Resistance of *Rosa* Species and Cultivars to *Pratylenchus penetrans*

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Abstract. Methods to screen for resistance to root-lesion nematode *Pratylenchus penetrans* in *Rosa* were modified to screen-rooted materials. Sixty days after rooting, plants were transplanted into 50-mL pots filled with river sand and each inoculated with 500 *P. penetrans* in 400 μ L water 10 days later. The inoculated plants were fertilized weekly and incubated in a growth chamber or a greenhouse for 5 months when nematodes were extracted from the sand and root system and enumerated. When used for screening of the 131 *Rosa* accessions, this approach allowed the observation of a large variation in host suitability. While a majority of the accessions supported the multiplication of *P. penetrans*, previously reported resistance of *R. multiflora* 'K1' and *R. virginiana* to *P. penetrans* was confirmed. *Rosa laevigata anemoides* allowed a significantly lower nematode multiplication than the currently prevalent rootstock *R. corymbifera* 'Laxa'.

The root-lesion nematode, *Pratylenchus penetrans* (Cobb, 1917) Filip'jev & Schuurmans Stekhoven, 1941, is a biotrophic plant parasite on a wide range of hosts mainly in temperate climates. It is one of the principal nematode species infecting ornamental plants. The nematode causes destruction of the root system, which results in loss of vigor in young plants. Serious losses in roses caused by *P. penetrans* were reported in California (Sher, 1959), Canada (Johnson and McClannahan, 1974), Japan (Ohkawa and Saigusa, 1981), and Europe (Coolen and D'Herde, 1970; Coolen and Hendrickx, 1972; Corbett, 1973).

Satisfactory control of *P. penetrans* in the field is achieved by the application of fumigant or non-fumigant nematicides (Corbett, 1973; Richardson and Grewal, 1993). However, increasing concern about environmental contamination stimulates the use of alternative control strategies that make use of resistance and tolerance of species and cultivars (Dubois et al., 1990; Horst, 1983). Resistance and susceptibility relate to nematode reproduction, whereas tolerance describes host injury (Cook and Evans, 1987). Plants that are resistant (support fewer nematodes) or are tolerant (do not exhibit growth reduction) improve nematode management and crop development. The extractable population density in soil and roots of infected plants at the end of the growing season (Pf) and its ratio (Pf/Pi) to the initial population density (Pi) of nematodes are common measurements for comparison of host suitability of plants (Westcott and Zehr, 1991). Accessions

with Pf/Pi ratio less than one are considered resistant in that they do not support nematode reproduction. Measurements of plant growth characters are indicators of tolerance (Cook and Evans, 1987).

Resistance and tolerance to nematodes have been important components in a few rose screening and breeding programs (Dubois et al., 1990; Horst, 1983). *Rosa eglanteria* (syn. *R. rubiginosa*) and *R. chinensis* 'Major' were found to be the least suitable *Rosa* hosts of *P. penetrans* (Coolen and Hendrickx, 1972; Ohkawa and Saigusa, 1981). In a previous study, we detected partial resistance to *P. penetrans* in *R. virginiana*, which supported significantly lower multiplication of the nematode than the control cultivar *R. corymbifera* 'Laxa' (Peng and Moens, 2002a). In the present study, we evaluated the host suitability of 131 accessions of *Rosa* sp. for *P. penetrans*.

Materials and Methods

Inoculum preparation. The population of *P. penetrans* used in this study was isolated in a mist chamber (Seinhorst, 1950) from maize roots collected in a field at Lokeren, Belgium. Traditional species identification using morphology and morphometrics was confirmed with rDNA-RFLP patterns compared to those obtained by Waeyenberge et al. (2000). Isolated nematodes were sterilized with a mixture of malachite green and streptomycin sulfate for 10 min (Peng and Moens, 1999) and were cultured monoxenically on carrot disks (O'Bannon and Taylor, 1969) for 70–75 d. After that period they were extracted from the carrot disks during 1 week in the mist chamber and collected as inoculum. The adult to juvenile ratio of the inoculum varied between 1 and 1.2. The sequence of extraction, sterilization, and culturing on carrot disks was repeated to build up and to maintain the nematode population. The isolate used in this study was shown to be pathogenic on *Rosa* after its inoculation on

seedlings of *Rosa corymbifera* 'Laxa' (Peng and Moens, 2002b).

