partial resistance is a prerequisite to withstand customer rejection and to regain the economic basis to breed and produce roses (NOACK 2003). Increased disease resistance is especially necessary for garden roses to inspire confidence from the amateur gardener and for use in public areas.

Podosphaera pannosa (syn. *Sphaerotheca pannosa*) is the most common disease on roses worldwide. It attacks plants grown in the open air, such as garden roses, and greenhouse roses grown for cut flower production. Pathotypes of rose powdery mildew were described by LINDE and DEBENER (2003) and LEUS et al. (2006). A dominant resistance gene *Rpp1*, for race-specific resistance to rose powdery mildew, has been reported (LINDE and DEBENER 2003; LINDE et al. 2004). Resistance to powdery mildew varies among rose species and cultivars and is often pathotype-specific. Results of natural and artificial infections show that only a few cultivars are highly resistant (LINDE and SHISHKOFF 2003).

Breeding programs for vegetatively propagated crops in particular, face two problems in early selection: frequently progeny are grown in conditions different from those of normal production and the first selection is done at an unreplicated stage (BRADSHAW et al. 2003). In commercial garden rose breeding, seedlings are often grown in greenhouses during the first year of selection. Disease resistance is only recorded in later years when selected plants are grown on the field. Resistance to powdery mildew is difficult to evaluate in field circumstances because the extent of infection varies substantially from year to year. In addition, it is very difficult to interpret results on reported resistances as pathotypes can differ between tests and on different moments and locations. Controlled screening in an early selection stage would therefore enhance selection efficiency.

Standardised artificial inoculation with powdery mildew conidia on detached rose leaves is possible by use of an inoculation tower (LINDE and DEBENER 2003) or by spraying a conidial suspension under controlled greenhouse conditions. During spraying, a higher temperature is needed to avoid damage to the conidia by water (YAN 2005). Both methods are laborious on large numbers of seedlings. An extrapolation of lab tests to field circumstances is inappropriate.

In this study we thoroughly evaluate the possible use of inoculation plants in an early selection stage to screen powdery mildew resistance under controlled greenhouse conditions. Preliminary results already showed the effectiveness of inoculation plants during a negative selection

process (LEUS et al. 2003). In the study presented here no selection was applied, seedlings were followed during the whole growing season and greenhouse results of seedlings were compared to field performance one year later.

Materials and Methods

Cross breeding

In the rose breeding program at ILVO, parent plants of garden roses were grown under greenhouse conditions. Crosses were made from April to June 2003 to allow the hips to ripen during summer. Every cross was made by hand pollination. To emasculate the flower, and avoid self-pollination, the anthers were excised with a pair of tweezers. In the morning of the following day the plant was pollinated by direct contact with the pollen of a chosen father plant or by applying the pollen to the stigma with a paint brush. Ripe hips were harvested in October 2003. Seeds were removed from the hips and directly sown in trays. After a stratification period of three to four months at 2 °C, the trays were put in a heated greenhouse. In March 2004, when the seedlings developed two or more leaves, they were planted out in soil beds in the greenhouse. During the next months the seedlings were observed and selection started. The following winter, selected seedlings were cloned by winter grafting on the rootstock *Rosa canina* 'Pfänder'. About five grafts were made of every seedling. In spring the clones were planted in the field.

Greenhouse inoculation

In 2004, the offspring of 7 different parental combinations were planted in two identical compartments within the same greenhouse block. The same fertilisation and climate conditions were maintained in both compartments. The two neighbour compartments were chosen in the middle of the block to minimize environmental differences as much as possible. The parental combinations used and the number of seedlings in each of the families are listed in Table 1. Seedlings were planted in seedling beds in rows of four or six; with a between distance of 30 cm. Each row consisted of seedlings of the same family and rows were randomised in different planting beds. In Compartment 1 (C1), seedlings were subjected to a high infection pressure using artificial inoculation. In Compartment 2 (C2; control compartment) no artificial inoculation took place. Artificial inoculation in C1 was performed by planting rootstocks of *R. canina* 'Pfänder' in between the seedlings every 2 m. This rose genotype is very susceptible to powdery mildew and was inoculated on March 29, 2004 after planting in between the seedlings by dusting a conidial mixture of powdery mildew collected from infected greenhouse roses.

