Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins

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The non-pathogenic *Pseudomonas fluorescens* strain WCS417r has been shown to induce systemic resistance in radish against *Fusarium oxysporum* f.sp. *raphani*. In this paper we investigate the involvement of pathogenesis-related (PR) proteins in this *Pseudomonas*-induced resistance. For comparison, salicylic acid (SA) and isonicotinic acid (INA) were used as inducers. It appeared that in our rock wool artificial soil system both strain WCS417r and SA could induce resistance, whereas strain WCS417r-B4, a lipopolysaccharide-mutant, and INA could not. The induced resistance was expressed as a reduction in the percentage of diseased plants, but not in the disease severity. Western blot analyses revealed that in the control plants PR-2 is constitutively present in the intercellular fluid of leaves and PR-3 in the roots. Treatment with either of the inducing agents, with or without pathogen-inoculation, induced no further accumulation of cross-reacting homologues of PR-1–5. We concluded that in our system induction of systemic resistance by strain WCS417r and SA is not associated with accumulation of PRs proteins. This demonstrates that accumulation of PR proteins is not a prerequisite for the expression of induced systemic resistance.

INTRODUCTION

Systemic resistance of plants against pathogens can be induced by a variety of biotic and chemical agents. Ross [*21*] introduced the term systemic acquired resistance (SAR) to describe induction of resistance in tobacco against a range of viruses by prior inoculation with the necrosis-causing tobacco mosaic virus. Subsequently, induction of systemic resistance by plant pathogens has been described in over 100 host–pathogen systems (for review, see [*23*]). Although this has not been generally tested, SAR seems to be aspecific in terms of the range of pathogens against which the plant is protected [*7*]. It is often assumed that the inducing organism should cause necrosis in order to induce SAR [*31*], although Roberts [*20*] demonstrated that this is not a prerequisite.

Chemical agents that do not cause any visible symptoms have also been shown to induce resistance. White [*32*] was able to induce resistance in tobacco against tobacco mosaic virus by injecting acetylsalicylic acid into the leaves. Métraux *et al.* [*16*] found that foliar or drench application of isonicotinic acid (INA) induces systemic resistance in cucumber against *Colletotrichum lagenarium*. Since then, resistance induced by salicylic acid (SA) and INA was demonstrated in a variety of plant–pathogen

Abbreviations used in text; ICF, intercellular fluid; INA, 2,6-dichloro-isonicotinic acid; LPS, lipopolysaccharide; PDA, potato dextrose agar; PR, pathogenesis-related; SA, salicylic acid; SAR, systemic acquired resistance.

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combinations (for review, see [*13*] and [*25*]). In the majority of cases the term SAR is also used, thus broadening the basic concept in which the inducing agent is a pathogen.

In all cases of SAR induced by necrotizing pathogens or chemical agents described so far, a concomitant accumulation of pathogenesis-related (PR) proteins was observed. However, the relationship between SAR and PR proteins may not always be direct [*34, 35*]. Currently, five families of PR proteins, numbered 1–5, have been classified (for review, see [*12*]). Within each family, members of the so-called class I are generally localized in the vacuole, whereas class II proteins occur extracellularly. Proteins of the PR-2 family have β-1,3-endoglucanase activity, and those of the PR-3 family have endochitinase activity. Evidence is accumulating that PR proteins possess direct antifungal activity *in vitro* [*14, 19, 22, 30*]. However, evidence on their biological role *in vivo* is scarce. High-level constitutive expression of the PR-1 gene in transgenic tobacco plants reduces disease severity caused by *Peronospora tabacina* and *Phytophthora parasitica* [*1*]. Broglie *et al.* [*4*] reported that transgenic tobacco plants, constitutively accumulating high levels of class I chitinase from bean, were more resistant to *Rhizoctonia solani*. Benhamou *et al.* [*3*] established increased chitin breakdown from the *Rhizoctonia* cell wall in transgenic canola plants accumulating the same protein. Van den Elzen *et al.* [*26*] found that tomato plants constitutively expressing both class I β-1,3-glucanase and class I chitinase genes exhibit enhanced resistance against *Fusarium oxysporum* f.sp. *lycopersici*. Plants expressing either gene alone are not significantly protected. These results suggest that the accumulation of PR proteins is causally related to the onset of SAR. However, definite evidence for the role of PR proteins in SAR is still lacking.

