Occurrence of Pathotypes of *Diplocarpon rosae* on Roses in Belgium

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Abstract

Monospore cultures of black spot (*Diplocarpon rosae* Wolf) 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. For this purpose a screening protocol including scoring after artificial infection was developed on *in vitro* plantlets. Differences between the examined isolates proved that there exist different pathotypes of *Diplocarpon rosae* in Belgium.

Keywords: black spot; *Diplocarpon rosae*; *Rosa* spp.; monospore cultures; pathotypes

INTRODUCTION

Black spot, caused by *Diplocarpon rosae* Wolf, is world wide one of the most important diseases on outdoor grown roses. In rose breeding, resistance to diseases such as black spot, is one of the major challenges (DE VRIES & DUBOIS 2001). To increase the selection efficiency for more resistant rose cultivars by crossbreeding, and to establish phenotypic data for molecular research, the development of a reliable bioassay for screening of parent plants and seedlings is essential. 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 black spot 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 black spot were evaluated for the existence of different pathotypes in Belgium by the use of *in vitro* rose plants.

MATERIALS AND METHODS

Monospore cultures. Isolates of black spot were collected on different cultivars at 6 locations in Belgium (A, B, C, D, E and F). First fungal development was established in the lab by growing black spot leaf discs (diameter 5 mm) on potato dextrose agar. Monospore cultures were obtained by streaking a conidial suspension prepared from the leaf discs, on potato dextrose agar. Single emerging conidia were isolated and put on a disinfected rose leaf on agar. Established monospore cultures were multiplied and maintained on *in vitro* plantlets.

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 & SKOOG 1962) [1 MS micro- and macro-elements including vitamins (Duchefa), 30 g/l sucrose, 7 g

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Lab M plant tissue agar, 0.05 mg/l α-naphtalene acetic acid (NAA) and 1 mg/l 6-benzylaminopurine (BAP), pH 5.8.]

Infection. Conidia for infection were collected on infected *in vitro* plantlets and conidial suspensions were prepared (2.10^4 conidia/ml). For infection, newly transferred plants in test tubes were used. Every leaf of the test plants was inoculated with 10 μl of conidial suspension (mostly 3 to 4 leaves). For every cultivar and species at least 15 plants were infected to perform the test, what makes that over 45 leaves were scored for every combination of monospore culture and rose cultivar or species.

Scores. 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 a suspected beginning of infection. Score 2, 3 and 4 were given to leaves with development of characteristic leaf spots and development of acervuli in increasing area. To score a binocular (64×) was used.

Classification. A class was given to each combination of rose cultivar or species and monospore isolate. This class was given according to the highest occurring scores, on condition that at least 10% of the scored leaves belong to this class.

For example if only score 0 and 1 appeared this was called class R (resistant) (less than 10% of the leaves in a higher class). If the highest scores (> 10% of the leaves) were 2 and 3 this is called class I (intermediate) and for scores 4 and 5 the class is S (susceptible).

RESULTS

Results presented in Table 1 show the classes attributed to the combination between the monospore culture and test species or cultivar. It is clear that differential reactions appear between different black spot isolates on some of the rose species and cultivars. These differential reactions point out the different pathotypes. Figure 1 shows the distribution of the classes of resistant, intermediate and susceptible reactions as a percentage of the tested leaves in each combination. By this, individual combinations can be compared. For example the infection of Melrose gives the same intermediate response for all tested isolates. On Ville du Roeulx isolate A can not establish infection, isolates B, C and D give a intermediate result, while this cultivar seems to be highly susceptible to isolates F and E.

DISCUSSION

Different pathotypes could be found in the isolates collected in Belgium. Monospore isolates E and F proved to be the most aggressive.

