Received: 3 February 2022 | Accepted: 17 May 2022

DOI: 10.1111/ppa.13596

Plant Pathology WILEY

ORIGINAL ARTICLE

Factors influencing *Ralstonia pseudosolanacearum* infection incidence and disease development in rose plants

Jan van der Wolf[1] | Pieter Kastelein[1] | Leo Polelij[1] | Marjon Krijger[1] | Odette Mendes[1] | Nasim Sedighian[1,2] | Caitilyn Allen[3] | Peter Bonants[1] | Viola Kurm[1]

[1]Wageningen University & Research, Business Unit Biointeractions & Plant Health, Wageningen, Netherlands

[2]Department of Plant Protection, Shiraz University, College of Agriculture, Shiraz, Iran

[3]University of Wisconsin-Madison, Madison, Wisconsin, USA

Correspondence
Jan van der Wolf, Wageningen University & Research, Business Unit Biointeractions & Plant Health, PO Box 16, 6700 AA Wageningen, Netherlands.
Email: jan.vanderwolf@wur.nl

Funding information
Dutch Ministry of Economic Affairs

[Correction added on 21 June 2022, after first online publication: the spelling of co-author 'Caitilyn Allen' has been corrected in this version.]

Abstract

Glasshouse experiments were conducted to study infection and disease development in rockwool-grown rose plants inoculated with *Ralstonia pseudosolanacearum*. A *R. pseudosolanacearum* strain isolated from rose plants was more aggressive than strains from anthurium or curcuma. The three rose cultivars tested, Avalanche, Red Naomi, and Armando, differed in susceptibility. At 20°C, the rose strain caused hardly any symptoms over a 6-week period, whereas at 28°C typical wilt symptoms were observed within 2 weeks after stem inoculation of Armando, the most susceptible cultivar. Inoculating roots with the rose strain resulted only in weak atypical symptoms. Nevertheless, inoculating roots of cv. Armando at a relatively low inoculum dose of 10^4 cfu/ml led to high densities in the base of stems in one out of two experiments. *R. pseudosolanacearum* occasionally spread from stem inoculated plants with symptoms in rockwool slabs. This limited spread resulted in a low infection incidence, and only of plants directly adjacent to the plants with symptoms.

KEYWORDS
bacterial wilt, cultivar resistance, dissemination, rifampicin resistant mutant, symptomless infections, virulence

1 | INTRODUCTION

Bacteria belonging to the *Ralstonia solanacearum* species complex (RSSC) are the causative agents of bacterial wilt on a range of important crops such as banana, potato and tomato, as well as on ornamental plants like gerbera, sunflower and geranium (Norman et al., 2009). Its wide host range, aggressiveness, easy dissemination and persistence in the environment makes the RSSC one of the most destructive groups of bacterial pathogens in agricultural systems (Genin, 2010). Phylogenetic analysis divides the RSSC into four phylotypes. In 2014, phylotypes I and III were reclassified as *R. pseudosolanacearum*, phylotype II as *R. solanacearum*, and phylotype IV as *R. syzygii* (Safni et al., 2014).

Pathogens within the RSSC are occasionally found in ornamental glasshouse crops in Europe, such as *R. solanacearum* in pelargonium (Janse et al., 2004), and *R. pseudosolanacearum* in curcuma (Bergsma-Vlami et al., 2018) and anthurium (Anonymous, 2016). In 2016, *R. pseudosolanacearum* was described for the first time as a pathogen of rose (Tjou-Tam-Sin et al., 2016). The pathogen can cause wilting of shoots and flower stalks, chlorosis and early abscission of leaves, stunting and black necrosis of pruned branches that results in dieback. On the susceptible rose cultivar Armando, symptoms occurred as early as 10 days after stem inoculation when plants were grown in potting soil at 28°C. Symptoms developed more slowly or not at all if plants were grown at a lower temperature (20°C).

A *R. pseudosolanacearum* strain isolated from rose also caused symptoms in tomato, tobacco, eggplant and sweet pepper (Tjou-Tam-Sin et al., 2017).

Rose bacterial wilt outbreaks have occurred in different European countries, but all rose isolates cluster in a monophyletic clade of phylotype I, suggesting a single introduction (Bergsma-Vlami et al., 2018). In 2019, bacterial wilt was found in various rose cultivars in Korea (Kim et al., 2019). In this case, the isolates also belonged to a monophyletic clade of phylotype I. It was not reported if the European and Korean pathogens belong to the same haplotype.

Rose cultivars differed significantly in susceptibility to the strain of *R. pseudosolanacearum* isolated from rose, with cv. Armando more susceptible than cv. Red Naomi (Tjou-Tam-Sin et al., 2017). Further, inoculation of wounded stems resulted in a significantly higher disease incidence and severity than soil drench inoculation with the pathogen (Tjou-Tam-Sin et al., 2017).

The infection route of *R. pseudosolanacearum* during rose cultivation has not yet been fully clarified. It is known that rose plants can become infected aboveground during pruning and clipping with contaminated pruning shears, or underground if plants are exposed to contaminated irrigation or nutrient solution or contact infected roots of neighbouring plants. For other host plants it has already been shown that not only water, soil and plant debris, but also contaminated machinery and clipping tools can be sources of infection and dissemination of RSSC (Álvarez et al., 2010; Fortnum & Gooden, 2008; McCarter & Jaworski, 1969).

In this study, we evaluated factors influencing the infection and disease development of *R. pseudosolanacearum* in rose plants grown in rockwool, a widely used substrate in commercial picking rose cultivation in the Netherlands. We studied the role of inoculation methods (stem vs. root inoculation), bacterial densities during root inoculation, rose cultivar, the virulence of the bacterial strain and growth temperature on disease incidence and severity. The aggressiveness of the strains was also determined in tomato, which is used as a reference host to determine the virulence of RSSC (Anonymous, 2018). Pathogen dissemination in rockwool slabs and the risks for infection of neighbouring plants were also evaluated. High microbial background in underground plant parts often complicates pathogen detection and enumeration by dilution plating on the semiselective medium. Therefore, for this experiment, we used a virulent rifampicin resistant variant of *R. pseudosolanacearum* PD7123, derived via homologous recombination from a spontaneous mutant.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and bacteria growth conditions

The following strains of *R. pseudosolanacearum* were included: PD7123 (=IPO4001, phylotype I) isolated from a rose plant in the Netherlands (Tjou-Tam-Sin et al., 2017); PD2272 (=IPO4009, phylotype I) isolated from *Curcuma longa*, PD3205 (=IPO4017, phylotype I) isolated from anthurium. In addition, *R. solanacearum* strain PD2763 (phylotype II) isolated from potato, in the Netherlands, was used. All strains were grown for 48 h on yeast extract peptone glucose agar (YPGA) medium (Sukroongreung et al., 1984) at 25°C. Stock solutions of bacterial suspensions with an optical density (OD) at 600 nm of 0.1 (approximately 10^8 cfu/ml) were prepared in sterile 0.01 M phosphate buffer (PB) (per L: 1.2 g $Na_2HPO_4.12H_2O$ and 0.22 g $NaH_2PO_4.2H_2O$, pH 7.2). The bacterium *Acidovorax cattleya* strain NBC430 (working collection, Naktuinbouw, Roelofarendsveen, NL), used as extraction and amplification control in the TaqMan assays, was grown in tryptic soy broth medium for 48 h at 25°C (30 g/L; Becton Dickinson & Co.).

2.2 | Generation of a rifampicin resistant strain by homologous recombination

A rifampicin resistant strain of PD7123 (IPO4144) was generated by homologous recombination for use in experiments on the spread of the pathogen in rockwool slabs as previously described (Milling et al., 2009; Swanson et al., 2005). Genomic DNA was isolated from UW551-rif, a spontaneous rifampicin-resistant variant of phylotype IIB *R. solanacearum* strain UW551, originally isolated from geranium (Williamson et al., 2002) and was used to naturally transform PD7123. Briefly, strain PD7123 was incubated for 1 day at 28°C in minimal medium (25 mM KH_2PO_4, 3.8 mM $[NH_4]_2SO_4$, 45 µM $FeSO_4.7H_2O$, 0.517 mM $MgSO_4$, pH 7.0) supplemented with 1.8% (vol/vol) glycerol, 0.2% (wt/vol) glucose, 0.2% (wt/vol) galacturonic acid (pH 7.0) and 20 mM 4-morpholine ethane sulfonic acid (MES, pH 7.0). One hundred microlitres of bacterial suspension and 15 µl of UW551-rif genomic DNA (2.2–4.5 µg) were mixed and incubated on a sterile nitrocellulose membrane filter (GSWP02500; Millipore) placed on top of a CP plate (per L: 1 g Bacto casamino acids [BD 223050], 10 g Bacto peptone [BD 211677] and 18 g agar). Suspensions were allowed to dry before growing bacteria for 1 day at 28°C on the inverted plates. The next day, bacteria grown on the filter were resuspended in 500 µl water, plated and grown for 3–4 days on the same medium containing 50 mg/L rifampicin to select transformants. Transformed colonies were subcultured on CPG + TZC plates (per L: 1 g Bacto casamido acids [BD 223050], 10 g Bacto peptone [BD 211677], 10 g D-(+) glucose monohydrate, 0.01% tetrazolium chloride, and 18 g agar) to eliminate untransformed cells and to confirm the stability of the mutation; two single colony cultures were stored at −80°C. One of the resulting strains, named IPO4144, was used in experiments with rose plants.

