POWDERY MILDEW ON ROSES: PATHOTYPE SCREENING

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Abstract

Monospore cultures of rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) were established from isolates collected on outdoor grown roses at different locations in Belgium. These monospore cultures were tested on rose cultivars and species with different levels of natural resistance to examine the possible existence of different pathotypes. Therefore a screening protocol including scoring after artificial infection was developed on *in vitro* plantlets. The calculated disease indexes of the collected monospore cultures on different rose cultivars and species showed that up to now in the collected isolates no evidence of distinct pathotypes could be found. The reaction of plants towards the disease differed greatly between the examined cultivars and species. The test was validated for *in vivo* circumstances indicating that *in vitro* rose plants can be used for pathotype and resistance screening.

1. Introduction

Powdery mildew (*Sphaerotheca pannosa* var. *rosae*) is world wide one of the most important diseases on indoor and outdoor grown roses. The fungus is an obligate parasite, the use of water can harm the spores (Sivaplana, 1993 and 1994). Therefore infections were made with brushes and *in vitro* plants were used to keep monospore cultures well isolated. A study was published by Bender and Coyier (1984) pointing on the existence of pathotypes of *Sphaerotheca pannosa* on rose in the United States.

In rose breeding, disease resistance is one of the major challenges. The development of a reliable bioassay for screening of parent plants and seedlings is indispensable to increase the selection efficiency for more resistant rose cultivars by crossbreeding and to establish phenotypic data for molecular research. Pathotypes of a fungus can cause differential reactions on plant species and cultivars with different levels of resistance. Differences in pathotypes can influence a screening towards resistant plants. It is this knowledge on differential reactions towards fungal isolates that can discriminate the existence of pathotypes. For blackspot (*Diplocarpon rosae*), another important rose disease, it has been shown that different pathotypes can provoke differences in resistance in rose species and cultivars (Debener *et al.*, 1998; Yokoya *et al.*, 2000).

In the present study monospore cultures of powdery mildew were evaluated for the existence of different pathotypes by the use of *in vitro* rose plants.

2. Material and methods

2.1. Monospore cultures and infection

Isolates of powdery mildew of outdoor grown roses were collected on different cultivars at 5 places in Belgium (A, C, E, F and G) (Fig. 1). First inoculation of cultures in the lab was done by transferring spores on *in vitro* plants in test tubes. To avoid the use of water, multiplication of the monospore cultures and infection of the test plants was established with a paint brush sterilised in 70% ethanol. Monospore cultures were prepared after transfer on *in vitro* plants by use of the eyelash method according to Bender and Coyier (1984). One single spore was taken with an eyelash and transferred onto new *in vitro* plantlets.

The monospore cultures were multiplied on *in vitro* plants before use for infection. Spores for infection were collected on infected plants preferably ten to twenty days after inoculation for multiplication. For infection, newly transferred plants in test tubes were used. Every leaf of the test plants was infected with a paint brush. For every cultivar and species at least 15 plants were infected to perform the test. Plants with not more than five fully expanded leaves were most practical to score.

2.2. Plant material

The monospore cultures were evaluated on 7 rose cultivars and species: 'Wettra', 'Gomery', 'Melrose', 'Ville du Roeulx', 'Excelsa', *R. laevigata anemoides* and *R. wichuraiana*. The plants were grown on a Murashige and Skoog medium (Murashige and Skoog, 1962) [1 MS micro- and macro-elements including vitamins (Duchefa), 30g/l sucrose, 7g Lab M plant tissue culture agar, 0.05mg/l α -naphthalene acetic acid (NAA) and 1mg/l 6-benzylaminopurine (BAP), pH 5.8].

2.3. Disease index

Each leaf was scored 4 weeks after inoculation. A score ranging from 0 (no infection) to 5 (leaf died) was used. Score 1 was given to leaves with mycelium growth only, without the development of spores. Score 2, 3 and 4 is made of leaves with spore development ranging from start of sporulation up to coverage of the whole leaf with spores. To be able to detect leaves with score 1, a binocular was used (64x). Afterwards the disease index (DI%) of each plant was calculated according to Liu *et al.* (1996). The average DI% was calculated only on the infected plants. Plants with DI% 0 were considered as not well infected, except for those cultivars/species where no single plant was infected, there the average DI% was considered to be 0.

$$DI\% = \frac{\sum_{i=1}^5 (\# \text{ leaves score } i \times i)}{\text{total \# leaves} \times 5} \times 100$$

While scoring it was observed that morphology of the fungus on the different plant cultivars and species could differ. Some species showed an altered balance between formation of mycelium and spores, therefore the formation of mycelium and spores was evaluated morphologically by use of a binocular.

