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Induction of systemic resistance by *Pseudomonas fluorescens* in radish cultivars differing in susceptibility to fusarium wilt, using a novel bioassay

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Abstract

Pseudomonas fluorescens-mediated induction of systemic resistance in radish against fusarium wilt (*Fusarium oxysporum* f. sp. *raphani*) was studied in a newly developed bioassay using a rockwool system. In this bioassay the pathogen and bacterium were confirmed to be confined to spatially separate locations on the plant root, throughout the experiment. Pathogen inoculum obtained by mixing peat with microconidia and subsequent incubation for four days at 22 °C, yielded a better percentage of diseased plants than a microconidial suspension drench, an injection of a microconidial suspension into the hypocotyl, or a talcum inoculum. *Pseudomonas fluorescens* strain WCS374 applied in talcum or peat, but not as a suspension drench, induced systemic resistance. A minimal initial bacterial inoculum density of $\geq 10^5$ CFU WCS374 root^{-1} was required to significantly reduce the percentage diseased plants. At least one day was necessary between bacterization of strain WCS374 in talcum on the root tips and inoculation of the pathogen in peat on the root base, for an optimal induction of systemic resistance. Strain WCS374 induced systemic resistance in six radish cultivars differing in their susceptibility to *F. oxysporum* f. sp. *raphani*. Significant suppression of disease by bacterial treatments was generally observed when disease incidence in the control treatment, depending on pathogen inoculum density, ranged between approximately 40 to 80%. Strains WCS374 and WCS417 of *Pseudomonas fluorescens* induced systemic resistance against fusarium wilt, whereas *P. putida* WCS358 did not. This suggests that the induction of systemic resistance by *Pseudomonas* spp. is dependent on strain-specific traits.

Abbreviations: CFU = colony forming units; IFC = immunofluorescence colony-staining; ISR = induced systemic resistance; PBS = phosphate buffered saline; SAR = systemic acquired resistance.

Introduction

Infection of plants with a necrosis-causing pathogen or by treatment with an abiotic agent (e.g. isonicotinic acid or salicylic acid) enhances plant resistance to subsequent challenge infections of a variety of viral, bacterial, and fungal pathogens [Kuć, 1982; Sequeira, 1983; White, 1979]. This phenomenon of induction of disease resistance provides systemic protection in the plant, and is referred to as systemic acquired resistance (SAR) [Ross, 1961]. SAR has been demonstrated in a large variety of plant-pathogen systems [Sequeira, 1983]. Development of SAR correlates with the expression of a set of genes including those which encode for pathogenesis-related proteins [Linthorst, 1991; Ward *et al.*, 1991].

In studies on fluorescent *Pseudomonas* spp.-mediated disease suppression, induction of systemic disease resistance is a relatively new topic [Tuzun and Kloepper, 1994]. For this induction of resistance, the term induced systemic resistance (ISR) [Kloepper *et al.*, 1992] is commonly used. In this concept, ISR

involves the activation of the plant's defense mechanisms, which lead to a systemic protection. Whether ISR and SAR have the same characteristics is not yet clear. Van Peer *et al.* [1991] presented evidence for ISR in plants by a pseudomonad plant growth-promoting rhizobacterium against a fungal soil-borne plant pathogen. In their bioassay, *Pseudomonas fluorescens* WCS417 suppressed wilt disease in carnation caused by *Fusarium oxysporum* f. sp. *dianthi*, when the bacterium was inoculated onto the roots one week prior to stem-inoculation with the pathogen. Wei *et al.* [1991] demonstrated ISR in cucumber against *Colletotrichum orbiculare* by pseudomonad plant growth-promoting rhizobacteria. Alström [1991] showed that bacterization of bean seeds with *P. fluorescens* S97 resulted in ISR to halo blight caused by *Pseudomonas syringae* pv. *phaseolicola*. Recently, *Pseudomonas* spp.-mediated ISR was also observed against viral pathogens [Lui *et al.*, 1992; Maurhofer *et al.*, 1994]. In all of these studies, the pathogen and the resistance inducing pseudomonad were applied at spatially separated locations on the plant, excluding direct antibiosis and competition as mechanisms of disease suppression.