Determination of optimum inoculation density. Optimum inoculation density was identified in an experiment conducted in a growth chamber. Fifty-day-old plants of the known susceptible standard *R. corymbifera* 'Laxa' were transplanted into 50-mL pots filled with sand (sizes between 150–212 μ m) and amended with 27.75 mg·mL⁻¹ soluble composed fertilizer M77 (Scotts, The Netherlands) according to Peng and Moens (2002a) and subjected to a 12-h photoperiod and temperature and relative humidity set at 22 °C and 85% during the light and 20 °C and 80% during the dark. Ten days later they were inoculated at their base with 125, 250, 500, or 1000 mobile stages including larvae and adults of *P. penetrans* that had passed through the filter paper placed under the carrot disks in the mistifier chamber. Nematodes were inoculated in 400 μ L water. Plants were placed in a completely randomized design; each treatment was replicated 8 to 10 times. Each week, every pot was watered (10 mL) twice and fertilized once (10 mL) with 7.5 mg·mL⁻¹ M77 fertilizer. Fifty days after inoculation, *P. penetrans* density was determined separately in the sand and the roots. Roots were cut into 1–2 cm fragments and macerated in 500 mL water in a Waring blender for 90 s. Nematodes were extracted from both the obtained nematode-root suspension or the sand with a fully automated nematode extractor based on centrifugation (Hendrickx, 1995). All the nematodes in the 10 mL extract were counted under a dissecting microscope.

Plant material. A total of 131 accessions of *Rosa* was obtained from different sources (Table 1). The collection was composed of 29 tea or hybrid tea rose cultivars, 11 floribunda roses, 9 hybrid musk roses, 7 shrub roses, 23 other modern roses, 35 accessions of a total of 24 botanical *Rosa* species or subspecies, and 17 other old garden roses. The accessions were classified according to the American Rose Society (ARS) Approved Horticultural Classification (Liberta and Young, 2000). The rootstocks *R. corymbifera* 'Laxa' and *R. canina* 'Pollmeriana' (Pf/Pi = 15.8 and 24.2, respectively) were added as standards with known susceptibility.

As seeds were not available for the majority of the screened accessions, each accession was propagated by dipping hardwood cuttings (two leaves) in a mixture of active carbon and 2000 ppm IBA and planted in a shaded greenhouse in a 1 peat : 1 sand (v/v) mixture previously pasteurized with steam at 80 °C in late June. Forty days later, the majority of the plants were successfully rooted. After another 20 d, the rooted plants with 3–4 roots and 4–6 new leaves were uprooted. The peat was washed off and plants were transplanted singly into 50-mL plastic pots (see above).

Screening. From Aug. 1998 to Jan. 1999, plants of 59 accessions were screened in a growth chamber (conditions as above). From Aug. 1999 to Jan. 2000, another 72 accessions were screened in the greenhouse (20 to 22 °C) along with 'Barcarolle', 'Pailine Sport', 'Pink Prosperity', 'Rose Romantic', and *R. laevigata*

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Table 1. Host reaction of *Rosa* accessions to *Pratylenchus penetrans* 5 months after inoculation with 500 nematodes in 400 μ L water in the 1998–2000 screening.