2.4. Greenhouse validation test

Validation of the *in vitro* test was done on cuttings of 'Wettra', 'Gomery', 'Melrose', 'Ville du Roeulx' and 'Excelsa' in a greenhouse. Ten week old cuttings were

infected with a painting brush using an inoculum collected from infected roses. For each cultivar at least 6 plants were used. All leaves were infected and scored separately, four weeks after inoculation. Disease indexes were calculated for each plant.

3. Results

All monospore cultures could infect the five test cultivars (Table 1). Although a large variation occurs, the differences in DI% of the monospore cultures on the same cultivar do not seem to point to different pathotypes. When all cultivars are considered, none of the monospore cultures seem to show enhanced or minor virulence. Within the species, up to now only the isolates F1 and F2 were able to establish a minor infection on *R. wichuraiana*.

However, both *in vitro* and *in vivo* the cultivars showed a clear difference concerning their resistance. 'Wettra' and 'Gomery' turned out to be the most sensitive cultivars whereas 'Melrose' and 'Ville du Roeulx' are more resistant (Fig. 2). The species are more resistant than the cultivars, but here a distinction can be made between the resistant *R. wichuraiana* and the partial resistant 'Excelsa' (cultivar closely related to *R. wichuraiana*) and *R. laevigata anemoides* (Table 1). On 'Excelsa' the fungus mainly developed mycelium and hardly any spores were produced. Whereas on *R. laevigata anemoides* the fungus developed a lot of conidia but the mycelium could not spread and grew mostly rather restricted.

In general, *in vitro* plants showed a higher disease index compared to cuttings (Fig. 2). However, cultivars with a higher resistance *in vitro*, also have a higher resistance *in vivo*. This indicates that *in vitro* plantlets can be used for resistance and pathotype screening.

4. Discussion

No different pathotypes could be found in the isolates collected in Belgium up to now. Only isolate F1 and F2 were able to establish infection on *R. wichuraiana*. The possibility that isolate F is a different pathotype, will be looked at in more detail. The number of infected plants was small and the DI% in the performed tests on *R. wichuraiana* was very low. It would as well be useful to include in a future screening on the existence of different pathotypes some isolates of greenhouse roses. Bender and Coyier (1984) found 5 different pathotypes of *Sphaerotheca pannosa* out of 9 monospore cultures collected in Oregon (United States). Recently also in Germany different pathotypes were distinguished (Marcus Linde, personal communication).

Numerous other authors described pathotypes of powdery mildew. On cucurbits for instance pathotypes of *Sphaerotheca fuliginea* (Bardin *et al.*, 1997) and of *Sphaerotheca fusca* (Vakalounakis and Klironomou, 1995) were described.

Between cultivars and species differences in natural resistance were found. The ranking of the cultivars that was found after artificial infection was in agreement with the general knowledge on resistance of the tested cultivars and species. Results found *in vitro* were confirmed in the greenhouse test, what proves that an *in vitro* screening procedure can be used for pathotype screening.

Because disease resistance is more often found in species, the use of species is interesting in rose cultivar breeding. As the genetic background of rose cultivars is very narrow, the introduction of wild species can not only be interesting for disease resistance, but for other characteristics as well. The difficulty in this breeding work is the ploidy of cultivars (mostly $2n=4x$) that differs from these of most wild species ($2n=2x$ to $8x$) (De Vries, 2000; De Vries and Dubois, 1996; De Vries and Dubois, 2001; Gudín, 1995).

The occurrence of differences in morphological development of the fungus on different species/cultivars indicates different mechanisms of partial resistance in 'Excelsa' and *R. laevigata anemoides*. These differences in resistance will be looked at in more detail in the near future.

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Table 1. Average disease index and total mean for different rose cultivar/species four weeks after infection with collected monospore isolates ('⊗' combination not tested). The development of mycelium and conidia was examined for each combination of monospore culture and cultivar or species. The morphology was expressed as '+' (well developed) and '-' (poorly developed). Number of plants tested, n>15.

Cultivar	Average Disease Index (%)									Morphology	
										(+/ -)	
	monospore isolate								total mean	mycelium	conidia
	A	C1	C2	E	F1	F2	G1	G2			
'Wettra'	28	⊗	33	51	29	⊗	60	58	43	+	+
'Gomery'	42	⊗	65	21	38	42	32	49	41	+	+
'Melrose'	22	27	52	39	⊗	20	25	17	29	+	+
'Ville du Roeulx'	24	⊗	23	33	14	42	6	38	26	+	+
<i>R. laevigata an.</i>	26	40	21	17	16	7	21	12	20	-	+
'Excelsa'	7	7	11	12	30	11	7	11	12	+	-
<i>R. wichuraiana</i>	0	0	0	0	7	5	0	0	1.5	-	-

Place	Code of isolates
Melle	A
Lede	C1 - C2
Laakdal	E
Lesdaire	F1 - F2
Heers	G1 - G2

Figure 1. Places of collection of powdery mildew isolates in Belgium

Figure 2. DI%, calculated after scoring of infection on *in vitro* plants and on cuttings of different rose cultivars