Powdery mildew infection on individual plantlets was scored regularly from the first appearance of symptoms until there was no further spread of infection in C1, or until the end of the growing season in C2.

Seedlings were scored individually on a 0 to 3 scale adapted from NICOT et al. (2002): score 0: no powdery mildew; 1: a single colony per plant; 2: different colonies on different leaves; 3: colonies on most leaves. Using the mildew severity score, the disease index (DI) per offspring group and for the whole compartment was calculated at every time point using following formula:

$DI = [\sum(i * x_i)]/(\text{highest score possible x total amount of plants})] * 100$; with i = 0–3 (and x_i is the number of leaves with rating i) and the highest score possible in our scale = 3.

Field evaluation

The families grown in both compartments in 2004 were multiplied by winter grafting and planted together in the same field in March 2005. Five plants per genotype were planted in 13 rows. Families were planted subsequently and blocks of families were planted randomised in different rows. There was also a randomisation between families from both compartments. In this field experiment, natural infection was scored three times on each genotype: on June 23, July 28 and August 28, 2005. Powdery mildew was scored on a 0 to 3 scale as mentioned above for the greenhouse screening; one conclusive score was given for each genotype by two evaluators. The DI was calculated for each genotype and also for the families as described above for the greenhouse evaluation.

Table 1. Number of seedlings planted in two different greenhouse compartments with (C1) and without inoculated plants (C2) for each family.

Seed parent	Pollen parent	Number of seedlings in C1	Number of seedlings in C2
'Cassandra'	'Melissa'	20	18
'Melissa'	'Cassandra'	20	20
'Kanegem'	'Melrose'	19	19
'Melglory'	'André Brichet'	44	48
'Melglory'	seedling 94-70	17	20
'Trier2000'	'Melissa'	48	48
'Johann Strauss'	'Apricot Nectar'	18	16
Total		186	189

Statistical analysis

Correlations were calculated using SPSS11.5. Correlations were calculated between the DI of individual seedlings in the greenhouse compartments and the DI in the field. The same calculation was made for the different families. Scores for families without or with powdery mildew infection in the greenhouse compartments were compared to their field performance.

Results

Greenhouse inoculation

In Compartment 1, the first visual symptoms of powdery mildew infection were observed on April 14, 2004, 16 days after introduction of inoculation plants. The earliest infection in Compartments 2 started one month later (May 12, 2004) (Fig. 1). For most families, the disease