In addition to necrosis-inducing pathogens and selected chemicals, non-pathogenic biotic agents have also been shown to induce resistance. Reports are available on induction of systemic resistance by plant growth promoting root colonizing *Pseudomonas* spp. in several plant–pathogen combinations [*2, 28, 32*]. In this context, the term induced systemic resistance [*6*] is commonly used. It is assumed that the plants already possess some level of resistance against the pathogen involved and that the inducing agent activates this resistance. Nothing has been reported yet on the range of pathogens against which this induced systemic resistance is operative in the plant species investigated. Neither is information available on the possible involvement of PR proteins in this induced systemic resistance. Maurhofer *et al.* [*15*] reported that resistance induced by the root-colonizing *Pseudomonas fluorescens* strain CHA0 in tobacco against the spread of tobacco necrosis virus in the leaves is correlated with induction of PR-1 proteins, β-1,3-glucanases and endochitinases. However, plants with bacteria showed stress symptoms, suggesting that strain CHA0 is pathogenic.

In this paper we address the question whether PR proteins may be involved in systemic resistance induced by the non-pathogenic *P. fluorescens* strain WCS417r against *F. oxysporum* f.sp. *raphani* in radish (*Raphanus sativus* L.). Using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis we investigated whether strain WCS417r induces PR proteins. Known inducers of PR proteins (*Pseudomonas syringae* pv. tomato [*25*]) and resistance (INA and SA) were used for comparison. As a negative control we used *P. fluorescens* strain WCS417r-B4, which lacks the O-antigenic side chain of the surface lipopolysaccharide (LPS) and is unable to induce resistance [*10*].

MATERIALS AND METHODS

Growth of plants

Radish plants (*Raphanus sativus* L. cv. Saxa*Nova, moderately resistant to *Fusarium oxysporum* f.sp. *raphani*) were grown in a rock wool system as described by Leeman *et al.* [*11*], allowing for spatial separation of an induction treatment and a challenge inoculation of the same root system. All rock wool cubes were drenched in a nutrient solution (2 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$ and trace elements). After germination on quartz sand, seedlings were laid horizontally on two cubes, which were individually enclosed in plastic bags. Roots were positioned through an incision in the bags, so that the lower part contacted one cube, and the upper part the other. Subsequently, both parts were covered with small cubes to prevent dehydration. Plants were grown in a greenhouse providing 70% RH, and a photoperiod of 16 h supplemented with SON-T lamps. The day/night temperature regime was 24 °C/22 °C. Nutrient solution was supplied once a week.

Growth of microorganisms

Rifampicin-resistant *Pseudomonas fluorescens* strain WCS417r [*9*] and its mutant B4, which lacks the O-antigenic side chain of the LPS [*10*], were used. As a necrotizing pathogen and inducer of PR proteins *Pseudomonas syringae* pv. tomato DC3000 (obtained from Dr A. J. Slusarenko, Institute Plant Pathology, Zürich, Switzerland) was employed. After growth for 24–48 h at 27 °C on King's medium B agar plates [*5*], the bacterial cells were suspended in 0·01 M $MgSO_4$ and adjusted to a concentration of 10^9 cells ml^{-1}.

Fusarium oxysporum Schlecht. f.sp. *raphani* Kendric and Snyder (formerly *F. oxysporum* Schlecht.:Fr. f.sp. *conglutinans* [(Wollenw.) Snyder & Hansen] race 2 Armstrong & Armstrong) strain WCS600 was maintained on potato dextrose agar (PDA). A conidial suspension ($\pm 10^6$ conidia ml^{-1} 0·01 M $MgSO_4$) was prepared after growth of the fungus on liquid malt (2%) medium for 2 weeks. The suspension was added to sterilized peat (0·1 ml g^{-1}) and the fungus was allowed to germinate and grow into the peat for 2 days at 22 °C. The number of cfu reached was determined by plating a peat suspension diluted in 0·01 M $MgSO_4$ on PDA. The peat was mixed with quartz sand to obtain an inoculum density of 10^5 cfu of *F. oxysporum* g^{-1}.