2.3 | Virulence in tomato

The virulence of the wild-type *R. pseudosolanacearum* strains and the rifampicin resistant mutant IPO4144 was confirmed in tomato (*Solanum lycopersicum* 'Moneymaker'). Plants were grown from

seeds in potting soil (potting mixture WUR no. 4) at 21°C. The seedlings were inoculated at the third true leaf stage (around 4 weeks old) by injecting approximately 25 µl of bacterial suspension into the leaf axis of the second true leaf. Bacterial inocula were prepared from bacteria grown on YPGA in 0.01 M phosphate-buffered saline (PBS; NaCl 8 g, KCl 0.2 g, $Na_2HPO_4.2H_2O$ 2.9 g, KH_2PO_4 0.2 g, in 1 L demineralized water, pH 7.2) and adjusted to a density of 10^6 cfu/ml. Control plants were injected with 0.01 M PBS. Five plants were inoculated per strain, resulting in 60 tomato plants in total for each experiment. After inoculation the plants were kept in the greenhouse at a day/night regime of 16 h/8 h, 20°C/20°C (2017) or 28°C/23°C (2018 and 2019) and a relative humidity of 70%–80%. Symptom development was checked after 7, 14 and 20 days and scored according to the scoring scheme provided in Table 1. The area under the disease progress curve (AUDPC) was calculated for each plant according to the method of Shaner and Finney (1977).

2.4 | Virulence in rose

2.4.1 | Plant growth

In 2017, we studied differences in susceptibility of rose cultivars Avalanche and Red Naomi. In 2018 and 2019, experiments were also conducted with cultivar Armando. Plants were grown in 100 × 15 × 7.5 cm Vital NG2.0 rockwool slabs (Grodan) in a glasshouse in Wageningen, Netherlands. Rooted rose cuttings were received from commercial growers in 7.5 × 7.5 cm rockwool blocks and 2 weeks later cut to a length of approximately 20 cm. Between 1 and 4 weeks after arrival, rockwool blocks containing young plants were placed on gaps cut in the plastic covering of rockwool slabs for further root growth. On each slab, five plants were placed at a distance of approximately 20 cm. Slabs were placed crosswise on 100 cm-wide benches separated by distances of 60 cm.

Each plant was individually watered with nutrient solution (Yara Substrafeed Package E1) through an irrigation pin placed in the rockwool block in which they were planted as a cutting. The amount of irrigation water received varied depending on the developmental stage of the plants. Excess nutrient water was drained via crevices cut at the sides of the slabs. Mildew was controlled with Nimrod (Adama) and evaporated sulphur. Pest insects (whiteflies and spider mites) were controlled with Carex (Nufarm), Calypso (Bayer Crop Science) and/or Scelta (Certis). In all cases, the directions of use on the labels of the crop protection products were followed. Branches with flowers ready for picking were trimmed to prevent infestations with thrips and to avoid plants from becoming too large. After inoculation, plants were observed for symptom development weekly.

2.4.2 | Inoculation and sampling

In 2017, rose cuttings were received in the second week of August. Plants were stem- or root-inoculated on 14 September. At 81 days postinoculation (dpi), plants were sampled for analysis. In 2018, rose cuttings were received in the last week of March. On 15 May, plants were inoculated and on 10 July, at 56 dpi, the experiment was terminated and plants and substrates were sampled. In 2019, rose cuttings were received in the first week of April. On 13 May, plants were inoculated and at 52 dpi plants and substrates were sampled.

Rose plants were root inoculated by pouring 50 ml of inoculum (at a bacterial density of 10^4, 10^6 or 10^8 cfu/ml in PB) over the rockwool block in which each was planted as a cutting. For stem inoculation, the woody stem base was wounded 1–2 cm above soil level by stabbing a scalpel through the stem to make a vertical incision prior to injection with 20–50 µl of a 10^8 cfu/ml PB bacterial suspension per plant (until droplets were released from both sides of the wound) with a syringe and needle (21 G × 2″; 0.8 × 50 mm) into the incision of the stem.

Plants were observed weekly for symptom expression. Plants were rated using a modification of the disease index described by Tjou-Tam-Sin et al. (2017), with additional categories (in particular 4 = total death of plants): 0 = healthy plant, 1a = first appearance or mild symptoms of bacterial wilt, 1b = first appearance of leaf necrosis, 1c = first appearance of leaf chlorosis, 2a = progressing wilting, 2b = leaf necrosis protruding from the leaf margins towards the leaf midribs, 2c = yellowing of leaves and early leaf drop sometimes accompanied by a browning or blackening of petioles (die back), 3a = irreversible total wilt, 3b = death of one or more branches, followed by dark necrosis of stems, 4 = total death of plants (Figure 1). Disease incidences were calculated as the percentage of plants with symptoms out of the total inoculated plants.

TABLE 1 Symptom scoring scheme for tomato inoculated with strains of *Ralstonia pseudosolanacearum*

Score	Symptoms
0	No leaves affected
0.5	Yellowing inoculated leaf
1	1%–25% of leaves affected (yellowing, wilt or partial necrosis)
2	26%–50% of leaves affected (yellowing, wilt or partial necrosis)
3	51%–75% of leaves affected (yellowing, wilt or partial necrosis)
4	76%–100% of leaves affected (yellowing, wilt or partial necrosis)
5	All leaves completely wilted or dead

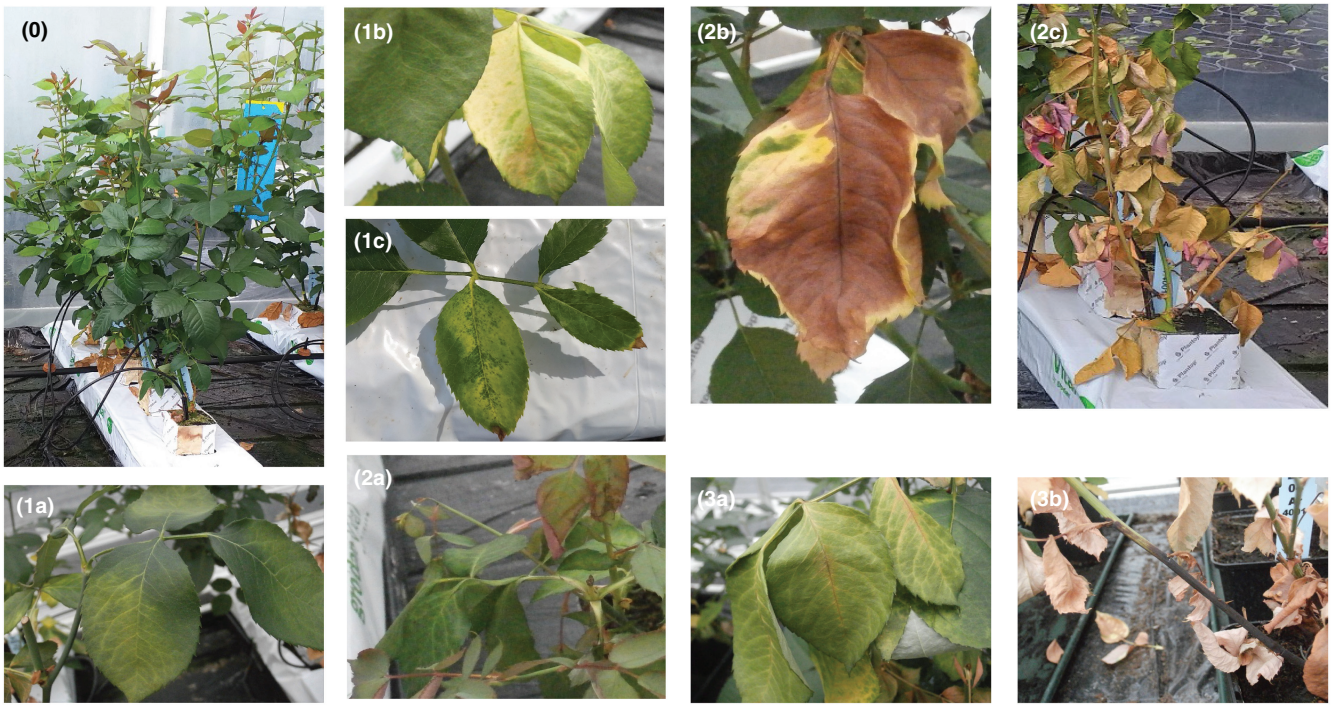

FIGURE 1 Symptom expression after inoculation of cv. Armando inoculated with *Ralstonia pseudosolanacearum* strain PD7123 isolated from rose plants. 0 = healthy plant, 1a = first appearance of bacterial wilt, 1b = first appearance of leaf necrosis, 1c = first appearance of leaf chlorosis, 2a = progressing wilting, 2b = leaf necrosis protruding from the leaf margins towards the leaf midrib, 2c = yellowing of leaves and early leaf drop sometimes accompanied by a browning or blackening of petioles (die back), 3a = irreversible total wilt, 3b = death of one or more branches, followed by dark necrosis of stems.