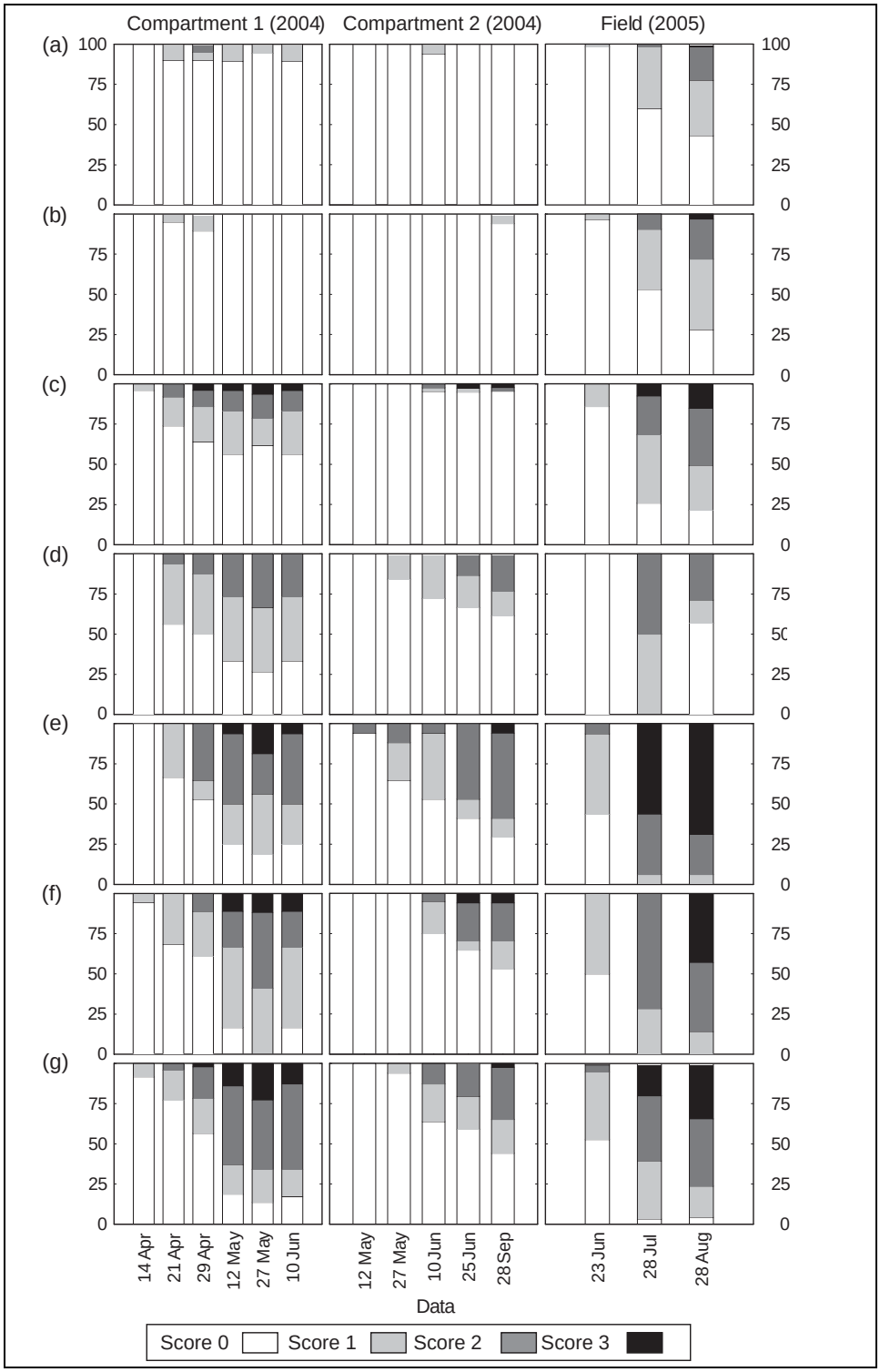


Fig. 1. Distribution of powdery mildew resistance scores in function of time for the evaluations of the families in Compartment 1 (with artificial inoculation), in Compartment 2 (without artificial inoculation) and in the field. (a) 'Cassandra' x 'Melissa', (b) 'Melissa' x 'Cassandra', (c) 'Trier2000' x 'Melissa', (d) 'Kanegem' x 'Melrose', (e) 'Johann Strauss' x 'Apricot Nectar', (f) 'Melglory' x seedling 97-40, (g) 'Melglory' x 'André Brichet'.

appeared even more than one month later in C2 compared to in C1 (Fig. 1). A uniform spread of the disease was obtained in C1. At the end of the season, 69 % of the seedlings in C1 were infected by powdery mildew compared to 25 % of the seedlings in C2. The overall mean DI for C1 and C2 was maximal 32 and 18, respectively. Score distributions for the families in both greenhouse compartments are presented in Fig. 1.

The progeny of 'Cassandra' x 'Melissa' and its reciprocal cross showed very good resistance in both greenhouse compartments; a very low disease incidence was observed on only a few seedlings (Fig. 1a, b). In the F_1 of 'Trier2000' x 'Melissa' 32.7 % of the progeny became infected by powdery mildew in C1, while almost no plants of this cross were infected in C2 (Fig. 1c). On seedlings of 'Kanegem' x 'Melrose', only scores ranging from 0 to 2 were observed in both greenhouse compartments (Fig. 1d). For the F_1 of 'Johann Strauss' x 'Apricot Nectar', 'Melglory' x seedling 94-70 and 'Melglory' x 'André Brichet', seedling infection was uniformly distributed among all possible scores. Seedlings of 'Johann Strauss' x 'Apricot Nectar' were very susceptible in both greenhouse compartments (Fig. 1e). The highest infection rates were observed in the progeny of 'Melglory' x seedling 94-70 and 'Melglory' x 'André Brichet' under the high infection pressure conditions of C1 (Fig. 1f, g).

Greenhouse selection versus field evaluation

In total, 148 clones originating from C1 and 182 clones from C2 were evaluated for powdery mildew resistance under natural infection in the field in 2005. No significant differences within families originating from the two different greenhouse compartments were observed in the field scores (data not shown).