<i>Rosa</i> accessions	Breeder	Group ^z	Source ^y	n	Pf/Pi mean	Difference in Pf/Pi		Maximum Pf/Pi in one of the three replicates
						Within groups ^x	Between all accessions ^w	
Barcarolle	Laperrière, 1959	HT MR	DPGB	5	1.49	A	a	3.42
Aruba	Spek, 1996	HT MR	DPGB	3	1.69	A	a	4.04
G.D. Jardins de Bagatelle	de Bagatelle, 1986	HT MR	DPGB	3	1.89	A	ab	5.06
Red Unique		HT MR	DPGB	3	1.95	A	ab	3.04
Frederyk Chopin	Zyla, 1973	HT MR	DPGB	3	2.27	A	ab	4.04
Pavarotti	deRuiter, 1993	HT MR	DPGB	3	2.38	A	ab	2.76
Dame de Coeur	Lens, 1958	HT MR	DPGB	3	2.47	A	ab	4.78
Pailine Sport	RvS-Melle, 1989	HT MR	DPGB	7	2.49	A	ab	7.24
Velvet Fragrance	Fryer's Nursery Ltd., 1988	HT MR	DPGB	3	2.97	A	ab	4.28
Isabelle	RvS-Melle, 1989	HT MR	DPGB	3	3.07	A	ab	4.54
Christine	McGredy, 1918	HT MR	DPGB	3	3.09	A	ab	3.96
Rossini		HT MR	DPGB	3	3.66	A	a–c	5.04
Gypsy Curiosa		HT MR	DPGB	3	3.97	AB	a–c	6.74
Inka	Tantau, 1978	HT MR	DPGB	3	4.01	AB	a–c	5.42
Dream	Dramm, 1938	HT MR	DPGB	3	4.03	AB	a–c	6.24
Limona	Kordes, 1993	HT MR	DPGB	3	4.17	AB	a–c	8.44
Josée	RvS-Melle, 1987	HT MR	DPGB	3	5.07	AB	a–c	5.22
Orange Unique		HT MR	DPGB	3	5.67	AB	a–c	12.78
Gomery	RvS-Melle, 1985	HT MR	DPGB	3	5.77	AB	a–c	7.36
Verosa		HT MR	DPGB	3	5.83	AB	a–c	6.50
Texas	Kordes, 1993	HT MR	DPGB	3	5.91	AB	a–c	6.44
Kiss		HT MR	DPGB	3	5.97	AB	a–c	11.50
Pascali	Lens, A. Dickson, 1963	HT MR	DPGB	3	6.11	AB	a–c	9.36
Auguste Renoir	Meilland, A. A., 1992	HT MR	DPGB	3	7.38	A–C	a–c	11.34
Helena	RvS-Melle, 1988	HT MR	DPGB	3	8.53	A–C	a–c	12.48
Sweet Shot		HT MR	DPGB	3	8.97	ABC	a–c	16.12
Madelon	de Ruiter, G., 1987	HT MR	DPGB	3	13.74	BC	a–c	17.02
Red Calypso		HT MR	DPGB	4	17.00	C	a–f	23.88
Katrien	RvS-Melle, 1990	HT MR	DPGB	3	32.76	D	e–g	35.56
Ville du Rœulx	RvS-Melle, 1989	F MR	DPGB	5	1.64	A	a	3.48
Mercedes	Kordes, R., 1974	F MR	DPGB	3	2.30	AB	ab	5.66
Rose Romantic	Kordes, 1984	F MR	DPGB	12	2.83	AB	ab	8.14
Modern Fire		F MR	DPGB	3	2.99	AB	ab	4.02
Floranje	RvS-Melle, 1985	F MR	DPGB	4	4.30	AB	a–c	6.84
Miracle	Verbeek, 1962	F MR	DPGB	3	4.37	AB	a–c	5.38
Red Velvet	Kordes, 1940	F MR	DPGB	3	5.85	AB	a–c	7.50
Gaetane	RvS-Melle, 1985	F MR	DPGB	3	7.75	AB	a–c	9.46
Bonica	Meilland, F., 1958	F MR	DPGB	3	9.81	A–C	a–c	13.06
Vanilla	Kordes, 1994	F MR	DPGB	4	12.05	BC	a–e	20.26
Melglory	RvS-Melle, 1982	F MR	DPGB	3	19.39	C	a–f	28.24
Pink Prosperity	Bentall, 1931	HMSk MR	DPGB	8	1.81	A	a	6.01
Lavender Lassie	Kordes, 1960	HMSk MR	DPGB	5	2.05	A	ab	3.93
Felicia	Pemberton, 1928	HMSk MR	DPGB	3	3.19	A	a–c	4.52
Plaisanterie	Lens, 1996	HMSk MR	DPGB	3	3.23	A	a–c	5.78
Diamond Rose	Lens, 1995	HMSk MR	DPGB	3	5.97	A	a–c	9.86
Ravel	Lens, 1987	HMSk MR	DPGB	4	6.43	A	a–c	8.70
Jacqueline Humery	Lens, 1995	HMSk	DPGB	3	6.75	A	a–c	9.46
Moonlight	Pemberton, 1913	HMSk MR	DPGB	3	6.82	A	a–c	7.86
Mozart	P. Lambert, 1937	HMSk MR	DPGB	6	7.68	A	a–c	11.52
Carmenetta	Central Exp. Farm, 1923	S MR	DPGB	3	2.16	A	ab	2.65
William Shakespeare	Austin, David, 1987	S MR	DPGB	3	2.31	A	ab	2.46
Fredica	INRA, 1974	S MR	INRA	3	2.59	A	ab	4.88
Rush	Lens, 1983	S MR	DPGB	3	3.97	A	a–c	5.90
Heidtraum	Noack, W., 1991	S MR	DPGB	3	5.26	AB	a–c	7.16
Graham Thomas	Austin, David, 1983	S MR	DPGB	6	5.39	AB	a–c	6.92
Red Wing		S MR	DPGB	3	15.99	B	a–e	18.69
× 8914	Lens, 1989	MR	DPGB	4	1.74	A	a	3.71
Pink Surprise	Lens, 1980	Min MR	DPGB	3	1.93	A	ab	3.46
Flash	Hatton, 1938	LCI MR	DPGB	3	1.95	A	ab	3.28
× 8843	Lens, 1988	MR	DPGB	6	2.27	A	ab	4.34
Swany	Meilland, Mrs. Marie-Louis, 1978	Min MR	DPGB	3	2.54	A	ab	6.71
Lena	Dickson, R., 1906	T MR	DPGB	3	2.55	A	ab	4.58
Enigma		MR	DPGB	3	3.17	A	ab	3.58
New Dawn Somerset	Somerset Rose Nursery, 1930	LCI MR	DPGB	3	3.20	A	a–c	6.31
Max Graf	Bowditch, 1919	HRg MR	DPGB	3	3.67	A	a–c	12.08
Dr Huey	Thomas, 1914	LCI MR	INRA	3	3.94	A	a–c	5.34
Veilchenblau	Schmidt, J.C., 1909	HMult MR	DPGB	3	4.29	A	a–c	5.77
Prairie		MR	DPGB	3	4.56	A	a–c	6.78
Marstem		MR	INRA	3	5.10	A	a–c	11.60
Kathleen Harrop	Dickson, A., 1919	B MR	DPGB	3	6.20	A	a–c	7.96