Stems were sampled by cutting a section of about 6–8 cm around the inoculation point (stem base) and at the top of the side branches (stem top). Thorns were removed and stem pieces were sterilized in 70% ethanol for 2 min and dried before processing. For sampling of roots, the stem base was jiggled out of the rockwool slab and rockwool attached to the roots was removed. Root samples were rinsed in water, sterilized in 70% ethanol for 2 min and dried before processing. The samples were weighed, transferred to a bag (BioReba AG) and crushed with a hammer and then PB was added in a volume of approximately two times the weight of the sample and mixed through the crushed tissue. After 1–5 min to allow bacteria to diffuse out of tissues into the fluidal phase, two aliquots of the macerate were collected for detection of *R. pseudosolanacearum*. A 1 ml aliquot was used immediately for serial dilution plating, and another 1 ml aliquot was transferred to a 1.2 ml collection tube (Qiagen), centrifuged for 15 min at 5800 × *g* (Sigma) at room temperature, and 980 µl supernatant was removed from the tube before storage of the pellet at −20°C until further processing for a direct TaqMan assay. For detection of the pathogen in the nutrient solution by TaqMan, approximately 50 ml of liquid was squeezed from the rockwool material separated from most of the root material. Coarse particles were removed after sedimentation. Bacteria were concentrated by centrifugation in two steps, first for 10 min at 9000 × *g*, after which the pellet was suspended in 1 ml of PB, and then for 10 min at 6000 × *g*. The pellet was suspended in approximately 20 µl of water and stored at −20°C until use.

2.5 | Detection of *R. pseudosolanacearum*

Detection of *R. pseudosolanacearum* in plant material and rockwool was done in two ways, by dilution plating on SMSA or by a (Bio-) TaqMan assay.

2.5.1 | Dilution plating on SMSA

Samples were spread-plated undiluted or 100-fold diluted in PB on selective medium South Africa (SMSA) agar (Elphinstone et al., 1996) with modifications to improve the selectivity as suggested by M. Bergsma-Vlami (Netherlands Food and Consumer Product Safety Authority [NVWA], Wageningen, Netherlands, personal communication). The modified medium contained (per litre) casamino acids 1 g, Bacto peptone 10 g, glycerol 5 ml and bacteriological agar No. 1 (Oxoid) 17 g. After autoclaving the medium for 15 min at 121°C and cooling to 50°C, the following antibiotics were added: 2,3,5, triphenyltetrazoliumchloride 50 mg, crystal violet 5 mg, polymyxin B-sulphate 100 mg, bacitracin 75 mg, chloramphenicol 9 mg, penicillin G 0.84 mg and cycloheximide 100 mg.

2.5.2 | TaqMan assay

Bacterial DNA was extracted from drain water samples with a DNeasy PowerWater Kit (Qiagen) from plant and rockwool samples using the sbeadex maxi plant kit (LGC Genomic GMGH) using a KingFisher extraction robot (Thermofisher Scientific) in accordance with the manufacturer's instructions. To check for efficiency of extraction and amplification, an internal control (IC) was used based on supplying a suspension of *A. cattleya* (Acat NBC430) to each sample prior to extraction as described by Bonants et al. (2019).

In 2017, samples were analysed by a TaqMan assay based on 16S rDNA sequences as described by Weller et al. (2000) but using a modified TaqMan (MGB) probe to increase specificity of the assay (Table 2). In 2018 and 2019, a TaqMan assay was used based on endoglucanase (*egl*) sequences as described by Vreeburg et al. (2016, 2018), but with some modifications as explained below. The *egl*-based assay was used in later experiments because it showed a higher diagnostic sensitivity than the modified Weller assay in samples with a high level of nontarget DNA (Sedighian et al., 2020).

TaqMan assays were performed using a final volume of 25 µl in a CFX96 thermocycler (Bio-Rad). *R. pseudosolanacearum* was detected as described (Vreeburg et al., 2018), but with the following modifications. The assay consisted of 5 µl PerfeCTa Multiplex qPCR ToughMix 5× (Quantabio), 300 µM Acat 2-F and Acat 2-R primers, 100 µM Acat 2-Pr TexasRed-labelled probe with either 300 µM RS-I-F and RS-II-R primers, 200 µM RSP-55 T VIC-labelled probe, or 300 µM Rs-pan1-F5 and Rs-pan1-R3 primers, 300 µM TP-Rsol-pan1-81 and TP-Rsol-pan1-81-alt2 probes, both labelled with FAM. PCR-grade water was added to a final volume of 20 µl and 5 µl of extracted DNA was added for each sample to a total volume of 25 µl. PCR-grade water alone served as negative control. For the positive control, 5 µl of a 10-fold dilution series of genomic DNA of *R. pseudosolanacearum* strain PD7123 was used (1 ng–1 fg). Each positive control was supplemented with 2 pg of *A. cattleya* DNA as an internal amplification control. The cycling conditions were as follows: initial denaturation for 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Samples with C_t values lower than 30 were considered positive, with values between 35 and 30 as suspect, and above 35 as negative.

2.5.3 | Bio-TaqMan assay

To further enhance the sensitivity and specificity of the assays, dilution plating on SMSA was combined with a TaqMan assay into a so-called Bio-TaqMan assay. For this, the SMSA plate was flooded with 3 ml of Ringer's solution and the bacterial colonies were dislodged from the agar with the aid of an L-shaped spreader. Depending on the number of colonies, 1 ml of the undiluted suspension, or 1 ml of the suspension diluted to a slightly cloudy appearance, was transferred to a 1.2 ml collection tube. DNA was extracted from the suspensions with the sbeadex maxi plant kit. Before DNA extraction, *A. cattleya* DNA was supplemented to each sample as described for the TaqMan assay.

2.6 | Data analysis

Analysis of vaiance (general ANOVA; GenStat, 18th edition; VSN International Ltd) with AUDPC values was used to analyse the effect of *R. pseudosolanacearum* strain or rose cultivar on disease severity. Duncan's multiple range test was used to evaluate the significance of differences between pairs of averages.

3 | RESULTS

3.1 | Virulence of *R. pseudosolanacearum* in tomato

The virulence of selected *R. pseudosolanacearum* strains was evaluated in tomato during each glasshouse experiment. If tomato plants were grown at 20°C, most plants inoculated with the rose strain PD7123 wilted within 20 dpi, while plants inoculated with curcuma isolate PD2272 showed elongation of the internodes, and most plants inoculated with anthurium isolate PD3205 developed leaf

TABLE 2 Probes and primers for the TaqMan assays used in this study

Genetic region	Primer	Sequence (5′–3′)	5′ dye	Reference
16S rDNA	RS-I-F	GCATGCCTTACACATGCAAGTC		Modified after Weller et al. (2000), Vreeburg et al. (2016)
	RS-II-R	GGCACGTTCCGATGTATTACTCA		
	Probe RSP-55 T	AGCTTGCTACCTGCCGG-NFQ-MGB	VIC	
egl	Rs-pan1-F5	CGCGAACGAGCTGTC		Vreeburg et al. (2018)
	Rs-pan1-R3	TCACGTTGCCGTARTAG		
	TP-Rsol-pan1-81	CGGGTTCGTCAACGCCGTGAC		
	TP-Rsol-pan1-81-alt2	CGGGTTTGTCAACGCCGTGAC	FAM	
Acat NBC430	Acat 2-F	TGTAGCGATCCTTCACAAG		Bonants et al. (2019)
	Acat 2-R	TGTCGATAGATGCTCACAAT		
	Acat 2-Pr	CTTGCTCTGCTTCTCTATCACG	TXR	

TABLE 3 Development of symptoms and colonization of plants after stem-inoculation of tomato (cv. Moneymaker) with three different strains of *Ralstonia pseudosolanacearum*

			Symptom development (%)			Density in stems	
Trial	Strain	No. of plants	Wilt	Epinasty	Internodium elongation	Average (log cfu/g)[a]	*SD*
2017, 20°C (20 dpi)	PD7123	5	80	20	0	n.d.	–
	PD2272	5	0	0	100	2.0	1.7
	PD3205	5	20	80	0	8.0	0.0
	Water control	3	0	0	0	0.0	–
2018, 28°C/23°C (20 dpi)	PD7123	5	100	0	0	5.6	0.5
	PD2272	5	0	0	0	5.2	0.4
	PD3205	5	60	0	0	5.2	0.4
	Water control	3	0	0	0	0.0	0.0
2019, 28°C/23°C (29 dpi)	PD7123	3	100	0	0	8.0	0.0
	PD2272	3	100	0	0	7.0	1.7
	PD3205	3	100	0	0	8.0	0.0
	Water control	3	0	0	0	0.7	1.6

Note: Strains: PD7123 from rose, PD2272 from curcuma and PD3205 from anthurium. Plants were grown in the glasshouse at 20°C (2017), or at 28°C/23°C (2018 and 2019). dpi, days postinoculation.