Genotypes, in which no infection was observed in both greenhouse compartments in 2004, had an average DI of 23.3 (SE±1.5) in the field, while genotypes on which powdery mildew infection was seen in the greenhouse showed an average DI of 45.0 (SE±1.9) in the field. This difference between both groups was significant (t-test, $p<0.001$). The same comparison of average DI in the field, grouping genotypes without and with infection symptoms was made for two greenhouse compartments separately. For plantlets originating from C2, the average DI in the field was 23.5 (SE±3.0) and 44.1 (SE±2.5) in the greenhouse, while for plantlets from C1, the average DI was 23.3 (SE±1.8) in the field and 46.5 (SE ±3.2) in the greenhouse. The observed differences were statistically significant (t-test, $p<0.001$) for both greenhouse compartments.

For the families, correlations between the DI scored in both greenhouse compartments in 2004 and the field scores of 2005 were high (Table 2). The correlations between the DI of individual plants in the greenhouse compartments and on the field were lower (Table 2). Correlations for individual genotypes within one offspring group ranged between $r=0.25$ and $r=0.45$ ($p<0.01$) for susceptible families. In the case of families with a good resistance, no correlation could be calculated because too few infections were observed under the low infection pressure conditions.

Maximum scores for individual genotypes as evaluated in the greenhouse compartments (2004) and in the field (2005) are presented in Table 3. Data in this table show that for C1, 35.8 % and for C2, 29.1 % of the plants had equal scores in the greenhouse as in the field. When genotypes with the scores 0 and 1 (potentially good resistant) and scores 2 and 3 (susceptible) were grouped, 66.9 and 58.2 % of the plants respectively were placed in the same class in both years.

Table 2. Correlation coefficients (Pearson) between the DI of individual seedlings and the DI of families as seedlings in a greenhouse compartment with (C1) or without inoculation plants (C2) in 2004, and in the field in 2005.

	Correlation for individual seedlings	
	C1	C2
C1	1	
C2	–[z]	1
Field	0.55**	0.41**
	Correlation for families	
	C1	C2
C1	1	
C2	0.85*	1
Field (June 23)	0.83*	0.81*
Field (July 28)	0.97**	0.91**
Field (August 28)	0.77*	0.74^{NS}

NS, *, ** not significant or significant at the 0.05 or 0.01 level, respectively
[z]Correlation can not be made

Table 3. Distribution of the number of rose genotypes in function of powdery mildew infection score (maximum score from all scoring dates) in Compartment 1 (C1) or 2 (C2) in 2004 and in the field in 2005

	Scores				
	0	1	2	3	
Field scores	Number of genotypes C1 (with inoculation plants)				Total
0	**13**	1	2	0	16
1	22	**16**	7	0	45
2	14	16	**14**	7	51
3	5	5	16	**10**	36
Total	54	38	39	17	148
Field scores	Number of genotypes C2 (without inoculation plants)				Total
0	**37**	3	0	0	40
1	39	**2**	7	0	48
2	41	11	**9**	3	64
3	12	5	8	**5**	30
Total	129	21	24	8	182

Twenty-seven percent of the total number of genotypes in C1 showed resistance (scores 0 and 1) in the greenhouse but turned out to be susceptible (scores 2 and 3) in the field, compared to 37.9 % in C2. Only 6.1 (C1) and 3.8 % (C2) of the genotypes were susceptible in the greenhouse compartments but showed resistance in the field (Table 3).

The progeny of the crosses 'Cassandra' x 'Melissa' and 'Melissa' x 'Cassandra' appeared to be the most resistant plants also in the field. More than 40 % of the clones remained uninfected (Fig. 1a, b). In 'Trier2000' x 'Melissa', 21.5 % of the progeny were powdery mildew free in the field during the growing season (Fig. 1c).

The F_1 of 'Johann Strauss' x 'Apricot Nectar' reached the highest DI of all groups tested (Fig. 1e). Very high scores were already reached by the end of July. The DI was also high in two crosses with 'Melglory' as a parent. In these crosses almost all plants were infected in July (Fig. 1f, g) and infection increased until the end of August. All seedlings of 'Kanegem' x 'Melrose' were uninfected on the first scoring date, however were diseased by the second scoring date in the field (Fig. 1d). No genotypes resulting from this cross obtained score 3 in either the greenhouse or the field. The scores decreased later in the season, probably due to regrowth of the plants without new infection.