Bacterization of seed tubers of potato with *P. putida* WCS358 increased potato tuber yield [Bakker *et al.*, 1986; Geels and Schippers, 1983b] and root development [Bakker *et al.*, 1987] in high frequency cropping of potato, and suppressed fusarium wilt of radish, caused by *Fusarium oxysporum* f. sp. *raphani* [Raaijmakers, 1994]. Seed bacterization of radish and of seed tubers of potato with *P. fluorescens* WCS374 resulted in significant plant growth-promotion in high-frequency radish- and potato cropping soil, respectively [Geels and Schippers, 1983b; Geels *et al.*, 1985]. In these studies on strains WCS358 and WCS374, pseudobactin-mediated iron deprivation of deleterious microorganisms or of *F. oxysporum* f. sp. *raphani* was considered the mode of action for the observed growth-promotion. Strain WCS374 also significantly suppressed fusarium wilt of radish in commercial greenhouse trails [Leeman *et al.*, 1991b, 1995]. *P. fluorescens* WCS417 suppressed *Gaeumannomyces graminis* var. *tritici* in field trials [Lamers *et al.*, 1988], induced systemic resistance in carnation against fusarium wilt [Duijff *et al.*, 1993; Van Peer *et al.*, 1991], suppressed this disease in carnation by competition for iron [Duijff *et al.*, 1993; Van Peer *et al.*, 1990], and increased growth of tomato by internal colonization of the roots, displacing deleterious indigenous endophytic pseudomonads [Van Peer and Schippers, 1989].

The objective of this investigation was to study whether ISR is involved in the suppression of fusarium wilt of radish by *Pseudomonas* spp. A rockwool cube bioassay was developed with special attention for (1) the delivery system for pathogen and bacterial inoculum, (2) the remaining of the pathogen and bacterium at spatially separate locations on the plant root, (3) the bacterial inoculum density, (4) the required time interval between bacterization and pathogen inoculation, and (5) the influence of the level of resistance of radish cultivars on disease suppression.

Materials and methods

Radish cultivars

Six radish (*Raphanus sativus* L.) cultivars (seed size 2.50–2.75 mm) differing in their susceptibility to fusarium wilt were used: Hilo (susceptible, Nunhems Zaden BV, Haelen, The Netherlands), Marabelle (susceptible, Nickerson-Zwaan BV, Barendrecht, The Netherlands), and Fanal, Fuego, Saxa*Nova and Stellar (moderately resistant, S&G Seeds BV, Enkhuizen, The Netherlands).

Microbial cultures and inocula

The wilt pathogen of radish, *Fusarium oxysporum* Schlecht. f. sp. *raphani* Kendrick & Snyder (formerly called *Fusarium oxysporum* Schlecht. f. sp. *conglutinans* [(Wollenw.) Synder & Hansen] race 2 Armstrong & Armstrong), was isolated from an infected radish using Komada's agar [Komada, 1975], modified by Gams and Van Laar [1982]. This isolate of *F. oxysporum* f. sp. *raphani* is coded as strain WCS600 in our culture collection. The fungal culture was maintained on modified Komada's agar. The pathogen was cultured in aerated 2% malt extract (Difco) (7 days, 22 °C).

Pseudomonas fluorescens WCS374 and *P. putida* WCS358 were originally isolated from the rhizosphere of potato [Geels and Schippers, 1983a, b]. *P. fluorescens* WCS417 was isolated from the rhizosphere of wheat grown in a field suppressive to take-all, caused by *G. graminis* var. *tritici* [Lamers *et al.*, 1988]. For the bioassays, bacterial inocula were prepared by scraping overnight cultures (27 °C) from King's B agar [King *et al.*, 1954] plates in 0.01 *M* $MgSO_4$.

Growth of plants

Plants were maintained in the greenhouse at temperatures of 22 °C at night, 24–33 °C during the day, at a relative humidity of approximately 70% and supplemented with Son-t lighting for 16 h day^{-1}. Plants were watered twice a week and once a week they received modified half strength Hoagland's [Hoagland and Arnon, 1938] nutrient solution (5 m*M* $Ca(NO_3)_2$, 2 m*M* KNO_3, 1 m*M* KH_2PO_4, 1 m*M* $MgSO_4$, and trace elements, pH 7) containing 10 μM Fe-EDDHA (Sequestreen, 5% ferric iron of which 80% is bound as Fe-ethylenediamine di(o-hydroxyphenylacetic acid), Ciba-Geigy).

ISR-rockwool bioassay

In the ISR-rockwool bioassay, radish seeds were sown in sand and after 5 days transferred to MM40/40S rockwool growth cubes (Rockwool/Grodan BV, Roermond, The Netherlands). Each bioassay element consisted of two polythene bags containing four rockwool cubes each (8×8×4 cm). The bags were placed opposite each other and at the touching sides the bags were vertically incised at three places. The roots of three radish seedlings were placed horizontally on top of two opposing cubes and through the vertical incisions in the bags (Fig. 1). The roots were covered by rockwool cubes to prevent dehydration and exposure to light. The same day (t = 0), approximately 1.5 cm of the root tips were inoculated with a *Pseudomonas* inoculum (carriers: peat, talcum or suspension, see *Delivery systems for the bacterial inoculum*). The disease control treatment consisted of 0.01 *M* $MgSO_4$ or the uninoculated carrier mixed with 0.01 *M* $MgSO_4$ (1:1 w/v). Two days later (t = 2 days), unless indicated otherwise, approximately 1.5 cm of the roots at the base were inoculated with the pathogen in peat (0.2 g $root^{-1} \approx 2 \times 10^5$ CFU $root^{-1}$, see *Delivery systems for the pathogen inoculum*). In the experiments on the influence of the level of resistance of radish cultivars on ISR, the pathogen inoculum density in the peat was adjusted with sterile peat to 2×10^3, 2×10^4, or 2×10^5 CFU $root^{-1}$, prior to inoculation.