Induced resistance assay

Subsequent to the transfer of the radish seedlings to the rock wool cubes, the induction treatment was carried out by covering the lower part of the roots with approximately 0·5 g of talcum suspension. This suspension consisted of a mixture (1:1, w/v) of talcum (Merck) and either 0·01 M $MgSO_4$ (control treatment), a bacterial suspension, or a solution of SA (2 mM) or INA (Ciba-Geigy AG, Basel, Switzerland; 104 μM, formulated as 25% active ingredient with a wettable powder carrier) in 0·01 M $MgSO_4$. SA and INA solutions were adjusted to pH 6.

Three days later the plants were treated by placing $\pm 0{\cdot}2$ g per plant of the peat/sand mixture alone (control) or containing *F. oxysporum*, on the upper part of the roots.

For each treatment eight replicates of six plants were used. The percentage of

diseased plants per replicate was determined about 20 days after challenge inoculation. The plants were inspected for external (wilting) and internal (browning of the vascular tissue) symptoms, the latter after cutting the main root and the bulb longitudinally. All plants showing symptoms, either external or internal, were scored as diseased. To express the severity of the disease, four classes were distinguished, depending on the part of the plant to which the symptoms extended. Treatment effects were established after analysis of variance and a subsequent t test (Fisher's least significant difference test, $\alpha = 0{\cdot}05$). Two independent experiments were performed.

To check for root colonization by the introduced *P. fluorescens* strains, root material was collected and washed to remove talcum or peat. Three samples of $\pm 0{\cdot}3$ g root material from the upper part as well as from the lower part were taken per treatment. The samples were shaken vigorously for 30 s in 5 ml of 0·01 M $MgSO_4$ containing glass beads. Serial dilutions were plated on King's medium B supplemented with rifampicin (150 mg l^{-1}), cycloheximide (100 mg l^{-1}), ampicillin (40 mg l^{-1}) and chloramphenicol (13 mg l^{-1}).

Inoculation with P. syringae

Radish seeds were sown in potting soil and plants were grown under the same conditions as described above. Four weeks after sowing, the two lowest true leaves were each infiltrated with 15 µl of a suspension of *P. syringae* using a syringe without needle. Twelve days after inoculation the inoculated and younger leaves were harvested.

Protein extraction and western blot analyses

Intercellular fluid (ICF) of leaves was collected by vacuum infiltration with a phosphate buffer (20 mM, pH 7·0) followed by centrifugation (1000 ***g*** for 10 min). Proteins in the ICF from the *P. syringae*-inoculated leaves were precipitated overnight with 80% acetone at −20 °C.

Leaf proteins soluble at low pH were isolated by extracting frozen plant material in a citrate/phosphate buffer (0·05 M citric acid, adjusted with 0·1 M Na_2HPO_4 to pH 3·0; 2 ml g^{-1} plant fresh wt), centrifugation (30000 ***g*** for 30 min) and subsequent dialysis of the supernatant against ultra pure water. Protein concentrations were determined using the BCA method [*24*].

About 50 µg of protein per sample from the induced resistance assay was loaded onto a SDS-PAGE gel. The gels were stained with Coomassie brilliant blue R250. From samples from *P. syringae* inoculated plants 5 µg was loaded. Electrophoresis was carried out according to Laemmli [*8*] in a Bio-Rad Mini-protean II system, using 15% separating gels.

For immunodetection, the proteins were electroblotted onto a nitrocellulose membrane (0·2 µm) in a 25 mM Tris, 192 mM glycine, 20% (v/v) methanol transfer buffer for 60 min at 100 V. The blots were blocked in TBS/Tween (10 mM Tris, 20 mM NaCl, 0·5% Tween 20, pH 8·0) containing 5% defatted milk powder, and incubated with primary antibodies diluted with TBS/Tween containing 1% BSA. Antigens were visualized after incubation with goat anti-rabbit IgG conjugated to alkaline phosphate (Bio-Rad) and with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium as substrate, respectively.