Discussion

Greenhouse inoculation

The use of inoculation plants to screen powdery mildew resistance on greenhouse seedlings was evaluated positively. Inoculation plants introduced the pathogen homogenously and earlier in the growing season, and resulted in a higher infection pressure compared to a control compartment relying on natural infection. Results presented here confirm a preliminary test conducted in 2002. In this preliminary test a comparison was made between trials in greenhouse compartments with and without inoculation plants, evaluated using negative selection. In the compartment with inoculation plants many more seedlings were discarded due to powdery mildew susceptibility (Leus et al. 2003). Inoculation plants have previously been used to infect tomato and tobacco seedlings with *Oidium neolycopersici* (Achuo et al. 2004; Matsuda et al. 2005) in greenhouse conditions and for crown rust evaluation of perennial ryegrass in the open air (Reheul and Ghesquiere 1996). In the apple breeding program at East-Malling natural infection is used to evaluate powdery mildew resistance. However, if not enough inoculum is present, seedlings are dusted with conidia from mildewed shoots at monthly intervals during the growing season (Alston 1977). The use of inoculation plants in our evaluation methods assured that sufficient inoculum was continuously present. Additional inoculation during the growing season was not necessary.

Several methods to inoculate plants with powdery mildew have been published. Although water can damage powdery mildew conidia (Sivaplana 1993, 1994; Nicot et al. 2002), conidial suspensions of powdery mildew have been used to spray apple (Battle and Alston 1996) and rose plants (Yan 2005). To avoid the use of water, an inoculation tower can be used for standardised inoculation of detached leaves. This system has been used for the identification of powdery mildew isolates on apple by Urbanietz and Dunemann (2005) and on rose by Linde and Debener (2003). When this method is used for resistance screening, a large number of repetitions is needed to draw conclusions on the level of resistance of rose genotypes. Thus for large groups of plants this method is too labour intensive. The inoculation tower is ideal to test specific genotypes with characterised powdery mildew isolates. Mass cultures of monoconidial isolates are difficult to grow because the powdery mildew fungus is an obligate parasite and cannot be stored for a very long period. Also, pathotype identification in greenhouse and field tests remains problematic.

Resistance screening becomes more cost effective the earlier in the selection process that it can occur. In our study, seedlings were transplanted from seedlings trays to the greenhouse. An alternative method, leaving the plants at the place of sowing for evaluation of resistance before transplanting, would reduce the amount of labour required. When inoculation plants were used at the early stage, the seedlings developed a lot of young susceptible leaves. Later in the season, plants developed fewer young shoots; hence the lower observed DI. The optimal timing for evaluation of powdery mildew in a compartment with inoculation plants appeared to be around the end of May. For an efficient single screen this would have been the best moment. It was observed under natural infection (C2) that the DI was still increasing at the end of the season with more infection in areas adjacent to susceptible plants (data not shown). Therefore it can be concluded that the spread of the powdery mildew in C2 was not homogenous, while it was homogenous in C1. The spore-dispersal system in a greenhouse is very complex. Velocities of air movement are lower than in the open air. This implies that sedimentation of the fungal spores, in addition to impaction by air currents, plays a more important role in a greenhouse than in trials conducted under field conditions. Frinking et al. (1987) discussed the spread of fungal spores in a greenhouse where roses were grown; spores of *Lycopodium* sp. remained suspended for quite a long time in a closed glasshouse. Powdery mildew infection on cut roses can spread all over a greenhouse within a week (Pieters et al. 1994). Tests with powdery mildew on barley plants placed in a greenhouse showed that activities such as working in the greenhouse and sprinkling of water increase the amount of spores dispersed (Frinking and Scholte 1983).

Greenhouse selection versus field evaluation

Significant correlations were found between resistances of all seedlings in the greenhouse compartments and in the field. These results were similar to those obtained in apple. In apple breeding, yield potential can only be evaluated four years after germination due to the juvenile phase. For powdery mildew and scab, greenhouse selection is successful and can be evaluated on juvenile seedlings (Alston 1983). In recurrent-flowering roses flowering starts in the first growing year, where flowers appear after the formation of the first six or seven leaves. The juvenile period of these roses can be regarded, therefore, as absent or very short (Roberts and Blake 2003).

Within the families, correlations were also found for individual seedlings that showed infection on both loca-

tions. When infection was low, correlations for individual seedlings within the families could not be calculated.