continued on next page.

Table 1. Continued.

Rosa accessions	Breeder	Group ^z	Source ^y	n	Pf/Pi mean	Difference in Pf/Pi		Maximum Pf/Pi in one of the three replicates
						within groups ^x	between all accessions ^w	
Excelsa	Walsh, 1909	HWich MR	DPGB	3	6.57	A	a-c	9.16
Josephine Charlotte		MR	DPGB	3	7.23	A	a-c	13.82
Mevr. Nathalie Nypels	Leenders, M., 1919	Pol. MR	DPGB	3	7.73	A	a-c	13.82
× 8940	Lens, 1989	MR	DPGB	3	8.79	A	a-c	11.15
× 8913	Lens, 1989	MR	DPGB	3	10.95	A	a-c	11.63
Nèige d'été	Gailloux, G., 1984	Min MR	DPGB	3	11.38	A	a-d	17.94
Romantic Curiosa		MR	DPGB	4	11.48	A	a-d	15.86
Sander's White Rambler	Sander and Sons, 1912	HWich MR	DPGB	9	12.69	A	a-e	32.56
Yesterday	Harkness, 1974	Pol MR	DPGB	3	14.08	A	a-e	25.06
<i>R. indica</i> CE33		Sp OGR	INRA	3	1.53	A	a	2.50
<i>R. indica</i> CE35		Sp OGR	INRA	3	2.57	A	ab	3.74
<i>R. indica</i> CE4		Sp OGR	INRA	5	2.60	A	ab	4.66
<i>R. indica</i> CE20		Sp OGR	INRA	3	2.73	A	ab	3.08
<i>R. indica</i> CE25-1		Sp OGR	INRA	3	5.29	A	a-c	7.58
<i>R. indica</i> ECE7-1-1		Sp OGR	INRA	3	5.82	A	a-c	8.56
<i>R. indica</i> 64-11-85-1		Sp OGR	INRA	3	42.60	A	g	91.60
<i>R. multiflora</i> K1		Sp OGR	INRA	5	0.88	A	a	2.48
<i>R. multiflora cathayensis</i>		Sp OGR	Lens	4	1.39	A	a	1.98
<i>R. multiflora</i> K2		Sp OGR	INRA	5	5.08	A	a-c	7.62
<i>R. multiflora</i> 'Alfred Dietrich'		Sp OGR	DPGB	3	8.43	A	a-c	11.99
<i>R. multiflora</i> 'Catholica angelica'		Sp OGR	DPGB	3	10.39	A	a-c	10.50
<i>R. multiflora</i> Thunberg		Sp OGR	INRA	3	13.34	A	a-e	17.62
<i>R. multiflora</i> Thunberg		Sp OGR	Lens	3	13.55	A	a-e	30.94
<i>R. glauca</i> Pourrett (syn. <i>R. rubrifolia</i>)		Sp OGR	Lens	3	0.75	A	a	1.19
<i>R. californica</i> Chamisso and Schlechtendahl		Sp OGR	Lens	3	1.10	AB	a	1.48
<i>R. sempervirens</i> Linnaeus		Sp OGR	Lens	3	1.24	AB	a	2.22
<i>R. setigera tomentosa</i> Torrey and Gray		Sp OGR	DPGB	3	1.46	AB	a	3.02
<i>R. pendulina pyrenaica</i> (Gouan) R. Keller		Sp OGR	Lens	3	2.09	AB	ab	4.57
<i>R. macounii</i> Greene		Sp OGR	Lens	3	2.42	A-C	ab	2.82
<i>R. arkansana</i> Porter		Sp OGR	DPGB	4	2.48	A-C	ab	4.02
<i>R. orientalis</i> Dupont ex Seringe		Sp OGR	DPGB	3	2.61	A-C	ab	3.33
<i>R. eglanteria</i> Linnaeus		Sp OGR	Lens	3	2.67	A-C	ab	3.49
<i>R. canina</i> Linnaeus		Sp OGR	Lens	3	2.68	A-C	ab	4.78
<i>R. holodonta</i> Stapf		Sp OGR	Lens	3	3.20	A-C	a-c	3.89