In this ISR-rockwool bioassay the bacteria on the root tips and the pathogen on the root base, were at spatially separate locations on the root system. Plants were watered cautiously to prevent splashing of the microorganisms from cube to cube. Three weeks (t = 23 days) after inoculation of the roots with the pathogen, the plants were harvested and the percentage of plants with fusarium wilt symptoms (browning and/or blackening of the xylem tissue in root and radish, and yellowing/browning of leaves, which turn brown and brittle) was recorded per replicate. Per experiment, each treatment was replicated twelve times (3 plants $replicate^{-1}$). The experiments were repeated at least two times.

Delivery systems for the pathogen inoculum

Several delivery systems for the pathogen inoculum were studied. Therefore, radish seeds were sown in sand and after 7 days transferred to MM40/40S rockwool growth cubes (Rockwool/Grodan BV). A washed microconidial suspension (10^7 CFU mL^{-1}, counted microscopically) was (1) injected into the hypocotyls of radish seedlings (10^4 CFU $plant^{-1}$), (2) applied as a suspension drench (5 mL $root^{-1} \approx 2.5 \times 10^5$ CFU mL^{-1} radish nutrient solution), or (3) mixed with sterile talcum (1:1 v/w) (Merck) or gamma sterilized peat (1:10 v/w, 50% final moisture) (Agrifutur s.r.l., Alfianello, Italy). The talcum inoculum was dried overnight in a Laminar Flow Cabinet, whereas the peat inoculum was incubated for four days at 22 °C. The talcum and peat inocula were stored at 6 °C. The number of CFU in the dried talcum and in the incubated peat was determined before use, by dilution plating on modified Komada's agar. Plant roots were inoculated with the pathogen in talcum (0.5 g talcum $root^{-1} \approx 2 \times 10^5$ CFU $root^{-1}$) or in peat (0.2 g peat $root^{-1} \approx 2 \times 10^5$ CFU $root^{-1}$).

Delivery systems for the bacterial inoculum

The bacterial suspensions (10^{10} CFU mL^{-1}) from overnight cultures were used in the ISR-rockwool bioassay as a drench (5 mL $root^{-1} \approx 2.5 \times 10^8$ CFU mL^{-1} radish nutrient solution), or mixed with sterile talcum (1:1 v/w, final density approximately 10^8 CFU g^{-1} talcum, 1 g talcum $root^{-1} \approx 10^8$ CFU $root^{-1}$, unless indicated otherwise) for direct use in bioassay, or mixed with sterile peat (1:10 v/w, 50% final moisture) which was incubated overnight at 27 °C (final density 10^9 CFU g^{-1} peat, 0.2 g peat $root^{-1} \approx 2 \times 10^8$ CFU $root^{-1}$). The number of CFU in the inocula was determined by dilution plating on King's B agar.

In the ISR-rockwool bioassay studying the initial bacterial inoculum density required for suppressing the disease, the bacterial inoculum density (ranging from 10^4 to 10^9 CFU $root^{-1}$) in the talcum was adjusted with sterile talcum, prior to inoculation. In all other ISR-rockwool bioassays, the talcum inoculum (1 g talcum