Specific antisera against tobacco PR-1a, b and c and PR-2a and b proteins [*27*] were

used as heterologous antisera for detection of PR-1 and -2 homologues, respectively, in radish. Antibodies against tobacco PR-3 (class I and II) and PR-5 (acidic thaumatin-like) proteins were a kind gift from Dr B. Fritig (CNRS Strasbourg, France). Dr M. H. A. J. Joosten (Wageningen Agricultural University, The Netherlands) provided us with antiserum against a basic PR-4 protein, accumulating in the ICF of *Cladosporium fulvum*-infected tomato.

RESULTS

Plants were grown in a rock wool system ensuring spatial separation of the resistance-inducing agent and the pathogen. For induction, control solution, *P. fluorescens* strain WCS417r, its LPS mutant B4, SA or INA were applied to the lower part of the root system, while *F. oxysporum* f.sp. *raphani* was inoculated 3 days later on the upper part. Controls were mock inoculated as described in Materials and Methods. At the end of both experiments, no rifampicin-resistant *Pseudomonas* colonies could be detected on the upper part of the root systems (detection limit: 4×10^2 cfu g^{-1} root fresh wt), indicating that the bacteria remained spatially separated from the pathogen throughout the experiment. Thus, any effect of bacteria on disease development cannot be ascribed to a direct antagonistic effect of the bacterium on the pathogen, but must be plant mediated.

Three weeks after inoculation with *F. oxysporum* clear symptoms were visible, ranging from yellowing of the leaves and reduced growth, to complete wilting of the shoot and, in the most severe cases, death of the plant. In less severely infected plants, only browning of the vascular tissue in the main root and bulb caused by phenol oxidation as a reaction to the presence of the pathogen could be observed after longitudinal cutting. In all treatments, a varying number of the inoculated plants remained symptomless.

All mock inoculated plants showed normal growth and tuber formation. No effects of the induction treatments with the *Pseudomonas* strains, INA or SA on plant growth and development were evident.

Table 1 shows that in both experiments treatment of the roots with strain WCS417r or SA resulted in a statistically significant reduction in the percentage of diseased plants compared to the control treatment, whereas treatment with strain WCS417r-B4 or

TABLE 1
Disease incidence in the induced resistance assay, 20 days after challenge inoculation with Fusarium oxysporum (means ± SE; $n = 8$)

Induction treatment	Percentage No. of diseased plants	
	Experiment 1	Experiment 2
Control	69 ± 7^{a}	52 ± 10^{A}
Pseudomonas fluorescens strain WCS417r	50 ± 9^{bc}	12 ± 23^{C}
P. fluorescens strain WCS417r-B4	58 ± 15^{ab}	43 ± 15^{A}
104 μM INA	56 ± 23^{ab}	35 ± 16^{AB}
2 mM SA	39 ± 12^{c}	25 ± 20^{BC}

Means with the same letter are not significantly different (Fisher's LSD test, $\alpha = 0{\cdot}05$) INA, isonicotinic acid; SA, salicylic acid.

INA did not. Although the percentages of diseased plants differed slightly between the two experiments, the effects of the induction treatments were qualitatively the same.

To determine the effect of the induction treatments on disease severity, only the diseased plants from both experiments were considered. Fig. 1 shows that there was no