[a]Determined by dilution plating on the semiselective medium SMSA.

FIGURE 2 Symptoms of tomato plants inoculated with strains of *Ralstonia pseudosolanacearum* grown at 20°C. Strain PD7123 was isolated from rose, PD2272 from anthurium and PD3205 from curcuma.

epinasty, defined as a downward curving of leaves as a result of disturbances in their growth (Table 3; Figure 2). At 20 dpi, only low densities of PD2272 were found in the stems, whereas high densities of PD3205 were detected. No dilution plating was conducted for wilted plants inoculated with PD7123, as we assumed that they contained large bacterial populations. At a 28°C/23°C day/night regime, inoculation of tomato plants with PD7123 resulted in severe wilting of plants in both years. However, in 2018 the plants inoculated with PD2272 and PD3205 developed fewer symptoms than in 2019 (Table 3). Correspondingly, bacterial densities in the stems in 2018 were 100- to 1000-fold lower than in 2019. The AUDPC was analysed for each experiment and for all three experiments combined (Table S1). Data from combined experiments showed a significantly higher mean AUDPC for PD7123 than for PD2272 and PD3205 ($p < 0.05$).

3.2 | Infections and disease development at 20°C in rose (2017)

If rose plants were grown at 20°C, about half the plants of rose cvs Avalanche and Red Naomi showed mild symptoms (leaf

TABLE 4 Presence of *Ralstonia pseudosolanacearum* in rose plants of two rose cultivars grown at 20°C for 81 days after stem inoculation with a strain isolated from rose (PD7123), curcuma (PD2272) or anthurium (PD3205), as determined by dilution plating on the semiselective medium SMSA

		Stem base			Branches		
Cultivar	Inoculum	No. plants	No. positive	%	No. plants	No. positive	%
Avalanche	Control[a]	0	—	—	2	0	0
	PD7123	5	5	100	2	2	100[b]
	PD2272	5	2	40	2	0	0
	PD3205	5	5	100	2	0	0
Red Naomi	Control[a]	5	0	0	2	0	0
	PD7123	5	5	100	2	1	50[c]
	PD2272	5	2	40	2	0	0
	PD3205	5	5	100	2	0	0

[a]Plants stem inoculated with phosphate buffer.
[b]One out of two plants with weak symptoms (disease score 1).
[c]Plant with weak symptoms (disease score 1).

chlorosis resulting in drop of leaves) at 81 dpi with *R. pseudosolanacearum* PD7123 (a rose isolate), but no symptoms developed after inoculation with the curcuma (PD2272) and anthurium (PD3205) isolates (Table 4). Nevertheless, dilution plating on SMSA demonstrated the presence of the pathogen in the stem base near the inoculation point in all five plants inoculated with PD7123 and PD3205, but only in two out of five for plants inoculated with PD2272. Systemic colonization of the plants resulting in detectable bacteria in side branches was found only for some plants of both cultivars inoculated with PD7123.

After stem inoculation, strain PD7123 systemically colonized plants when grown at 28°C, irrespective of the cultivar, reaching high densities in the stem base and also frequently in the branches and shoots (Table 5). Stem inoculation with PD2272 resulted in systemic colonization only in 1 year and only for cv. Armando. Inoculation with PD3205 resulted in a systemic colonization of cv. Armando in 2 years and in cv. Avalanche in 2018, the only year this cultivar was included in the studies.

In general, results of the TaqMan assay were congruent with the plating results; all samples with a density exceeding 10^4 cfu/g were positive in the TaqMan assay ($C_t < 30$), but some samples with low densities also gave positive TaqMan results.

3.3 | Infections and disease development at 28°C in rose (2018 and 2019)

3.3.1 | Comparison of cultivars

With rose plants grown at 28°C/23°C (day/night regime), the first symptoms were already visible at 7–14 days after stem-inoculation with PD7123 (Figure 3). In 2018, all stem inoculated plants of cv. Armando showed symptoms at 14 dpi and symptoms gradually became more severe, resulting in the death of some of the plants by 42 dpi. For cv. Red Naomi, 80% of the plants showed symptoms at 42 dpi, but the severity remained more or less stable during the course of the experiment. In 2018 cv. Avalanche was least susceptible, with a maximum of only 40% of the plants showing weak symptoms. In 2019, after stem inoculation with PD7123, the disease severity in the three cultivars followed the same trends as in 2018, but the disease incidences for the cultivars Avalanche and Red Naomi were higher in 2019 than in 2018 and the disease severity in both cultivars was also remarkably higher.

The AUDPC was analysed for each of the two experiments and for data from both experiments combined (Table S2). In 2018, but not in 2019, a significant higher mean AUDPC value was found for cv. Armando than for the cvs Avalanche and Red Naomi ($p < 0.05$).

3.3.2 | Relative virulence of strains

We compared the virulence of *R. pseudosolanacearum* PD7123 (rose), PD2272 (curcuma) and PD3205 (anthurium) following stem-inoculation of rose cvs Avalanche (2018) and Armando (2018 and 2019) (Figure 4). Inoculation of cv. Avalanche with PD7123 and PD2272 resulted in slightly higher disease severities and incidences than PD3205. In both years, inoculation of cv. Armando with PD7123 led to rapid disease development, with all plants becoming severely diseased, and most plants were dead at 42 dpi. Inoculating roses with PD3205 resulted in intermediate levels of disease severity and incidence; about 50% of the plants showed progressing chlorosis at 42 dpi, but death of branches was never observed. Symptoms remained largely absent after stem inoculations of cv. Armando with PD2272.

The AUDPC for cv. Armando was analysed for each of the two experiments and for data from both experiments combined (Table S3). In both years, a significant higher mean AUDPC value was found for PD7123 than for the other two strains, whereas in 2018, the mean AUDPC of PD3205 was higher than of PD2272 ($p < 0.05$).

After stem inoculation, strain PD7123 always systemically colonized plants, reaching high densities in the stem base, and also