<i>R. glauca</i> Pourrett		Sp OGR	Lens	3	3.24	A-C	a-c	4.53
<i>R. hemisphaerica</i> Hermann		Sp OGR	Lens	3	3.37	A-C		4.49
<i>R. spinosissima</i> Linnaeus		Sp OGR	DPGB	4	3.99	A-C		6.80
<i>R. gallica</i> Linnaeus		Sp OGR	Lens	3	5.21	A-C		9.07
<i>R. nitida</i> Willenow		Sp OGR	Lens	6	5.86	A-C		12.39
<i>R. palustris nuttalliana</i> Rehder		Sp OGR	DPGB	3	7.82	A-C		9.82
<i>R. corymbifera</i> 'Laxa'		Sp OGR	Standaert	4	15.85	A-C	a-e	22.64
<i>R. virginiana</i> Miller		Sp OGR	Lens	4	16.5	A-C	a-f	32.44
<i>R. hugonis</i> Hemsley		Sp OGR	Lens	3	22.83	BC	b-g	44.82
<i>R. canina</i> 'Pollmeriana'		Sp OGR	INRA	3	24.2	C	c-g	39.16
<i>R. laevigata anemonoides</i>		OGR	Lens	5	0.56	A	a	0.80
<i>R. johannensis</i>		OGR	INRA	6	1.37	A	a	2.93
<i>R. beggeriana</i> postnarjan		OGR	DPGB	3	2.32	A	a	5.65
<i>R. tomentosa cinerascens</i>		OGR	DPGB	3	2.44	A	ab	2.55
Jules Margottin	Margottin, 1853	HP OGR	DPGB	9	2.48	A	ab	5.00
<i>R. glauca carmenetta</i>		OGR	DPGB	3	2.87	A	ab	3.60
Königin von Dänemark	Booth, 1826	A OGR	DPGB	4	3.35	A	ab	5.16
<i>R. pendulina</i> × <i>R. tomentosa</i> var. 'Denudata'		OGR	Lens	3	5.38	A	a-c	11.22
<i>R. noisettiana</i> 'Manetti'		N OGR	INRA	4	6.08	A	a-c	10.22
<i>R. noisettiana</i> 'Manetti' CE2		N OGR	INRA	3	6.39	A	a-c	8.28
<i>R. virginiana</i> × <i>macrophylla</i>		OGR	Lens	3	6.53	A	a-c	7.79
Rosa × Mariae-Graebneriae	Ascheson and Graebner	OGR	DPGB	3	7.23	A	a-c	10.35
Coupe d'Hébé	Laffay, M., 1840	OGR	DPGB	3	9.26	A	a-c	10.62
<i>R. chinensis</i> Jacquin		Ch OGR	Lens	3	9.56	A	a-c	11.41
Gele Dorenbos		OGR	DPGB	3	10.27	A	a-c	13.21
<i>R. × inuoluta wilsonii</i> (Borrer) Baker		OGR	DPGB	3	31.95	B	d-g	44.96
<i>R. canina</i> 'Kiese'		OGR	Lens	3	37.16	B	fg	38.92

^zAccessions classified according to the American Rose Society (ARS) Approved Horticultural Classification (Liberta and Young, 2000) grouping accessions as Hybrid Tea (HT), Floribunda (F), Hybrid Musk (HMsk), Shrubs (S), or other modern roses (MR) including large-flowered climbers (LCI), Hybrid Wichurana (HWhich), Bourbon (B), Tea (T), Hybrid Rugosa (HRg), Polyantha (Pol), Miniature (M), and Hybrid Multiflora (Hmult). Other accessions are botanical species or subspecies (Sp.) or other old garden roses (OGR) including Hybrid Perpetual (HP), Alba (A), China (Ch), and Noisetta (N).