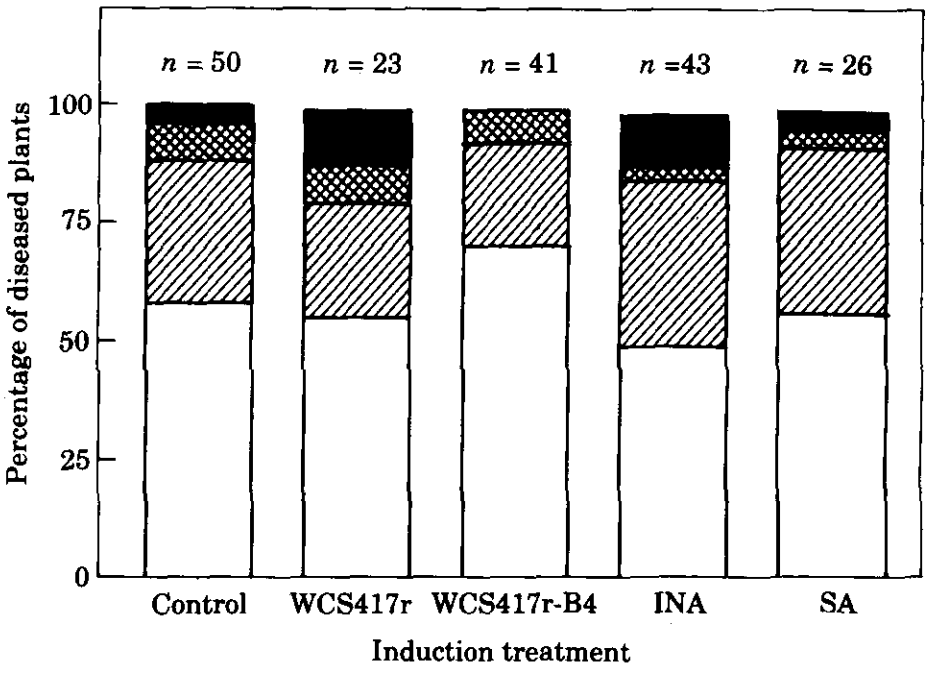

FIG. 1. Disease severity in the induced resistance assay. Diseased plants were scored in four distinct classes, depending on the plant part in which *Fusarium oxysporum* was detected. For each experiment, the relative contribution of each class was calculated. Given data are means of experiment 1 and 2. n = number of diseased plants in the two experiments. The total number of plants per treatment was 96. (□), Internal symptoms detected in the main root only; (▨), internal symptoms in both main root and bulb; (▩) external symptoms of wilting in one or more leaves; (■), death of the shoot. INA, isonicotinic acid; SA, salicylic acid.

effect of any induction treatment on the distribution of the diseased plants among the four classes of disease severity distinguished. Thus, the inducing agents did not affect plant colonization by the pathogen or the severity of symptoms once the plants were visibly infected by the pathogen.

The results presented in Table 1 and Fig. 1 are representative for a large number of independent experiments performed in our laboratory.

Root colonization by the introduced *Pseudomonas* strains was determined 3 weeks after the application of the bacteria. There was no significant difference between strains WCS417r and WCS417r-B4 with respect to colonization of the lower part of the roots (Table 2), indicating that the lack of resistance induction by the mutant strain cannot be attributed to poor root colonization.

Using SDS-PAGE we examined systemic induction of proteins in plants from the induced resistance assay. Fig. 2 shows separation of leaf proteins, soluble at pH 3·0, from mock-inoculated plants from experiment 1, collected 4 days after the induction

TABLE 2

Colonization of the lower part of the radish root system by rifampicin-resistant Pseudomonas *bacteria in the induced resistance assay, 3 weeks after treatment* (means ± SE, $n = 3$)

Treatment	Root colonization by rifampicin-resistant bacteria (10^6 cfu g^{-1} root fresh wt)	
	Experiment 1	Experiment 2
Control	0	0
Pseudomonas fluorescens strain WCS417r	5·6 ± 2·5	14·3 ± 9·7
P. fluorescens strain WCS417r-B4	3·7 ± 0·8	6·6 ± 2·7