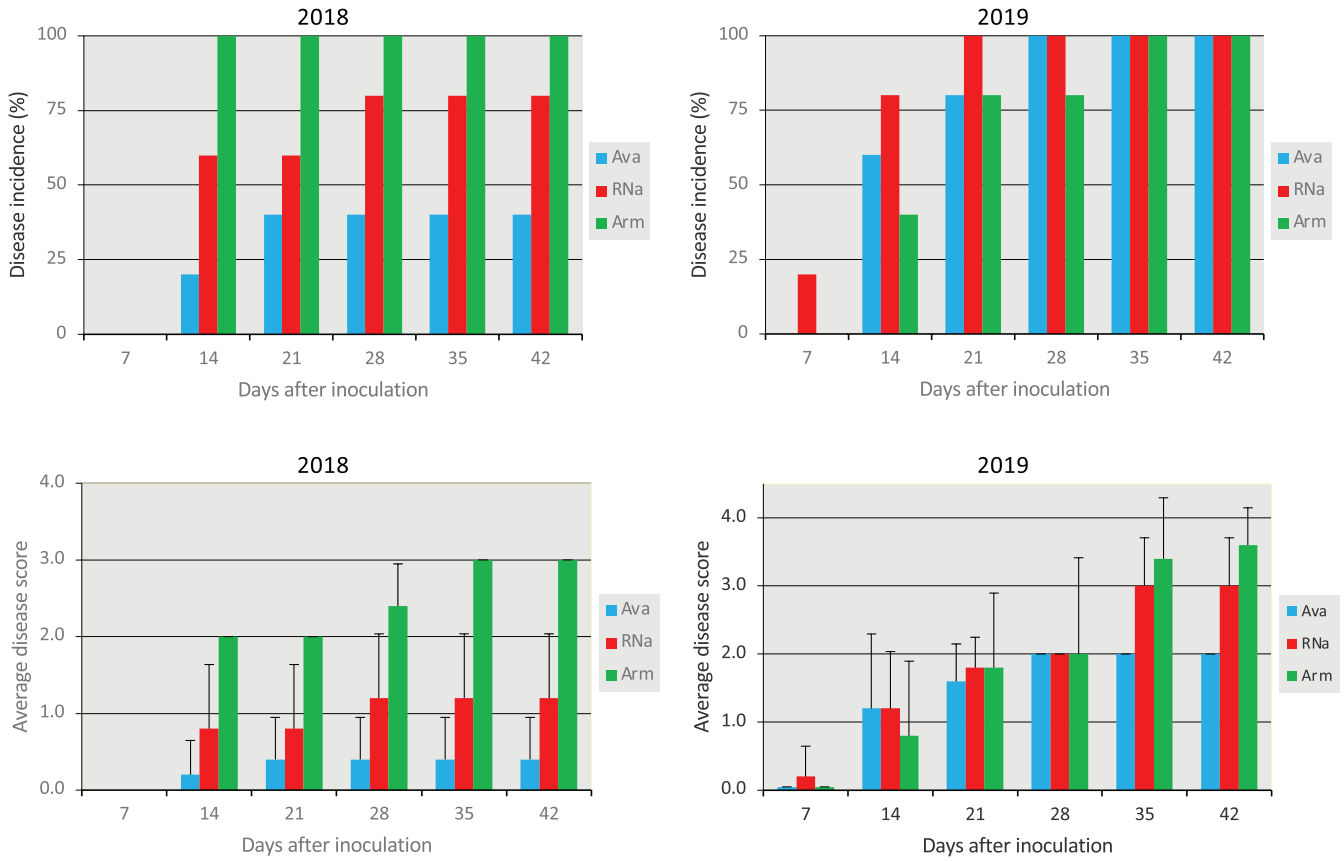

FIGURE 3 Disease incidences (upper figures) and disease severity scores (lower figures) of rose plants cultivars Avalanche (Ava), Red Naomi (RNa) and Armando (Arm) after stem inoculation with *Ralstonia pseudosolanacearum* PD7123 (rose strain) scored 7, 14, 21, 28, 35 and 42 days after inoculation. Plants were grown at 28/23°C day/night regime in the glasshouse in experiments conducted in 2018 and 2019. For the disease severity, standard error bars are provided to display variability. Calculations of the area under the disease progress curve for the disease severity, together with the analysis of variance, are found in Table S2.

frequently in the branches and shoots (Table 5). Systemic colonization of PD2272 and PD3205 was also detected, but the results varied by year; much higher densities were found in 2019 than in 2018.

3.3.3 | Root inoculations

In 2018, pouring PD7123 inoculum over the rockwool blocks in which plants of cvs Armando and Avalanche were grown caused few or no symptoms during the course of the experiment, not even at the highest inoculum density of 10^8 cfu/ml (Figure 5). However, under similar conditions in 2019, the first mild symptoms appeared after 3 weeks in cv. Armando at the 10^8 cfu/ml inoculum density and after 7 weeks all plants showed symptoms, although the disease scores remained rather low.

In 2018, after root inoculation, a low density of the pathogen was found at 49 dpi only in the stem base of one cv. Armando plant inoculated with 10^6 cfu/ml (results not shown). In addition, a single cv. Armando plant inoculated with 10^8 cfu/ml was infected with low densities in the roots (results not shown). In 2019 the experiment was repeated only with the most susceptible cultivar (Armando). Consistent with the observed disease incidence, the 2019 root inoculations resulted in an efficient and systemic colonization of every plant even at the lowest inoculum density of 10^4 cfu/ml, with stem base infection of each plant (Table 6). The AUDPC values for the three inoculum densities were not statistically different (Table S4). Unfortunately, the roots of mock-inoculated plants were also infected, probably due to cross-contamination, although the pathogen was not detected in the stem base.

3.3.4 | Pathogen spread

To study the dissemination of *R. pseudosolanacearum* from rose plants with symptoms and the risk of infecting neighbouring plants, we used three rockwool slabs each containing five rose plants of cv. Armando. The first plant in each slab was stem inoculated with rifampicin resistant *R. pseudosolanacearum* strain IPO4144 (PD7123-RIF1). Symptoms developed only in the stem-inoculated plants, resulting in plant death at 42 dpi (Table 7). A sensitive Bio-TaqMan assay detected the pathogen in the water sampled from the rockwool around the inoculated plants. In one of the three slabs, the plant directly neighbouring the plant with symptoms became systemically infected as evidenced by dilution plating of stem segment, and the roots of the next plant in the same slab were found to be infected.

TABLE 5 Relative virulence of three strains of *Ralstonia pseudosolanacearum* isolated from rose (PD7123), curcuma (PD2272) and anthurium (PD3205) by stem-inoculation of three different cultivars of rose with 10^8 cells/ml

					AUDPC[a]		Estimated cell density (log [cfu + 1]/g[b]						C_t value TaqMan assay[c]					
							Stem base		Branches		Shoots		Stem base		Branches		Shoots	
Treatment	Cultivar	Year	°C	*n*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Control	Avalanche	2017	20	5	0.0	0.0	n.d.[d]	–[d]	n.d.	–	n.d.	–	NT[d]	–	NT	–	NT	–
		2018	28/23	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.3	8.1	38.7	2.3	39.8	0.3
		2019	28/23	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NT	–	NT	–	NT	–
PD7123	Avalanche	2017	20	5	0.0	0.0	7.4	1.3	2.0[e]	0.0	n.d.	–	13.2	1.0	10.2	0.4	NT	–
		2018	28/23	5	12.6	14.5	8.0	0.0	1.6	3.6	1.3	2.5	19.2	1.5	33.2	5.8	30.8	5.6
		2019	28/23	5	41.2	0.3	7.2	0.4	7.8	0.4	8.0	0.0	21.6	1.1	21.6	0.9	22.8	1.3
PD2272	Avalanche	2017	20	5	0.0	0.0	1.4	1.3	0.0[e]	0.0	n.d.	–	32.6	5.1	n.d.	–	NT	–
		2018	28/23	5	7.2	9.9	2.4	0.5	0.0	0.0	0.0	0.0	27.9	0.4	32.8	4.2	39.7	0.8
PD3205	Avalanche	2017	20	5	0.0	0.0	8.0	0.0	0.0[e]	0.0	n.d.	–	19.6	1.6	27.4	2.7	NT	–
		2018	28/23	5	7.1	10.8	4.6	0.5	1.6	3.6	2.0	4.0	22.2	1.4	31.6	2.2	39.7	0.5
Control	Red Naomi	2017	20	5	0.0	0.0	0.0	0.0	0.0[e]	0.0	n.d.	–	35.0	1.8	n.d.	–	NT	–
		2018	28/23	3	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	–	38.3	2.9	38.3	1.6	NT	–
		2019	28/23	3	0.0	0.0	2.0	3.5	2.0	3.5	4.0	3.5	22.3[f]	–	22.3[f]	–	24.9	1.6
PD7123	Red Naomi	2017	20	5	0.0	0.0	8.0	0.0	4.0[e]	5.7	n.d.	–	9.1	1.5	24.6	6.8	NT	–
		2018	28/23	5	35.4	21.1	8.0	0.0	4.8	4.4	n.d.	–	17.7	3.5	24.5	8.2	NT	–
		2019	28/23	5	61.7	10.2	8.0	0.0	8.0	0.0	8.0	0.0	22.3	0.9	22.1	1.4	21.9	0.9
PD2272	Red Naomi	2017	20	5	0.0	0.0	1.8	2.0	0.0[e]	0.0	n.d.	–	32.6	5.1	n.d.	–	NT	–
PD3205	Red Naomi	2017	20	5	0.0	0.0	8.0	0.0	0.0[e]	0.0	n.d.	–	16.7	1.5	36.6	4.7	NT	–
Control	Armando	2018	28/23	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	38.3	2.0	n.d.	–	36.9	5.4
		2019	28/23	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NT	–	NT	–	NT	–
PD7123	Armando	2018	28/23	5	69.5	4.1	6.2	1.6	6.8	1.3	7.3	1.5	14.5	0.8	14.9	1.0	15.0	0.7
		2019	28/23	5	65.0	25.1	8.0	0.0	7.8	0.4	7.6	0.5	20.4	1.6	20.5	2.2	20.8	1.1
PD2272	Armando	2018	28/23	5	1.4	3.1	4.0	1.4	0.0	0.0	0.0	0.0	24.4	1.0	35.6	3.0	n.d.	–
		2019	28/23	5	4.3	6.5	7.2	0.4	8.0	0.0	6.4	3.6	23.5	4.4	26.0	4.9	22.9	2.0
PD3205	Armando	2018	28/23	5	27.5	23.2	8.0	0.0	2.6	3.7	5.5	1.7	19.5	1.2	28.1	6.2	27.3	3.5
		2019	28/23	5	29.4	19.1	8.0	0.0	8.0	0.0	8.0	0.0	25.2	3.4	28.6	2.0	27.6	2.8

Note: Glasshouse experiments were conducted in 3 years at 20°C or 28°C/23°C. The cell densities in stem base, branches and shoots, and C_t values of a TaqMan assay are shown at 42 days postinoculation (dpi).

[a]AUDPC calculated between 14 and 42 days.

[b]Counts after dilution plating on a modified SMSA medium.