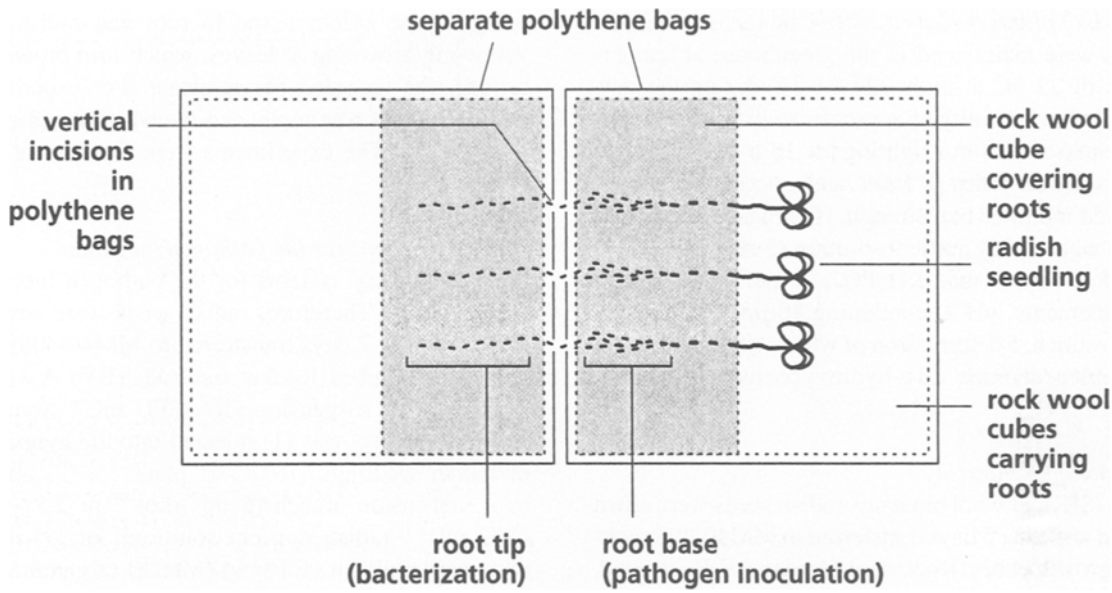

Fig. 1. Diagram of the induced systemic resistance (ISR)-rockwool bioassay (top view).

root$^{-1} \approx 10^8$ CFU root^{-1}) was used for delivery of the bacteria.

The possible role of already excreted metabolites was studied in the ISR-rockwool bioassay by washing the bacterial suspension twice in 0.01 *M* $MgSO_4$, before mixing it with talcum.

Root colonization

To study whether the bacterium and pathogen remained at spatially separate locations on the root in the ISR-rockwool bioassay, the root base (zone of pathogen inoculation) and the root tips (zone of bacterization) of cv. Saxa*Nova were checked for colonization by the pathogen and the introduced strain, just before inoculating the pathogen (t = 2 days), seven days afterwards (t = 9 days) and at harvest time (t = 23 days).

Root colonization by the pathogen was determined by dilution plating of root macerates (0.3 g root fresh weight as a sample of the entire root portion, ground in 5 mL 0.01 *M* $MgSO_4$ with pestle and mortar) on modified Komada's agar. The detection limit for *F. oxysporum* was 5.6×10^2 CFU g^{-1} root fresh weight.

Colonization by the introduced strain was determined in the same root macerates, by using immunofluorescence colony-staining (IFC) [Van Vuurde and Roozen, 1990], as modified by Leeman *et al.* [1991a]. The IFC method is based on mixing a root washing or tissue macerate (100 μL) containing bacteria through warm (40 °C) agar (400 μL) in 24 well macrowell plates (Greiner). For the detection of fluorescent pseudomonads, King's B agar supplemented with ampicillin (50 ppm), chloramphenicol (13 ppm) and cycloheximide (100 ppm) was used. After overnight incubation (27 °C), the agar containing macro-colonies of the introduced strain and other pseudomonads, or aerobic bacteria, was dried into a thin film, and afterwards consecutively rinsed with 500 μL 2 m*M* $FeCl_3$ (20 min), and 3 times (in total 45 min) 10 m*M* PBS (pH 7.2) containing 1.5 m*M* NaN_3. The agar containing the pseudomonads was incubated overnight with highly specific polyclonal antibodies conjugated with fluorescein-isothiocyanate (FITC) in PBS-NaN_3. After incubation with the antiserum, all colonies are stained orange with 500 μL 10 μ*M* ethidium bromide (15 min), followed by rinsing with PBS-NaN_3. In the target *Pseudomonas* sp. colonies the orange colour is exceeded by the bright fluorescent green of the bound antibody-FITC complex, which is made visible under a fluorescence microscope (Zeiss Axioskop 20) with incident blue light (exciter filter 490 nm, barrier filter 510 nm). The detection limit for the introduced *Pseudomonas* strain was 1.3×10^2 CFU g^{-1} root fresh weight.

Colonization of the root by the bacteria was routinely checked only at harvest time. Root parts (0.3 g root fresh weight) were shaken vigorously (30 sec) in 5 mL 0.01 *M* $MgSO_4$ containing 0.5 g of glass

beads (0.17 mm), followed by IFC on these root washings.

Data analysis

Data were analyzed for significance after arcsine square root (percentage diseased plants per replicate) or logarithmic (root colonization per replicate) transformations using analysis of variance followed by Fisher's least-significant-difference test ($\alpha = 0.05$), using SAS-software (SAS Institute, Cary, NC, USA). Repeated experiments demonstrated similar significant differences between treatments.

Results

Delivery systems for the pathogen inoculum

A percentage of 70–80% *Fusarium* wilted radish plants (cv. Saxa*Nova) in the rockwool system was obtained when peat inoculated with microconidia of the pathogen was applied on the roots (Fig. 2). When microconidia were delivered in talcum on the roots, significantly less plants were infected. No or very few diseased plants were observed when a suspension of microconidia was poured directly over the roots. Injection of microconidia into the stem of radish seedlings did not result in any disease symptoms. Plants treated with sterile peat, talcum or 0.01 *M* $MgSO_4$ showed no disease symptoms. Application of the pathogen in peat on the roots growing on rockwool was supposed to be most suitable for studying ISR and used throughout this study.