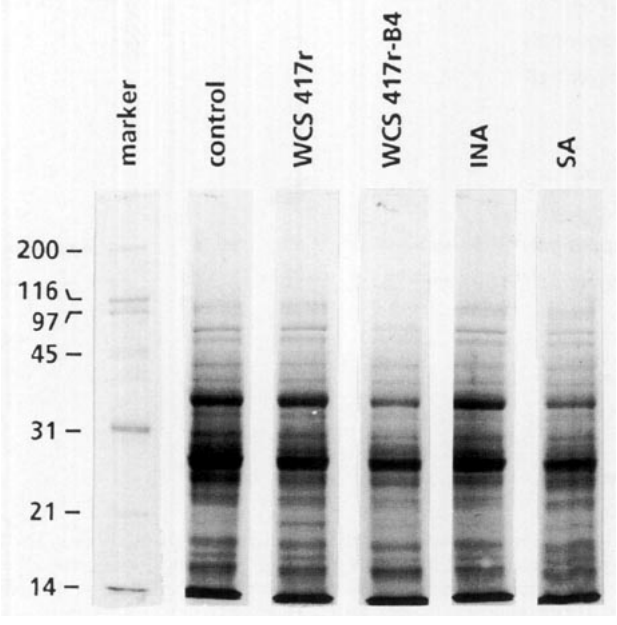

FIG. 2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins (± 50 μg per sample) soluble at pH 3·0 from radish-leaves in response to the induction treatments, separated on SDS-PAGE. The samples were collected from experiment 1, 4 days after treatment. Molecular mass markers are given in kDa. The gels were stained with Coomassie brilliant blue R250. INA, isonicotinic acid; SA, salicylic acid.

treatments. No differences could be observed in the protein patterns of the five treatments, indicating that no novel proteins were induced by WCS417r or SA. Protein patterns of inoculated plants, from other sampling days and from experiment 2 were similar.

To further investigate induction of PR proteins we analysed western blots of extracts collected 1, 8, 16 and 24 days after (mock) inoculation with the pathogen (i.e. 4, 11, 19 and 27 days after the induction treatment). Representative results are given in Fig.

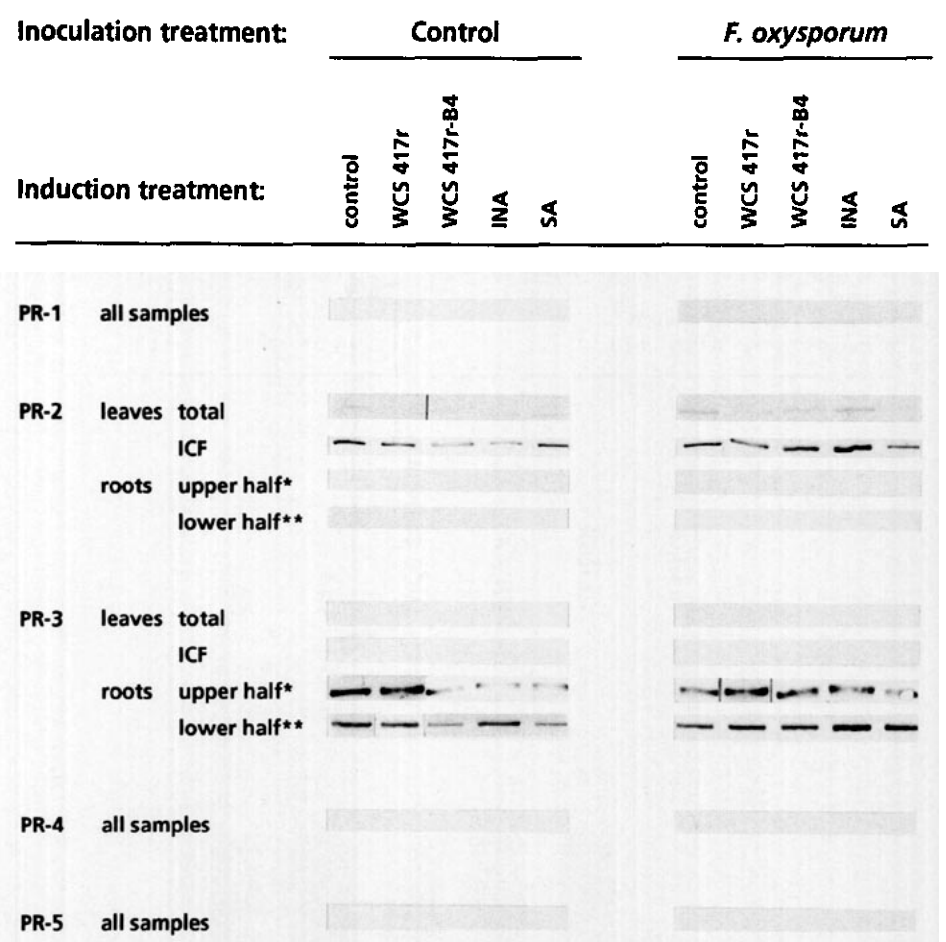

FIG. 3. Western blot analysis of pathogenis-related proteins from radish plants. The lower part of the root system was exposed to an induction treatment. Three days later, the upper part of the root system was (mock) inoculated with *Fusarium oxysporum*. Samples were collected 8 days after (mock) inoculation. Fifty micrograms of protein were loaded per lane. INA, isonicotinic acid; SA, salicylic acid; ICF, intercellular fluid. * Part of the root system which was (mock) inoculated with *F. oxysporum*; ** part of the root system to which the induction treatment was applied.