[c]In 2017 the TaqMan assay described by Weller et al. (2000) was used. In 2018 and 2019 the Nytor TaqMan assay described by Vreeburg et al. (2018) was used.

[d]n.d., not detected ($C_t > 40$); NT, not tested; –, could not be calculated.

[e]Branches of only two plants tested.

[f]Only one plant was tested.

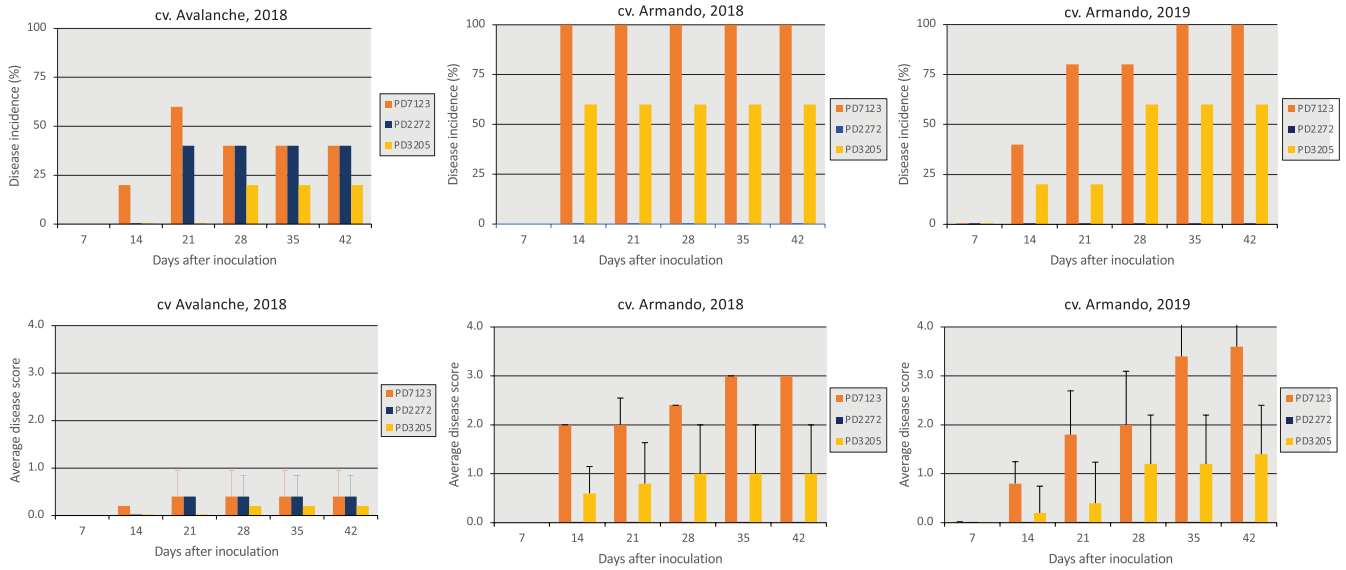

FIGURE 4 Disease incidences (upper figures) and disease severity scores (lower figures) for rose plants cv. Avalanche (2018) and cv. Armando (2018 and 2019) after stem inoculation with *Ralstonia pseudosolanacearum* PD7123 (rose strain), PD2272 (curcuma strain) and PD3205 (anthurium strain) at 7, 14, 21, 28, 35 and 42 days after inoculation. PD2272 did not show symptoms in cv. Armando. For the disease severity, standard error bars are provided to display variability. Calculations of the area under the disease progress curve for the disease severity, together with the analysis of variance, are found in Table S3.

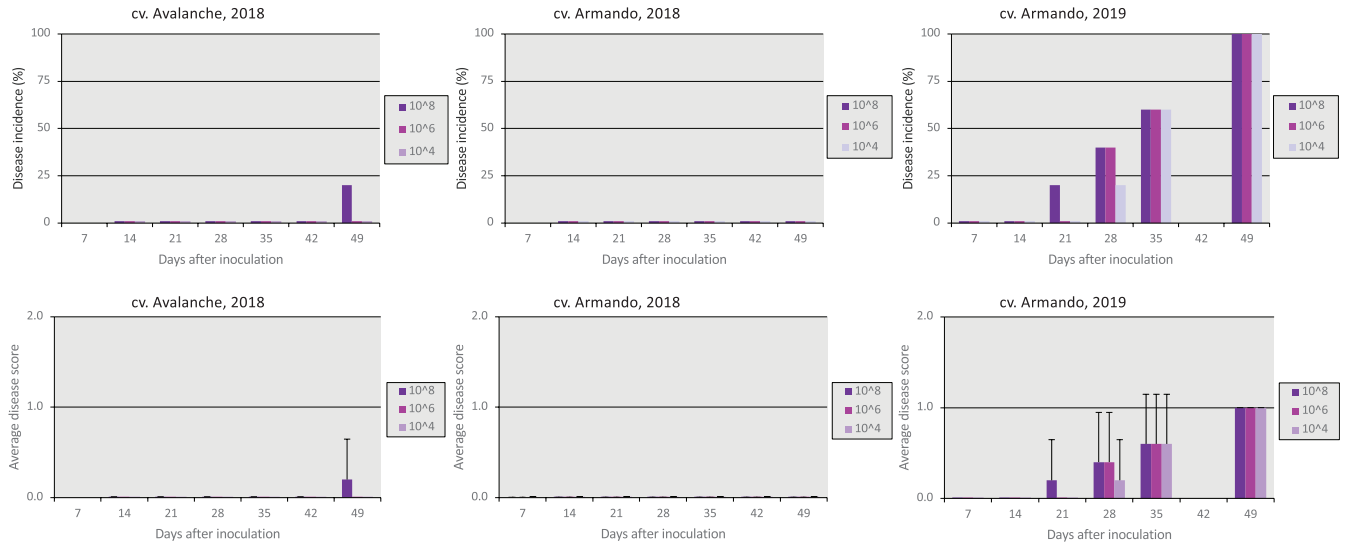

FIGURE 5 Disease incidences (upper figures) and disease severities (lower figures) for cultivars Avalanche and Armando of rose after root inoculation with *Ralstonia pseudosolanacearum* PD7123 (rose strain), in densities of 10^8, 10^6 or 10^4 cfu/ml during glasshouse experiments conducted in 2018 and 2019. For the disease severity, standard error bars are provided to display variability. Calculations of the area under the disease progress curve for the disease severity in 2019, together with the analysis of variance, are found in Table S4.

4 | DISCUSSION

This study demonstrates the influence of various factors, including cultivar, temperature, strain and inoculation method, on infection rate and disease expression of *R. pseudosolanacearum* in roses grown on rockwool, the substrate most commonly used in the Netherlands for rose cultivation (Hoog, 1998). The susceptibility of commercial rose cultivars to *R. pseudosolanacearum* varied greatly, consistent with a previous study that found cv. Armando more susceptible than cv. Red Naomi (Tjou-Tam-Sin et al., 2017). In our studies, stem inoculations of cv. Armando with an *R. pseudosolanacearum* strain from rose resulted in plant death under a 28/23°C day/night regime, while symptoms were moderate in cv. Red Naomi and very mild in cv. Avalanche. Nevertheless, all cultivars were systemically infected

TABLE 6 Infection levels and disease development after root inoculation of two rose cultivars with *Ralstonia pseudosolanacearum* PD7123 isolated from rose in glasshouse experiments conducted in 2018 and 2019 at 28°C/23°C

						Estimated cell density (log (cfu + 1)/g				C_t value in Nytor TaqMan			
				AUDPC[a]		Stem base		Roots		Stem base		Roots	
Treatment	Cultivar	Year	*n*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
0 (Control)	Avalanche	2018	3	0.0	0.0	0.0	0.0	0.0	0.0	n.d.[b]	–[b]	n.d.	–
	Armando	2018	3	0.0	0.0	0.0	0.0	0.0	0.0	37.4	4.6	n.d.	–
	Armando	2019	3	0.0	0.0	0.0	0.0	4.0	3.5	NT[b]	–	NT	–
10^8	Avalanche	2018	5	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	–	38.5	3.4
	Armando	2018	5	0.0	0.0	0.0	0.0	0.4	0.9	n.d.	–	35.4	3.2
	Armando	2019	5	18.1	11.0	6.0	0.0	6.0	0.0	NT	–	NT	–
10^6	Avalanche	2018	5	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	–	39.0	2.2
	Armando	2018	5	0.0	0.0	0.4	0.9	0.0	0.0	n.d.	–	n.d.	–
	Armando	2019	5	16.6	8.8	6.0	0.0	6.0	0.0	NT	–	NT	–
10^4	Avalanche	2018	5	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	–	39.1	1.9
	Armando	2018	5	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	–	38.8	2.6
	Armando	2019	5	15.2	7.6	6.0	0.0	6.0	0.0	NT	–	NT	–

Note: Three different densities of the pathogen were used. The cell densities in stem base, branches and shoots, and the C_t values of a TaqMan assay are shown at 42 days postinoculation).