Delivery systems for the bacterial inoculum and ISR

Strain WCS374 induced resistance when delivered in talcum or peat onto the roots growing on rockwool cubes compared with the non-bacterized carrier, and the pathogen was applied in peat two days after the bacterium (Fig. 3A). Bacterization by pouring a cell suspension over the roots in the rockwool cubes did not result in a significant reduction of disease. The percentage of diseased plants in the peat control was somewhat smaller (not significant) compared with both suspension control (0.01 *M* $MgSO_4$) and the talcum control. The root colonization by WCS374 at harvest time did not differ among the three delivery systems (talcum: 1.9×10^6, peat: 2.3×10^6, and suspension: 1.2×10^6 CFU g^{-1} root fresh weight). Bacterial cells of WCS374 washed twice before mixing with the talcum did not differ from unwashed cells in inducing resistance in radish (Fig. 3B). Application of the bacterium in talcum on the roots growing on rockwool, was most suitable for studying ISR and used throughout this study.

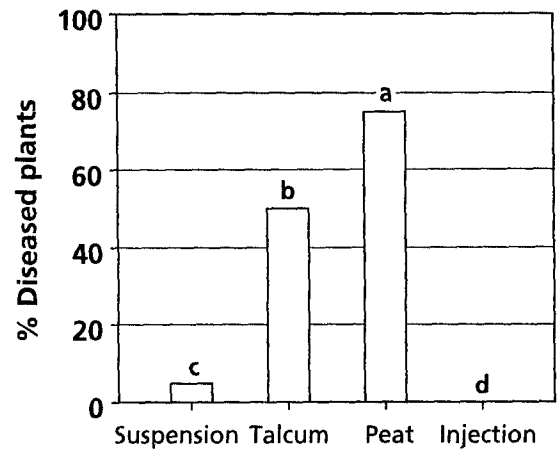

Fig. 2. Percentage of *Fusarium* wilted radish plants (cv. Saxa*Nova) in a rockwool system where the pathogen was applied on the roots of seedlings as a suspension drench (2.5×10^5 CFU mL^{-1} radish nutrient solution, in talcum (2×10^5 CFU $root^{-1}$), or in peat (2×10^5 CFU $root^{-1}$), or by injecting a suspension of microconidia into the hypocotyl of the seedlings (10^4 CFU $plant^{-1}$). (Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least-significant-difference test.)

Bacterial inoculum density and ISR

At initial inoculum densities of WCS374 in the talcum ranging from 10^5 to 10^9 CFU $root^{-1}$, resistance was induced, leading to the same level of disease (Fig. 3C). At a density of 10^4 CFU $root^{-1}$ there was no significant reduction of disease. Root colonization by WCS374 ranged from 1.3×10^6 to 2.5×10^6 CFU g^{-1} root fresh weight at harvest time.

Time interval between bacterization and pathogen inoculation required for ISR

For a significant induction of resistance by WCS374, an interval of at least one day between bacterization of the root tips and inoculation of the pathogen on the root base was required (Fig. 3D). However, simultaneous inoculation of both organisms did show a tendency of disease reduction. At a four day interval, the percentage of disease plants in the control treatment was significantly smaller compared with control plants inoculated at day 0, 1 or 2, and significant reduction of the percentage diseased plants by WCS374 was no longer observed.