3. All results from both experiments and the varying sampling times were similar (data not shown). Proteins cross-reacting with antisera against tobacco PR-1 and PR-5 and tomato PR-4 were not observed in any of the treatments. A class II-type PR-2 protein was constitutively present in the ICF of the leaves, and a PR-3 homologue was found in all root samples. The levels of PR-2 and PR-3 did not differ among the treatments.

To test whether radish plants are able to accumulate PR homologues upon infection with a necrotizing pathogen, we inoculated leaves with *Pseudomonas syringae* pv. tomato DC3000. At the moment of harvest, 12 days post-inoculation, the leaves showed clear lesions of about 10 mm in diameter. Fig. 4 shows accumulation of proteins in the ICF of leaves cross-reacting with the antibodies raised against tobacco PR-1, -2 and -5. PR-3

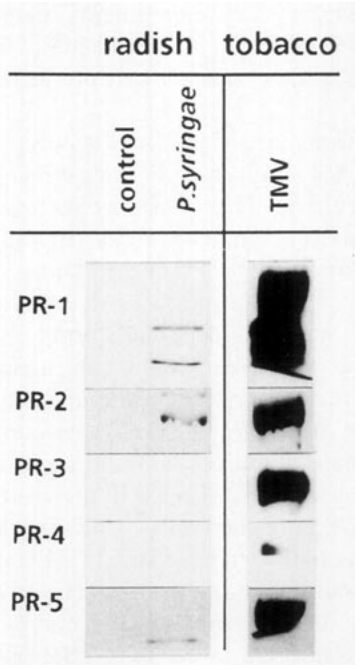

FIG. 4. Western blot analysis of pathogenesis-related proteins in the intercellular fluid (ICF) of radish leaves showing necrosis caused by *Pseudomonas syringae*. For comparison, ICF of tobacco mosaic virus (TMV)-infected tobacco plants was used [*26*]. For each sample about 5 μg of protein was loaded.

was not induced. No protein reacted visibly with the antibody raised against tomato PR-4. In contrast to Fig. 3, no background of PR-2 is visible in Fig. 4, because of the much smaller amounts of proteins used for loading of the gels.

DISCUSSION

We demonstrated that prior treatment of radish roots with *P. fluorescens* strain WCS417r or SA induces resistance against *F. oxysporum* f.sp. *raphani*. In our experimental set-up, a direct effect on the root surface of the bacteria or SA on the pathogen can be excluded, since the introduced bacterial strains remained spatially separated from the inoculated root parts, while diffusion of SA along the root system towards the inoculated root part is very unlikely. Moreover, 2 mM SA has been proven not to inhibit growth of *F. oxysporum in vitro* (unpublished results). Since Leeman *et al.* [*11*] showed that the bacterial strains used do not colonize the root internally, antagonism between the bacterial strains and the pathogen inside the root can be excluded as well.

We used the LPS mutant of strain WCS417r, WCS417r-B4, as a negative control for the bacterial induction treatment. Leeman *et al.* [*10*] demonstrated that the LPS of strain WCS417r is a main bacterial trait responsible for induction of systemic resistance in radish. Additionally, in the experiments performed in this study, no statistically significant reduction in disease incidence was found after induction with bacteria of

strain WCS417r-B4. However, in both experiments there was a slight reduction of disease incidence. This might indicate that other bacterial factors, besides the LPS, are involved in the induction of resistance. *Pseudomonas*-produced SA could be hypothesized as a possible factor [*17*].