[a]AUDPC calculated between 14 and 42 dpi.

[b]n.d., not detected (C_t > 40); NT, not tested, –, could not be calculated.

TABLE 7 Spread of *Ralstonia pseudosolanacearum* IPO4144 in rockwool slabs each with five plants of rose cultivar Armando

		Plant 1		Plant 2		Plant 3		Plant 4		Plant 5	
Slab	Sample	Plating[a]	TaqMan[b]	Plating	TaqMan	Plating	TaqMan	Plating	TaqMan	Plating	TaqMan
1	Stembase	***High***	***22.1***	0	NT	0	NT	0	NT	0	NT
	Roots	***High***	***28.0***	0	NT	0	NT	0	NT	0	NT
	Drainwater	NT	***22.1***	NT	***31.2***	NT	***34.2***	NT	39.8	NT	n.d.
2	Stembase	***High***	***27.2***	0	NT	0	NT	0	NT	0	NT
	Roots	***High***	***31.5***	0	NT	0	NT	0	NT	0	NT
	Drainwater	NT	***21.1***	NT	***30.9***	NT	***34.7***	NT	***32.0***	NT	35.0
3	Stembase	***High***	***30.9***	***High***	***28.2***	0	n.d.	0	NT	0	NT
	Roots	***High***	***32.5***	***High***	***28.6***	***Low***	***20.0***	0	NT	0	NT
	Drainwater	NT	***24.8***	NT	***30.6***	NT	***31.7***	NT	35.0	NT	***31.7***

Note: The first plant in each block was stem inoculated with the pathogen and developed symptoms resulting in plant death at 42 days postinoculation. None of the other plants developed symptoms, but using an enrichment TaqMan assay, dissemination of the pathogen in the drain water was found in the rockwool slabs up to the fifth plant in the row. Only in slab 3, infections in two plants (2 and 3), directly adjacent to the plant with symptoms, were found with plating on SMSA and with a Bio-TaqMan assay. Positive results are marked in bold and in italics. NT, not tested; n.d., no signal after 40 cycles.

[a]High: number of colonies on plates uncountable; low: number of colonies between 1 and 10.

[b]TaqMan values were considered positive at a C_t value <30, between 30 and 35 as suspected, and >35 as negative.

with the pathogen detected in stems and branches. Such symptomless or latent infections result in little or no direct crop losses, but they may contribute to unnoticed spread of the pathogen. Previous studies have demonstrated that RSSC strains are also easily disseminated from symptomless plants of geranium, potato and tomato (Pradhanang et al., 2000; Priou et al., 2001; Swanson et al., 2005).

We observed that temperature had a strong effect on *R. pseudosolanacearum* infection and wilt symptom development in rose as well as in tomato, as was previously found for tomato and tobacco (Bittner et al., 2016; Singh et al., 2014). A temperature dependency was previously reported for rose plants grown in potting soil, in particular following soil drench inoculation (Tjou-Tam-Sin et al., 2017).

At 20°C, the relatively low temperature prevailing during most of the year in commercial glasshouses for cutting roses in the Netherlands, hardly any symptoms were found in rose in the different studies. Nevertheless, the *R. pseudosolanacearum* rose isolate PD7123 did systemically colonize plants at 20°C. Wilting of tomato plants at 20°C was only observed after inoculation with the rose strain PD7123. The warmer temperature regime of 28°C/23°C strongly favoured disease development in rose, but for unknown reasons results varied considerably over the 2 years. In 2019, infections were more severe and disease development more pronounced than in 2018, in particular after root inoculation.

Strains of *R. pseudosolanacearum* isolated from various hosts differed in aggressiveness on both tomato and rose. The rose strain was most aggressive, the anthurium strain moderately aggressive, while the curcuma strain hardly caused symptoms. A previous study, conducted at 28°C, compared virulence of *R. pseudosolanacearum* isolates from *Casuarina equisetifolia* and *Pelargonium capitatum* and *R. solanacearum* strains from *Begonia* spp., *Solanum tuberosum* and *S. lycopersicum* (Tjou-Tam-Sin et al., 2017). That work found that rose plants harboured these strains from other host plants following stem inoculation. We found at 20°C, inoculating tomato plants with the anthurium strain caused only epinasty while the curcuma strain caused elongated internodes; only the rose strain wilted tomato. Differences in aggressiveness between strains in tomato have been reported previously, and symptoms varied from browning of the xylem and foliar epinasty to a full lethal generalized wilting (Buddenhagen & Kelman, 1964; Fraser et al., 2001). In 2018, even at high temperatures of 28°C/23°C, symptom development of particularly the curcuma strain in tomato was weak. Interestingly, the curcuma and anthurium isolates were aggressive on their hosts of origin, which allowed us to rule out the possibility that these strains lost virulence in storage (Stichting Control Food and Flowers, Bleiswijk, Netherlands, unpublished results). We conclude that not all *R. pseudosolanacearum* strains cause symptoms in tomato, which is used as a universal host in the EU protocol for RSSC diagnostics (Anonymous, 2018). A certain level of host specificity has been documented among RSSC phylotypes and even among strains within phylotype (Bocsanczy et al., 2022; Cho et al., 2018; Genin, 2010; Kumar et al., 2014). For example, *R. syzygii* (phylotype IV) strains isolated from potato were nonvirulent in tomato (Cho et al., 2018). Nevertheless, nonvirulent or hypovirulent strains may still cause systemic infections in tomato plants resulting in high population densities in plant tissues, thereby allowing reisolation to establish the identity of the pathogen.

Samples yielding relatively high pathogen densities, exceeding 10^4 cfu/g, all tested positive in the TaqMan assay, with low C_t values. However, we found only a weak correlation between dilution plating and TaqMan results for samples with low bacterial densities in the dilution plating assay. This could be explained by the presence of dead bacteria in samples.

We showed that an aggressive rifampicin-resistant mutant (IPO40144) of the rose strain PD7123 of *R. pseudosolanacearum* can spread in rockwool slabs from plants with severe symptoms to neighbouring plants via movement in the nutrient solution or via root contacts. Plant roots have previously been identified as a reservoir of *R. solanacearum* (Álvarez et al., 2010). However, we found infection of the two plants closest to the plant with symptoms occurred in 7–8 weeks, resulting in a systemic infection of only one plant in one of three slabs. In our studies, excess nutrient water was drained via crevices cut at the sides of the slabs. Adjacent plants may be more frequently infected if contaminated nutrient water can pass through all plants in a slab. The low infection incidence during the dissemination experiments may also be explained by decreased susceptibility of plants due to ageing or to shifts in the microbiome, as was found for tomato plants (Kwak et al., 2018; Winstead & Kelman, 1952). Nevertheless, the relatively low prevalence of infected plants growing in rockwool slabs near plants with symptoms indicates that this dissemination pathway is not the most relevant in the epidemiology. We speculate that rose plants become predominantly infected via the use of contaminated cutting knives.

Sampling material from symptomless rose plants may not be a reliable method to detect this pathogen because the incidence may be low and the distribution of the pathogen in the plants uneven, as was found for *R. solanacearum* in geranium (Swanson et al., 2005). Bacteria released from the roots of plants growing in rockwool into the nutrient solution will ultimately be collected in a drain well. It should be determined if nutrient solution sampled from the drain well can be used for testing purposes. Recently, a testing method for RSSC based on concentrating bacteria via filtration of drain water, enrichment for RSSC by incubation of concentrated bacterial cells in a semiselective broth, followed by a TaqMan assay, allowed the detection of one target cell per ml (Sedighian et al., 2020).

In conclusion, *R. pseudosolanacearum* infection rates and bacterial wilt disease development in rose depends on host and pathogen genetics as well as environmental variables. There is a high probability of symptomless *R. pseudosolanacearum* infections that go unnoticed in rose plants. Factors that increase the risks for symptomless infections are a low temperature, infections with strains hypovirulent for rose, a low susceptibility for the pathogen and root infections via a nutrient solution. Knowledge of the symptomology of RSSC in rose will be helpful to detect symptoms at an early stage.

ACKNOWLEDGEMENTS

The authors are grateful to Tjou-Tam-Sin and Maria Bergsma of the NVWA (Wageningen, Netherlands) for providing strains and for valuable advice on the preparation of SMSA. This research was funded by the Dutch Ministry of Economic Affairs in the Topsector Programme 'Horticulture and Starting Materials' via the project 'Prevention of *Ralstonia solanacearum* outbreaks in the Dutch agri- and horticulture' (grant 1605-082), and by the private partners Glastuinbouw Nederland, LTO Nederland, Plantum, Naktuinbouw, NAK, Gewascoöperatie Roos and Gewascoöperatie Gerbera.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest. This study does not contain studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jan van der Wolf https://orcid.org/0000-0002-9196-5419

REFERENCES

Álvarez, B., Biosca, E.G. & López, M.M. (2010) On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. In: Mendez-Vilas, A. (Ed.) *Current research, technology and education topics in applied microbiology and microbial biotechnology*, Vol. *1*. Badajoz: Formatex, pp. 267–279.