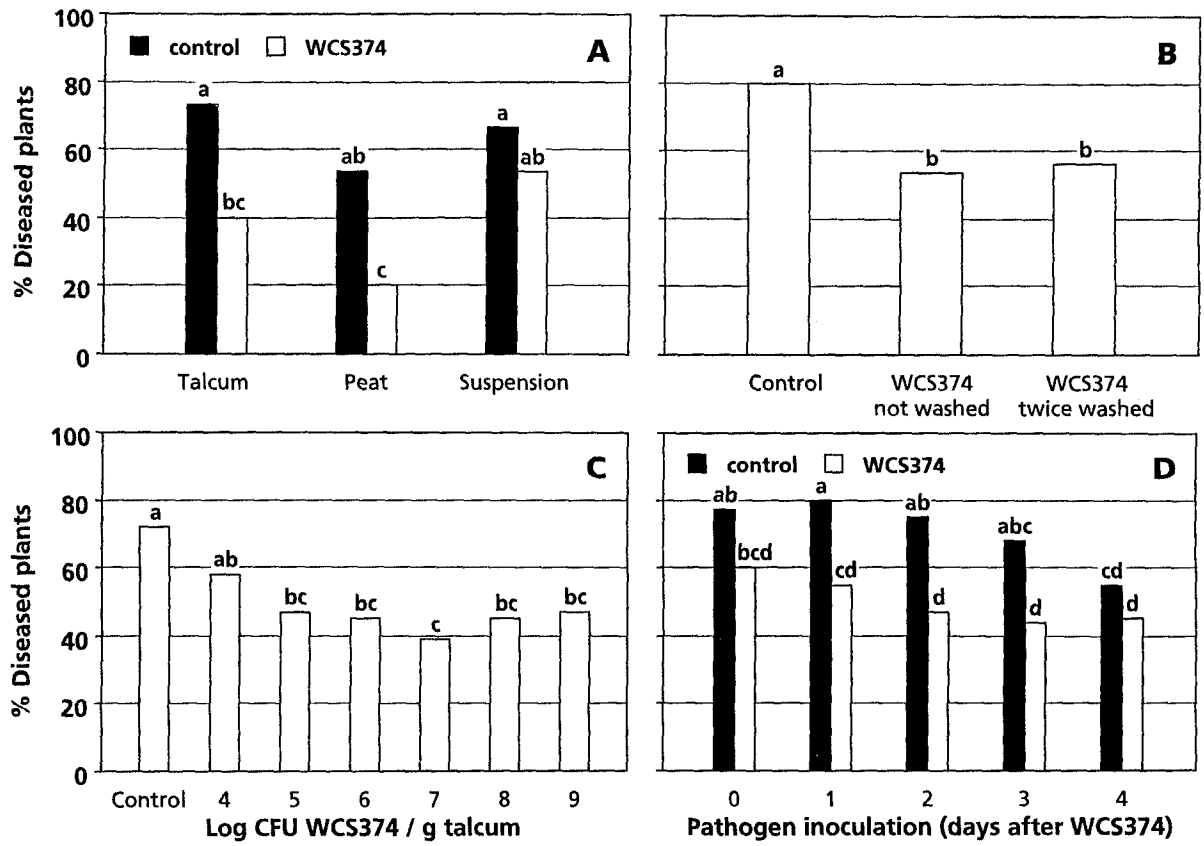

Fig. 3. Percentage of *Fusarium* wilted radish plants (cv. Saxa*Nova) in the ISR-rockwool bioassay where seedlings were treated on the root tips with: (A) talcum or peat with 0.01 *M* $MgSO_4$ or with WCS374 (10^8 or 2×10^8 CFU $root^{-1}$, respectively), or 5 mL 0.01 *M* $MgSO_4$ $root^{-1}$ without bacteria or with WCS374 (2.5×10^8 CFU mL^{-1} radish nutrient solution), (B) talcum with 0.01 *M* $MgSO_4$, with unwashed or two times washed cells of WCS374 (10^8 CFU $root^{-1}$), (C) talcum with 0.01 *M* $MgSO_4$ or with WCS374 (10^4, 10^5, 10^6, 10^7, 10^8, or 10^9 CFU $root^{-1}$), or (D) talcum with 0.01 *M* $MgSO_4$ or with WCS374 (10^8 CFU $root^{-1}$). The pathogen was inoculated on the root base in peat (2×10^5 CFU $root^{-1}$): (A, B, C) two days after bacterization, or (D) 0, 1, 2, 3, or 4 days after bacterization. (Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least-significant-difference test.)

ISR in radish cultivars differing in susceptibility to fusarium wilt

Induced resistance mediated disease suppression by strain WCS374 was obtained in all the six radish cultivars differing in susceptibility to fusarium wilt, depending on the *Fusarium* inoculum pressure (Fig. 4). Significant disease suppression by bacterial treatments were generally observed when disease incidence in the non-bacterized control treatment ranged between approximately 40 to 80%.

ISR by different Pseudomonas *spp.*

Resistance was induced by strains WCS374 and WCS417, but not by WCS358 (Fig. 5). At harvest time, no significant differences in root colonization were observed among the three introduced strains (1.6×10^6 to 3.7×10^6 CFU g^{-1} root fresh weight).

Root colonization

Root colonization by strain WCS374 delivered in talcum, as checked in macerates of the root tips, amounted to 1.6×10^8 CFU g^{-1} root fresh weight at the beginning of the experiment (t = 2 days), and decreased to 2.5×10^7 CFU g^{-1} at t = 9 days and 7.0×10^6 CFU g^{-1} at harvest time (t = 23 days). Throughout the experiment the population density of the pathogen on the root base approximated to 4.4×10^5 CFU g^{-1} root. Strain WCS374 delivered in talcum and the pathogen in peat remained at spatially separate locations on the root throughout the experiment. Contamination of the root base and root tips in the opposite rockwool cubes, with the bacterium or the wilt pathogen, respectively, was never detected. For strains WCS358 and WCS417 similar results were obtained (data not shown).