Resistance of radish plants against *F. oxysporum* is polygenic. The fungus penetrates the root and invades the cortex extracellularly and intracellularly, to the same extent in resistant and susceptible cultivars. The resistance reaction is believed to be localized in the vascular tissue [*18*]. We observed only a reduction in the percentage of diseased plants upon treatment with strain WCS417r or SA (Table 1), without any effect on the severity of the disease (Fig. 1). This indicates that induced resistance is effective particularly during the early stages of infection. It probably results in a more frequent failure of the fungus to reach or colonize the vascular tissue. This could occur at the level of root penetration, perhaps caused by a change in composition of root exudates under the influence of strain WCS417r and SA. Alternatively, upon infection a very rapid localization of the fungus might occur, resulting in a macroscopically invisible root lesion. Such localization might involve a hypersensitive death of one or a few epidermal or cortex cells, or lignification and/or phytoalexin accumulation in cortex or stele. Apparently, once the pathogen has caused visible symptoms in the main root, further colonization is not inhibited in plants in which resistance is induced. Research on the quantity and quality of root exudates and microscopic research on the first stages of root colonization as affected by strain WCS417r and SA, are in progress to test the above hypotheses.

SA and INA were used to compare induction of systemic resistance by strain WCS417r with previously described chemical induction in other plant species. SA induced resistance in our system against *F. oxysporum* (Table 1), but INA did not. The significance of this difference between the effects of SA and INA in radish is intriguing Métraux *et al.* [*16*] and Uknes *et al.* [*25*] found a concentration-dependent effect of INA on the induction of SAR. Although we applied a high concentration (104 μM), the use of talcum and of rock wool in our system may have reduced the activity of INA in the rhizosphere. Alternatively, different receptors for INA and SA can be hypothesized, the "INA-receptor" not being present in radish roots. Vernooij *et al.* [*29*] demonstrated that induction of SAR by INA does not require SA accumulation in *Arabidopsis*, indicating that either INA acts later than SA in the same pathway, or different signal transduction pathways are involved for INA- and SA-induction. Both possibilities imply different receptors for the two compounds.

We demonstrated that the antibodies against tobacco PR-1, -2, and -5 cross-reacted with radish PR proteins induced in leaves by the necrotizing *P. syringae* (Fig. 4). This induction of PR proteins is in line with previously reported effects of *P. syringae* on *A. thaliana* [*25*], which likewise belongs to the Cruciferae. Obviously, radish plants have a similar potential to accumulate PR proteins. It can be concluded that neither of our induction nor inoculation treatments induced further accumulation of PR-1–5. This distinguishes our system from all forms of SAR described so far. The difference is not only due to the use of a non-pathogenic biological inducer, but also because induction by SA is not accompanied by the accumulation of PR proteins in radish. SA has been reported to induce the accumulation of several PR proteins in many plant species [*13*]. Both SA and INA induce expression of PR-1–5 genes in tobacco [*31*] and PR-1, -2 and

-5 genes in *A. thaliana* [25]. Induction of radish-specific PR proteins, not cross-reacting with the antibodies used, cannot be excluded, although Coomassie brilliant blue stained gels showed no treatment effects on protein patterns in any of the samples (Fig. 2). The constitutive presence of a tobacco PR-3 homologue in the roots is probably related to the use of rock wool as a substrate. In roots of radish plants grown in soil, the PR-3 homologue was not detectable (results not shown).

These results demonstrate that induction of resistance is possible in plants in which no further accumulation of PR proteins occurs upon induction of resistance. This is the first report which demonstrates that accumulation of PR proteins is not a prerequisite for induction of systemic resistance. This holds for both chemical and biological induction. It remains to be investigated whether in our system induced systemic resistance has the same characteristics as the forms of SAR described so far with respect to the signal transduction pathways involved and the spectrum of the resistance. If this is the case, it can be postulated that the same pathway is activated in a later phase, no longer leading to accumulation of PR proteins. Accumulation of PR proteins in relation to SAR may then be an inessential side-effect. Whether they could exert an extra effect on disease reduction remains to be investigated. Otherwise, we are dealing with a different form of induced systemic resistance, not associated with accumulation of PR proteins.

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