When the results of the two greenhouse compartments (with and without inoculation plants) were compared to the field evaluation separately, comparable correlations were found. This indicates that in the compartment without inoculation plants (C2) the seedlings were evaluated in a representative way even though evaluations occurred later in the growing season. Correlations were weak for individual seedlings, but were much higher when mean scores of the families at both locations were compared. The lower correlation for individual seedlings might be caused by a scoring bias on individual plants, differences in disease development caused by environmental conditions or occurrence of different pathotypes. In the first year, only one plant of every genotype is available for selection while in the second selection year about five plants are evaluated in the field. Therefore it could be interesting to evaluate the families instead of individual seedlings. BLAZEK (2004) evaluated a pre-selection in apple seedlings for *Podosphaera leucotricha* and compared one year of greenhouse selection with ten years of orchard evaluation and also obtained better correlations for the families than for individual genotypes. JANSE et al. (1994) compared nursery and orchard resistance to powdery mildew in apple and concluded efficiency varies between families. One of the characteristics to be selected for in potato is disease resistance to *Phytophtora infestans.* Also in this case, the intense early-generation visual selection between seedlings in a greenhouse and spaced plants at a seed site can be replaced by discarding whole progenies at the unreplicated small-plot stage before starting conventional within-progeny selection (BRADSHAW et al. 2003). A disadvantage is the risk of eliminating potentially good clones in families that show segregation for resistance.

In general, seedlings showed similar levels of resistance or susceptibility in the greenhouse when compared to the clones grown in the field one year later. Due to a higher amount of inoculum in the compartment with inoculation plants, more seedlings in this compartment had comparable scores between the greenhouse and the field trials.

Some seedlings showed resistance in the greenhouse but turned out to be susceptible in the field, while only few seedlings susceptible in the greenhouse were resistant in the field. The selection under greenhouse conditions was useful to safely discard susceptible genotypes, nevertheless field evaluations will still be necessary. A thorough comparison of resistances in the test is impossible, because during different years and on different locations other pathotypes can appear and infection circumstances and conidia concentrations are different. However, since resistance breeding is a prerequisite for new cultivars, a ready-to-use selection protocol can be useful, notwithstanding possible disadvantages. If field performance is considered to be most important, it can be concluded that during our experiment rather inferior plants were maintained for further selection, while almost no interesting genotypes were discarded.

Evaluation of families through the inoculation of seedlings gives additional information on the resistance of parental combinations and conclusions on the resistance of genotypes tested can be made. The progeny of the crosses 'Cassandra' x 'Melissa' and 'Melissa' x 'Cassandra' were the most resistant, in both the greenhouse test and in the field. These genotypes are promising for further evaluation. Separate tests on the resistance of the parent plants could reveal if one or both parents are responsible for the resistance in the offspring. The plants of the cross 'Johann Strauss' x 'Apricot Nectar' showed the highest DI in the field compared to all the other crosses. This family was also very susceptible at an early stage under greenhouse conditions, both with or without artificial inoculation, suggesting this cross is very susceptible to powdery mildew, even when the amount of inoculum is low. Partial resistance can be recognised in all seedlings of the combination 'Kanegem' x 'Melrose'. The offspring showed only a moderate infection. No genotype of this family reached the maximal score either in the greenhouse compartments or in the field.

In conclusion, this study shows that greenhouse evaluation of garden rose seedlings at an early selection stage is representative for later field performance, especially when families are evaluated. This method can be the first step in a (pre-)screening procedure. The most virulent powdery mildew isolates characterised or mixtures of isolates should be used to test the most promising candidate varieties at a later stage of selection. The early presence of a sufficient quantity of homogeneously spread inoculum enables the early negative selection of non-resistant genotypes. In commercial breeding, this method can significantly reduce selection efforts and allows for a more economical and efficient manner to screen for resistance on a larger number of seedlings.

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Addresses of authors: Leen Leus (corresponding author), J. Van Huylenbroeck and E. Van Bockstaele, ILVO Unit Plant, Caritasstraat 21, 9090 Melle, Belgium, E. Van Bockstaele, Dept. Plant Production, Fac. of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium and M. Höfte, Dept. Crop Protection, Fac. of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium, e-mail: leen.leus@ilvo.vlaanderen.be.