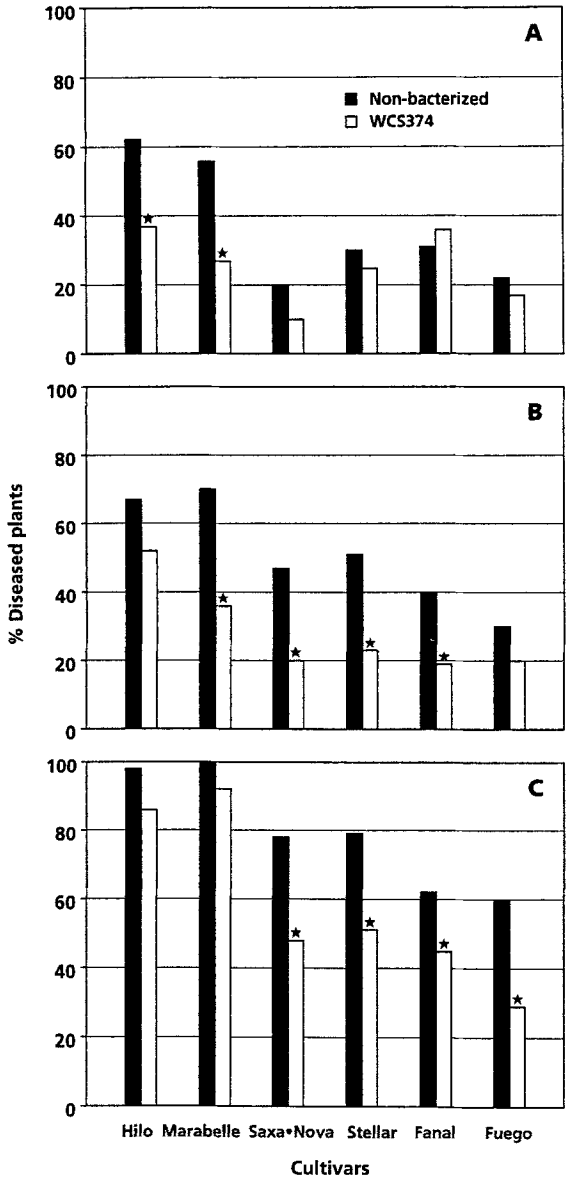

Fig. 4. Percentage of *Fusarium* wilted radish plants of six cultivars differing in their disease susceptibility in the ISR-rockwool bioassay where seedlings were treated on the root tips with talcum with 0.01 *M* $MgSO_4$ or with WCS374 (10^8 CFU $root^{-1}$), while the pathogen was inoculated on the root base in peat at (A) a low (2×10^3 CFU $root^{-1}$), (B) a moderate (2×10^4 CFU $root^{-1}$), or (C) a high (2×10^5 CFU $root^{-1}$) inoculum density. (Per inoculum density, bars with an asterisk are significantly different from the corresponding non-bacterized control at $P \leq 0.05$, analysis of variance.)

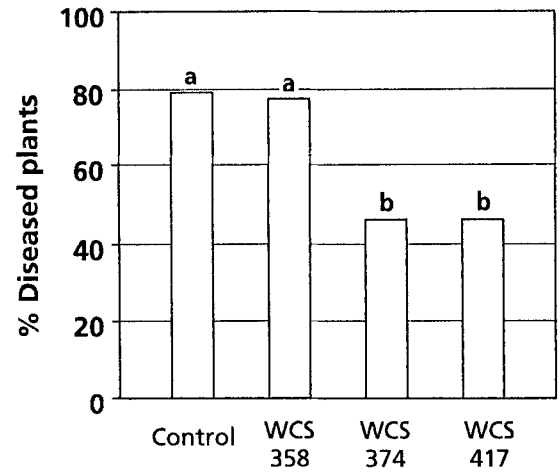

Fig. 5. Percentage of *Fusarium* wilted radish plants (cv. Saxa* Nova)in the ISR-rockwool bioassay where seedlings were treated on the root tips with talcum with 0.01 *M* $MgSO_4$ or with WCS358, WCS374, or WCS417 (10^8 CFU $root^{-1}$), while the pathogen was inoculated on the root base in peat (2×10^5 CFU $root^{-1}$) two days after bacterization. (Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least-significant-difference test.)

Discussion

ISR in radish against fusarium wilt (*Fusarium oxysporum* f. sp. *raphani*) was obtained by strains WCS374 and WCS417 of *Pseudomonas fluorescens* in a bioassay using a rockwool system, where the pathogen and bacterium are inoculated at spatially separate locations on the plant root. The fusarium wilt pathogen and introduced strains remained at spatially separate locations on the root system of radish, throughout the experiments. Because their populations could not be detected in root macerates of the uninoculated root parts, relocation of these microorganisms, either through the root interior or over the root surface, can be excluded. Relocation might have resulted in direct interaction of the introduced bacterium and the pathogen. Antibiosis and competition between the two organisms therefore can be excluded and the observed disease suppression can best be explained by induction of systemic resistance against the fusarium wilt disease.