^yDPGB: Dept. of Plant Genetics and Breeding, Agricultural Research Centre, Melle, Belgium. INRA: Station de Fréjus, Fréjus, France. Lens: Nurseries Louis Lens, Oudenburg, Belgium, Standaert: Standaert Nurseries, Brugge, Belgium.

^wMeans of accessions of a same species group followed by a common character are not significantly different according to Tukey honest difference (THD) test for unequal N ($P < 0.05$).

^xMeans followed by an overlapping alphabetic interval are not significantly different when accessions were compared altogether using THD test for unequal N ($P < 0.05$).

anemonoides, all accessions with a Pf/Pi < 1.0 during the 1998–99 screening, as well as with the randomly selected accession ‘Jules Magrotin’. In the greenhouse, 4 h of supplemental illumination were applied in winter.

For each accession, three to five plants were screened with 500 mobile stages of *P. penetrans* in 400 µL water (see above). They were placed in a completely randomized design, and fertilized and watered as described above. Five months after their inoculation, nematodes were extracted from the sand and the root system and counted separately (see optimum inoculation density).

The resistance of *R. laevigata anemoides* on which *P. penetrans* did not multiply (Pf/Pi averaged at 0.80) in the 2 years of screening was confirmed in a 0.5-L pot experiment in 2001. In mid-Sept. 2000, plants of *R. laevigata anemoides* were rooted as above, transplanted into 0.5-L pots filled with sand and inoculated with 500 mobile stages of *P. penetrans* in 400 µL water 10 d after transplanting in early Dec. 2000. Plants of *R. virginiana* and *R. corymbifera* ‘Laxa’ were infested similarly for comparison. Fifty milliliters of 7.5 mg·mL⁻¹ M77 were applied once, and 50 mL water twice every week to each plant. Nematodes were extracted (see above) and counted after 5 months of incubation in the air-conditioned greenhouse.

Statistics. For all of the experiments, Pf was calculated as the sum of numbers of nematode (including eggs) extracted from both sand and roots. The *t* test and ANOVA analysis were performed with the Statistica software package (StatSoft, 1999). Tukey honest difference (THD) test for unequal N (Sjotvoll/Stoline test) was used to compare the means ($P = 0.05$).

Results

Optimum inoculation density. Final nematode population on *R. corymbifera* ‘Laxa’ was inoculum density dependent ($F = 13.03$, $P < 0.0001$). The differences in Pf/Pi, however, were nonsignificant between the tested inoculum levels (Table 2). The standard deviation on the Pf/Pi averages was least at Pi = 500, which was then used during the screening.

Effects of accession on *P. penetrans* multiplication. During both screening periods, significant effects of the accessions on the multiplication of *P. penetrans* were observed ($F = 4.88$, $P < 0.0001$; and $F = 3.81$, $P < 0.0001$, during 1998–99 and 1999–2000, respectively). However, significant effects of screening times

on the nematode multiplication were not observed ($F = 0.0077$, $P = 0.9299$). Consequently, the screening data obtained during both periods were pooled and mutually compared; the result of this comparison was similar to those obtained when the data of each period were analyzed separately (data not shown).

Out of the 131 screened accessions, only 14 scored a Pf/Pi ≤ 1.81, smaller than the Pf/Pi on *R. canina* ‘Pollmeriana’ (Pf/Pi = 24.20). No accession had a Pf/Pi smaller than the Pf/Pi obtained for the susceptible rootstocks *R. corymbifera* ‘Laxa’ (Pf/Pi = 15.85) (Table 1). Conversely, two accessions, *R. canina* Kiese (Pf/Pi = 37.16) and *R. indica* 64-11-85-1 (Pf/Pi = 42.60), supported greater nematode multiplication than *R. corymbifera* ‘Laxa’, but no accession allowed better nematode multiplication than *R. canina* ‘Pollmeriana’.