Anonymous (2016) October 2016 Pest Report – the Netherlands, closing note. Finding of *Ralstonia solanacearum*, (race 1) in ornamental *Anthurium* plants for cut flower production. Netherlands Food and Consumer Product Safety Authority, Dutch Ministry of Economic Affairs. Available at: https://assets.ippc.int/static/media/files/pestreport/2015/10/26/Pest_report_August_2015_-_Finding_of_Ralstonia_solanacearum_race_1_in_ornamental_Anthurium_plants_for_cut_flower_production.pdf; https://english.nvwa.nl/documents/plant/plant-healtdh/pest-reporting/documents [Accessed 30 May 2022].

Anonymous. (2018) PM 7/21 (2) *Ralstonia solanacearum*, *R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex). *EPPO Bulletin*, *48*, 32–63.

Bergsma-Vlami, M., Van De Bilt, J., Tjou-Tam-Sin, N., Westenberg, M., Meekes, E.T.M., Teunissen, H.A.S. et al. (2018) Phylogenetic assignment of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* phylotype I) Isolated from *Rosa* spp. *Plant Disease*, *102*, 2258–2267.

Bittner, R., Arellano, C. & Mila, A. (2016) Effect of temperature and resistance of tobacco cultivars to the progression of bacterial wilt, caused by *Ralstonia solanacearum*. *Plant and Soil*, *408*, 299–310.

Bocsanczy, A.M., Bonants, P., Van der Wolf, J., Bergsma-Vlami, M. & Norman, D.J. (2022) Identification of candidate type 3 effectors that determine host specificity associated with emerging *Ralstonia pseudosolanacearum* strains. *European Journal of Plant Pathology*, *163*, 35–50.

Bonants, P., Griekspoor, Y., Houwers, I., Krijger, M., van der Zouwen, P., van der Lee, T.A. et al. (2019) Development and evaluation of a triplex TaqMan assay and next-generation sequence analysis for improved detection of *Xylella* in plant material. *Plant Disease*, *103*, 645–655.

Buddenhagen, I. & Kelman, A. (1964) Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology*, *2*, 203–230.

Cho, H., Song, E.-S., Lee, Y.K., Lee, S., Lee, S.-W., Jo, A. et al. (2018) Analysis of genetic and pathogenic diversity of *Ralstonia solanacearum* causing potato bacterial wilt in Korea. *The Plant Pathology Journal*, *34*, 23–34.

Elphinstone, J.G., Hennessy, J., Wilson, J.K. & Stead, D.E. (1996) Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *EPPO Bulletin*, *26*, 663–678.

Fortnum, B. & Gooden, D. (2008) Transmission and survival of *Ralstonia solanacearum* on tobacco machinery. *Beiträge zur Tabakforschung International*, *23*, 137–143.

Fraser, R., Hosein, F., Phelps, R. & Vincent-Sealy, L. (2001) The determination of the genetic diversity and relative aggressiveness among ten isolates of *Ralstonia solanacearum* in Trinidad: results from a pilot study. In: Boer, S.H. (Ed.) *Plant pathogenic bacteria*. Dordrecht: Springer, pp. 151–155.

Genin, S. (2010) Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist*, *187*, 920–928.

Hoog, J. (1998) *Teelt van kasrozen*. Aalsmeer: Proefstation voor Bloemisterij en Glasgroente, p. 218.

Janse, J.D., Van Den Beld, H., Elphinstone, J., Simpkins, S., Tjou-Tam-Sin, N. & Van Vaerenbergh, J. (2004) Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale* cuttings. *Journal of Plant Pathology*, *86*, 147–155.

Kim, Y., Lim, S., Kim, J.-W., Lee, H.-J. & Park, D. (2019) First report of *Ralstonia solanacearum* phylotype I causing bacterial wilt on *Rosa* spp. in Korea. *Plant Disease*, *103*, 1407.

Kumar, A., Prameela, T., Suseelabhai, R., Siljo, A., Anandaraj, M. & Vinatzer, B. (2014) Host specificity and genetic diversity of race 4 strains of *Ralstonia solanacearum*. *Plant Pathology*, *63*, 1138–1148.

Kwak, M.J., Kong, H.G., Choi, K., Kwon, S.-K., Song, J.Y., Lee, J. et al. (2018) Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology*, *36*, 1100–1109.

McCarter, S. & Jaworski, C. (1969) Field studies on spread of *Pseudomonas solanacearum* and tobacco mosaic virus in tomato plants by clipping. *Plant Disease Reporter*, *53*, 942–946.

Milling, A., Meng, F., Denny, T.P. & Allen, C. (2009) Interactions with hosts at cool temperatures, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* race 3 biovar 2. *Phytopathology*, *99*, 1127–1134.

Norman, D.J., Zapata, M., Gabriel, D.W., Duan, Y.P., Yuen, J.M.F., Mangravita-Novo, A. et al. (2009) Genetic diversity and host range variation of *Ralstonia solanacearum* strains entering North America. *Phytopathology*, *99*, 1070–1077.

Pradhanang, P.M., Elphinstone, J.G. & Fox, R.T.V. (2000) Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathology*, *49*, 403–413.

Priou, S., Salas, C., Mendiburu, F.D., Aley, P. & Gutarra, L. (2001) Assessment of latent infection frequency in progeny tubers of advanced potato clones resistant to bacterial wilt: a new selection criterion. *Potato Research*, *44*, 359–373.

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L. & Kappler, U. (2014) Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *64*, 3087–3103.

Sedighian, N., Mendes, O., Poleij, L., Bonants, P. & Van Der Wolf, J.M. (2020) Detection of *Ralstonia pseudosolanacearum* in drain water based on concentration, enrichment and the use of a duplex TaqMan assay test. *EPPO Bulletin*, *50*, 340–349.

Shaner, G. & Finney, R. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology*, *67*, 1051–1056.

Singh, D., Yadav, D., Sinha, S. & Choudhary, G. (2014) Effect of temperature, cultivars, injury of root and inoculums load of *Ralstonia solanacearum* to cause bacterial wilt of tomato. *Archives of Phytopathology and Plant Protection*, *47*, 1574–1583.

Sukroongreung, S., Schappert, K. & Khachatourians, G. (1984) Survey of sensitivity of twelve yeast genera toward T-2 toxin. *Applied and Environmental Microbiology*, *48*, 416–419.

Swanson, J.K., Yao, J., Tans-Kersten, J. & Allen, C. (2005) Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. *Phytopathology*, *95*, 136–143.

Tjou-Tam-Sin, N.N., Van De Bilt, J.L., Westenberg, M., Gorkink-Smits, P.P., Landman, N.M. & Bergsma-Vlami, M. (2017) Assessing the pathogenic ability of *Ralstonia pseudosolanacearum* (*Ralstonia*

solanacearum phylotype I) from ornamental *Rosa* spp. plants. *Frontiers in Plant Science*, *8*, 1895.

Tjou-Tam-Sin, N.N.A., Van De Bilt, J.L.J., Westenberg, M., Bergsma-Vlami, M., Korpershoek, H.J., Vermunt, A.M.W. et al. (2016) First report of bacterial wilt caused by *Ralstonia solanacearum* in ornamental *Rosa* sp. *Plant Disease*, *101*, 378.

Vreeburg, R., Bergsma-Vlami, M., Bollema, R., de Haan, E.G., Kooman-Gersmann, M., Smits-Mastebroek, L. et al. (2016) Performance of real-time PCR and immunofluorescence for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* in potato tubers in routine testing. *EPPO Bulletin*, *46*, 112–121.

Vreeburg, R.A.M., Zendman, A.J.W., Pol, A., Verheij, E., Nas, M. & Kooman-Gersmann, M. (2018) Validation of four real-time TaqMan PCRs for the detection of *Ralstonia solanacearum* and/or *Ralstonia pseudosolanacearum* and/or *Clavibacter michiganensis* subsp. *sepedonicus* in potato tubers using a statistical regression approach. *EPPO Bulletin*, *48*, 86–96.

Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N. & Stead, D.E. (2000) Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied and Environmental Microbiology*, *66*, 2853–2858.

Williamson, L., Nakaho, K., Hudelson, B. & Allen, C. (2002) *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. *Plant Disease*, *86*, 987–991.

Winstead, N. & Kelman, A. (1952) Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. *Phytopathology*, *42*, 628–634.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: van der Wolf, J., Kastelein, P., Poleij, L., Krijger, M., Mendes, O., Sedighian, N., Allen, C., Bonants, P. & Kurm, V. (2022). Factors influencing *Ralstonia pseudosolanacearum* infection incidence and disease development in rose plants. *Plant Pathology*, *00*, 1–14. https://doi.org/10.1111/ppa.13596