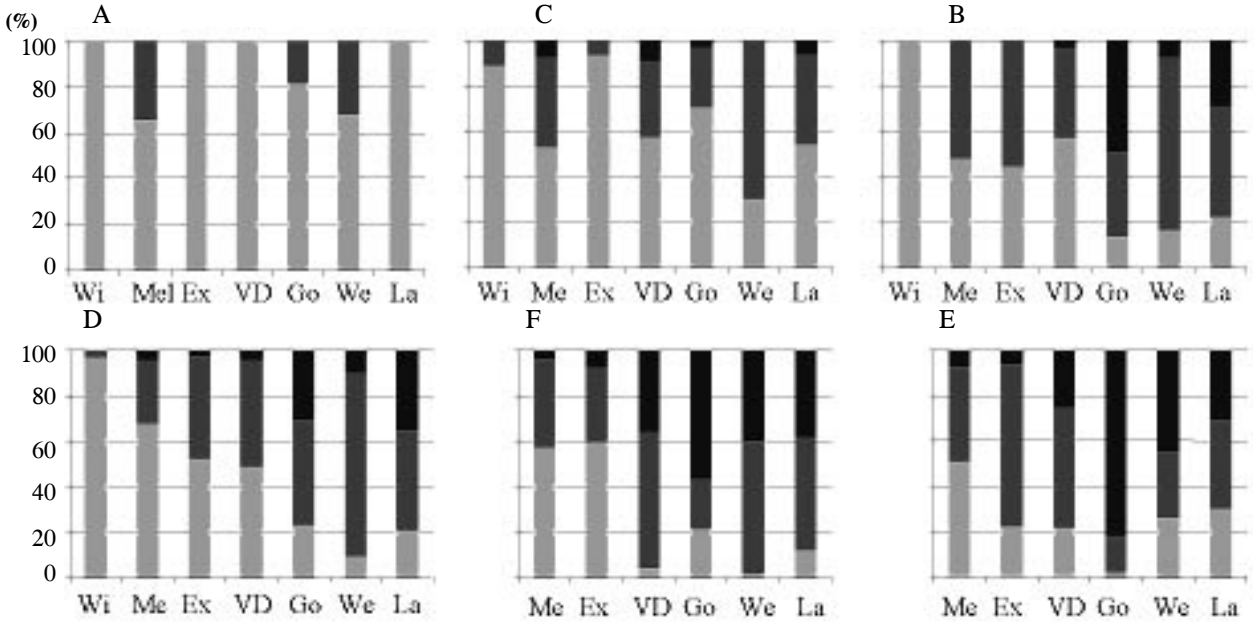

Figure 1. Percentage of leaves infected, for every combination of rose cultivar/species and monospore isolate, in three different classes: susceptible (black), intermediate (dark grey) and resistant (grey). Tested plants: *R. wichuraiana* (Wi), Melrose (Me), Excelsa (Ex), Ville du Roeulx (VD), Gomery (Go), Wettra (We) and *R. laevigata anemoides* (La)

Table 1. Interactions between monospore isolates of *Diplocarpon rosae* and rose cultivars and species ('/': not tested)

Cultivar or species	Classes (R = resistant, I = intermediate, S = susceptible)					
	Monospore isolate					
	A	B	C	D	E	F
Gomery	I	S	I	S	S	S
Wettra	I	S	I	S	S	S
Melrose	I	I	I	I	I	I
Ville du Roeulx	R	I	I	I	S	S
Excelsa	R	I	I	I	I	I
R. laevigata anemoides	R	S	I	S	S	S
R. wichuraiana	R	R	R	R	/	/

Differential reactions appeared in the cultivars Gomery, Wettra, Excelsa, but most of all in Ville du Roeulx and *R. laevigata anemoides* were all three responses: resistant, intermediate and susceptible reaction to the different monospore cultures could be observed.

Melrose a rose cultivar known for its good overall disease resistance showed a intermediate resistance towards all tested isolates. *R. wichuraiana* was the only rose with excellent resistance to all isolates in this test.

These results confirm earlier research on pathotypes in Germany and Great-Britain, were also a variation in occurring isolates could be shown (DEBENER *et al*. 1998; YOKOYA *et al*. 2000). Disease resistance was only found in the species *R. wichuraiana*. As is known the introduction of disease resistance in modern cultivars will have to be established by crosses with resistant wild species.

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