Inoculation of the radish root with the fusarium wilt pathogen in peat resulted in a level of disease, suitable to study induction of systemic resistance with this ISR-rockwool bioassay. The disease development, after inoculation with a microconidial suspension drench or after injection of the suspension into the hypocotyl, was remarkably small. This is surprising, since both methods of inoculation were successfully used in studying *Pseudomonas*-mediated ISR against fusarium wilt of carnation [Van Peer *et al.*, 1991; Duijff *et al.*, 1993]. The three week cultivation period

of radish in the ISR-rockwool bioassay, compared with twelve weeks for carnation, may be too short for a successful infection starting from microconidial suspensions. Moreover, resistance for the disease in the control plants appeared to increase while the radish plants aged, starting around 9 days after sowing.

Strain WCS374, when delivered in talcum or peat on the roots, was effective in inducing systemic resistance. The percentage of diseased plants was not reduced by WCS374 when delivered as a suspension drench. The absence of a significant induction of systemic resistance by the suspension drench, may be due to too much dispersal of the introduced strain WCS374. Immediately after delivery of the suspension drench, the inoculum is soaked into the rockwool cubes instead of being localized on the roots. The peat or talcum inoculum is delivered precisely onto the part of the root where it remains localized throughout the experiment.

Sterile peat applied on the root tips did not induce resistance but the disease was somewhat lower (non-significant), compared with both 0.01 *M* $MgSO_4$ or talcum. Therefore, talcum was chosen to be used as a carrier in the ISR-rockwool bioassay. Washing of the bacterial suspension had no effect on the induction of systemic resistance. ISR, apparently, was not mediated by microbial products released by WCS374 during its culturing on agar. At harvest time, WCS374 colonized the roots to the same extend, whether the strain was applied in talcum, peat or as a suspension. However, the timing of a certain minimum number of active cells on a part of the root may be of importance for a successful induction of resistance. The effective induction of systemic resistance by WCS374 applied in talcum or peat, compared with the ineffectiveness of the suspension drench, may also be explained by hypothesizing that the solid carriers have a beneficial effect on the plant, the bacterium/plant interaction or on the activity of the bacteria leading to ISR.

The WCS374-mediated ISR was independent of the initial bacterial inoculum density, when the number of cells delivered in talcum was $\geq 10^5$ CFU $root^{-1}$. This is best explained by assuming that triggering of the disease resistance in the plant is probably an on/off principle with a certain threshold.

Resistance could be induced by *P. fluorescens* WCS374 in all six radish cultivars tested, differing in susceptibility to fusarium wilt. The detection of suppression of disease depended on the *Fusarium* inoculum density applied, and was generally significant at inoculum densities which resulted in approximately 40 to 80% diseased plants in the non-bacterized control treatment. Van Peer *et al.* [1991] also demonstrated ISR against fusarium wilt of carnation in a moderately resistant cv. Pallas, and a susceptible cv. Lena, by *P. fluorescens* WCS417. However, disease suppression in their experiments was consistent with the moderately resistant cultivar and less consistent with the susceptible cultivar. It may well be, however, that in most of their experiments with carnation, inoculum density, and thus disease incidence was too high in cv. Lena to demonstrate ISR. Induction of systemic resistance by particular pseudomonad strains thus seems to contribute to the suppression of fusarium wilt in cultivars ranging from susceptible to resistant.

Of the strains tested in this study, strains WCS374 and WCS417 of *Pseudomonas fluorescens* induced systemic resistance against fusarium wilt, whereas *P. putida* WCS358 did not. Similarly, strain WCS417 significantly induced systemic resistance against fusarium wilt of carnation, whereas WCS358 did not [Duijff *et al.*, 1993; Van Peer and Schippers, 1992]. This suggests that *Pseudomonas* spp.-mediated ISR is dependent on strain specific traits.

Whether the observed ISR in this model is different from SAR is still not clear. Hoffland *et al.* [1995] demonstrated that ISR in the fusarium wilt of radish system is not associated with accumulation of pathogenesis-related proteins. This distinguishes ISR from SAR. Another difference between ISR and SAR could be the observation that *P. fluorescens* WCS374-mediated ISR was already detectable after a short induction interval of one day, compared with a minimum induction period of commonly 1–2 weeks for SAR to tobacco necrosis virus in tobacco [Dean and Kuć, 1985; Kuć, 1983]. On the other hand, the minimum induction period in the fusarium wilt of radish model may in fact be longer. After the inoculation of the pathogen, it may take some time before the pathogen actually penetrates the root. Until more data are available on the plant's biochemical responses associated with ISR, the interrelationship between this phenomenon and SAR remains unclear. Research on fluorescent *Pseudomonas*-mediated ISR in radish now is focused on the bacterial traits involved in the induction of disease resistance, and the biochemical responses of the plant.

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