Hybrid tea roses. All 29 tested cultivars supported multiplication of *P. penetrans*. The smallest multiplication was obtained on ‘Barcarolle’ (1.49). However, the Pf/Pi of 26 accessions did not differ from the Pf/Pi on this latter cultivar. Nematode reproduction obtained on ‘Katrien’ (32.76) was higher than on all other screened hybrid tea roses. The maximum Pf/Pi varied between 2.76 (‘Pavarotti’) and 35.56 (‘Katrien’).

Floribunda roses. *P. penetrans* multiplied well on all of the 11 screened hybrids. The Pf/Pi varied between 1.64 (‘Ville de Rœulx’) and 19.39 (‘Melglory’). Only on the latter cultivar and on ‘Vanilla’ (Pf/Pi = 12.05) did the nematodes multiply better than on ‘Ville de Rœulx’.

Hybrid musk roses. The lowest multiplication was observed on ‘Pink Prosperity’ (1.81), the greatest on ‘Mozart’ (7.68). Maximum Pf/Pi values varied between 3.93 (‘Lavender Lassie’) and 11.52 (‘Mozart’).

Shrub roses. The Pf/Pi on four out of seven tested shrub roses varied between 2.3 (‘William Shakespeare’) and 5.96 (‘Rush’) and was less than on ‘Red Wing’ (Pf/Pi = 15.99), which also showed the greatest maximum (Pf/Pi = 18.69).

Other modern roses. The average Pf/Pi obtained on modern roses other than hybrid tea, floribunda, hybrid musks and shrubs, was situated between 1.74 (‘× 8914’) and 14.08 (‘Yesterday’). On ‘Sander’s White Rambler’, the maximum Pf/Pi reached 32.56; the smallest value for the maximum Pf/Pi was observed on ‘Flash’ (3.28).

Rosa multiflora and R. indica. No significant differences in nematode multiplication were observed after multiple comparison of the accessions within the species, although varia-

tions were observed among these accessions. *Rosa multiflora* ‘K1’ did not allow nematode reproduction (Pf/Pi = 0.88) whereas on other accessions of this species great reproduction factors were registered (e.g., *R. multiflora* ‘Alfred Dietrich’ and ‘Catholica Angelica’). The majority of the accessions of *R. indica* allowed medium nematode multiplication, the Pf/Pi observed on *R. indica* 64-11-85-1 was extremely large (42.60). In one of the replicates of this accession, a Pf/Pi = 91.6 was scored.

Rosa species and subspecies other than R. multiflora and R. indica. One accession of *R. glauca* (syn. *R. rubrifolia*) did not support *P. penetrans* reproduction (Pf/Pi = 0.75). Another accession from the same nursery, however, did support nematode multiplication (Pf/Pi = 3.24). Poor multiplication of the nematode was also observed on *R. californica*, *R. sempervirens*, *R. setigera tomentosa*, and *P. pendulina pyrenaica* (Pf/Pi = 1.10–2.09) and good multiplication was observed on *R. canina* ‘Pollmeriana’. The reproduction factor on *R. virginiana* did not differ from the Pf/Pi obtained on *R. corymbifera* ‘Laxa’ and *R. canina* ‘Pollmeriana’. The maximum Pf/Pi in these accessions varied between 1.19 and 44.82.

Other old garden roses. Nematode multiplication deviated the other tested old garden roses in two categories. *Pratylenchus penetrans* did not multiply in any of the five replicates of *R. laevigata anemoides* (average Pf/Pi = 0.56) whereas very high nematode multiplication was observed on *R. xinuluta wilsonii* (Pf/Pi = 31.95 and *R. canina* ‘Kiese’ (Pf/Pi = 37.16). Other accessions supported the multiplication of nematodes, which was not different from that on *R. laevigata anemoides*.

In the experiment comparing the host suitability of *R. laevigata anemoides*, *R. corymbifera* ‘Laxa’, and *R. virginiana* in 0.5-L pots, the difference in multiplication of *P. penetrans* was significant ($F = 45.35$, $P < 0.0001$). The Pf/Pi on *R. laevigata anemoides* averaged 1.89 and was lower than that of *R. corymbifera* ‘Laxa’ but similar to that of *R. virginiana* (Table 3).

Discussion

Because cultural conditions can greatly influence the growth of plants on one hand and the survival, development and reproduction of nematodes on the other hand, it is necessary to conduct resistance-screening experiments in carefully defined conditions. In previous research we determined optimal conditions for screening of the host suitability of seed propagated *Rosa* species and cultivars to *P.*

Table 2. Multiplication of *Pratylenchus penetrans* at 50 d postinoculation in rooted plants of *Rosa corymbifera* ‘Laxa’ grown in sand in the growth chamber.

Pi	Pf ^a	Pf/Pi	SD of Pf/Pi
125	609 a	4.87 a	2.52
250	993 ab	3.97 a	2.00
500	1961 bc	3.92 a	1.12
1000	2731 c	2.73 a	1.44

^aMeans in a column followed by the same letter are not significantly different according to Tukey honest difference test ($P < 0.05$).

Table 3. Multiplication of 500 mobile stages of *Pratylenchus penetrans* 5 months after inoculation in 400 µL water onto *Rosa* species grown in sand (Dec. 2000–May 2001).

Accessions	N ^a	Pf/Pi	
		Mean ^b	95% confidence interval
<i>Rosa laevigata anemoides</i>	18	1.89 a	1.37–2.41
<i>R. virginiana</i>	10	2.69 a	1.65–3.73
<i>R. corymbifera</i> ‘Laxa’	13	19.74 b	13.92–23.55

^aN = number of plants.

^bMeans in column followed by the same letter are not significantly different ($P < 0.05$) according to Tukey honest difference (THD) test for unequal N ($P < 0.05$).

penetrans (Peng and Moens, 2002a). These conditions were used in the reported experiments on rooted hardwood cuttings. Only the inoculum density was modified here because the amount of the inoculum is critical for differentiation of the plant response among genotypes (Fassuliotis, 1985). The inoculum should be enough to establish a population (Kaplan, 1990) and be limited so that high dosages do not cause too much injury and mask potentially useful genetic material (Young, 1998). As the data obtained in the inoculum density experiment did not show differences in multiplication factor between densities ranging from 250 to 1000 and as the standard deviation was least at density $P_i = 500$, this latter quantity was selected for use in the screening tests.

It is accepted that experiments evaluating plant resistance to parasitic nematodes should have replicates ranging between six and ten because data are often highly variable (Kaplan, 1990). In the reported experiments we frequently observed important variation among replicates. However, less replicates with repeated screening as in reported experiments is acceptable (Kunde et al., 1968). In any case results obtained in pot experiments need to be confirmed in the field. A first selection in a limited number of pots reduces the extent of field experiments.

Nematode reproduction is the selection criterion in resistance screening tests. Reproduction on one accession can be evaluated by comparison with the reproduction on a known resistant accession (Verdejo-Lucas et al., 2000) or by assessing the total number of nematodes extracted from both soil and roots (Potter and Dale, 1994). We used the reproduction factor relating the final population density (found in the sand and the roots) to the inoculum density as suggested by Westcott and Zehr (1991).

When used for the screening of the 131 *Rosa* accessions, this approach allowed the observation of a large variation in host suitability. Previously reported resistance of *R. multiflora* 'K1' (Ohkawa and Saigusa, 1981; Santo and Lear, 1976; Schneider et al., 1995) and one accession of *R. virginiana* (Peng and Moens, 2002a) to *P. penetrans* was confirmed in this experiment. However, the resistance of *R. eglandaria* (Coolen and Hendrickx, 1972) was not confirmed in this and our previous experiment (Peng and Moens, 2002a); the species supported a nematode multiplication similar to that of *R. corymbifera* 'Laxa'. *Rosa laevigata anemoides* was the only species for which resistance was present in all of the tested plants, as none of the replicates scored a $P_f/P_i > 1$. However, in the 0.5-L pot experiments for which *R. laevigata anemoides* of another origin was used, the P_f/P_i averaged 1.89. Variations between different accessions were also observed in the species of *R. glauca*

and *R. virginiana*. Obviously, the screening of different accessions of the same species is a valuable strategy for searching sources of resistance. The majority of *Rosa* accessions in our experiment supported the multiplication of *P. penetrans*. It would therefore be of great interest to evaluate the accessions for their tolerance, i.e., the ability to limit the damage caused by nematode infection.

Although screenings using a local pathogenic nematode population are of practical importance for the regional application of resistance or tolerance, results obtained with a single population can not be generalized as differences in pathogenicity were observed among populations of *P. penetrans* (Peng and Moens, unpublished data) and *P. vulnus* (Pinochet et al., 1993). Therefore, further screening with populations of various origins would extend the use of resistance in *Rosa* sp. to *P. penetrans*.

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