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Induced Systemic Resistance (ISR) by *Pseudomonas* spp. Impairs pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots

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Abstract

The effect of induced systemic resistance (ISR) by *Pseudomonas* rhizobacteria on the pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots was investigated. Cucumber plants (cv. Corona) were grown in vermiculite, roots were split with one side bacterized with *Pseudomonas corrugata* strain 13 or *P. aureofaciens* strain 63-28 (bacterized roots) and the other distant side was treated with water (distant, induced roots). For the non-induced control, roots on the bacterized side were treated with buffer instead of the bacterial treatment. Intact, non-split roots were also treated with the bacteria or buffer as a control. Cucumber root tissue from these treatments were harvested and incubated with a zoospore suspension of *P. aphanidermatum* for three hours. Most of the zoospores in the suspension were stimulated to encyst or germinate. The numbers of germinated zoospores were significantly decreased on distant induced cucumber roots in comparison to non-induced controls. Germination was also reduced on intact bacterized roots, compared to controls. There was less attachment, germ tube production and penetration on roots bacterized or induced by the rhizobacteria compared to non-induced roots. Effects were significantly greater on bacterized roots (roots colonized by bacteria) compared to distant induced roots (roots with the opposite side bacterized). Systemic resistance induced by the two *Pseudomonas* spp. also reduced pathogen spread on split cucumber roots *in planta*. Crown infection from induced or bacterized roots was delayed for four to six days in comparison to the non-induced control. Results indicated that *Pseudomonas* spp. can exert both an indirect influence on *P. aphanidermatum* zoospore behaviour and infection via induced systemic resistance (ISR) and a local influence via antibiosis or local induced resistance.

Abbreviations: ISR – Induced systemic resistance; PAI – *P. aphanidermatum* inoculum; PGPR – Plant growth-promoting rhizobacteria; TSA – tryptic soy agar and TSB – tryptic soy broth.

Introduction

Pythium aphanidermatum (Edson) Fitzpatrick infects young cucumber roots, causing root and crown rot and death of mature plants. Recent reports showed that cucumber root disease caused by *P. aphanidermatum* is suppressed by strains of *Pseudomonas* spp. (McCullagh et al., 1996; Moulin et al., 1994, 1996; Paulitz et al., 1992). This suppression could be caused by many mechanisms, including competition for limiting nutrients, antibiosis, or induced resistance. Induced disease resistance is defined as a process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic

agents (Kloepper et al., 1992). This resistance may be local or systemic, a widely studied phenomenon called systemic acquired resistance (SAR) (Sticher et al., 1997). Induced resistance against plant diseases has been studied on more than 25 crop species by many research groups in past three decades (Tuzun and Kloepper, 1994). Systemic resistance induced by *Pseudomonas* spp. has been termed ISR (induced systemic resistance) to differentiate it from SAR, which may have different inherent mechanisms (Kloepper et al., 1992). Besides affecting host resistance, this activation process may also alter certain compounds in host plant exudates. Thus, ISR against *Pythium aphanidermatum* may indirectly influence one or more stages of zoospore behaviour prior to or during pathogen infection. Motile zoospores of the pathogen act as a primary inoculum (Royle and Hickman, 1964a,b) and zoospores are chemotactically attracted to root exudates. Several amino acids, polysaccharides or volatile compounds were demonstrated to stimulate chemotaxis and encystment (Donaldson and Deacon, 1993a, 1993b; Jones et al., 1991; Longman and Callow, 1987; Yung, 1970). ISR may also alter the post-infection colonization of the root by the pathogen.

Zhou and Paulitz (1994) used a split root system to show that *Pseudomonas* spp. rhizobacteria could exert a systemic influence on disease incidence of *P. aphanidermatum*, root and shoot biomass, and fruit yield of infected plants. In an earlier report (Zhou and Paulitz, 1993), the distribution of encysted zoospores on the rhizoplane was shown to be altered on cucumber roots treated with *Pseudomonas* spp. In addition, exudates from bacterial-treated roots were less attractive to *Pythium* zoospores. However, the authors used intact roots, where zoospores were in direct contact with bacteria or their metabolites including antibiotics. The precise mechanisms of induced systemic resistance by *Pseudomonas* spp. still remain largely unknown although a number of suggestions can be raised. In that context, we hypothesized that induced systemic resistance against cucumber root rot caused by *P. aphanidermatum* could be partially due to an indirect influence on zoospore behaviour and/or to a delay of pathogen infection and disease development in plants induced by *Pseudomonas* spp. Therefore, to provide new insights into the influence of ISR on zoospore behaviour and *Pythium* spread, we used the split root system to spatially separate *Pythium aphanidermatum* from *Pseudomonas* spp. in the experimental design. This study was undertaken to delineate the behaviour of *P. aphanidermatum* zoospores on *Pseudomonas*-induced cucumber roots. In addition, we also studied the influence of *Pseudomonas* spp. on the rate of colonization of inoculated roots by *P. aphanidermatum in planta* with a split root system.

Materials and methods

Media

Tryptic soy agar (TSA) (Difco, Detroit, MI, USA) and tryptic soy broth (TSB) were used for bacterial growth and storage. Bacterial strains were stored at $-80\,^{\circ}$C in nutrient broth (Difco, Detroit, MI, USA) with 10% glycerol. V-8 juice agar medium (V-8 juice supernatant 150 ml, $CaCO_3$ 3 g, agar 20 g, and distilled water 850 ml) was utilized for *P. aphanidermatum* growth or zoospore generation. In addition, *Pythium* selective medium (Mircetich and Kraft, 1973) was employed to recover *P. aphanidermatum* from inoculated root tissues.

Bacterial strains

Two strains of *Pseudomonas* spp., *Pseudomonas corrugata* 13 (Rankin and Paulitz, 1994) and *P. aureofaciens* 63-28 (provided by Agrium Inc. Saskatoon, SK, Canada), were selected as inducers. The bacterial strains were prepared by streaking each isolate from $-80\,^{\circ}$C storage onto a TSA plate. The plate was incubated at 25 °C for 36 h. A tube containing 15 ml of TSB was inoculated with a single colony of each strain from the TSA plate. The bacterial strain in the TSB tube was incubated on a shaker at 120 rpm at 25 °C for 48 h. Bacterial cells were washed by centrifuging at 3020*g* for 10 min, washing pellets and resuspending in 0.1 M $MgSO_4$ to a concentration of 10^8 cfu ml^{-1}.

Pathogen inoculum

Solid inoculum of *P. aphanidermatum* isolate 186 (provided by Dr. W. Jarvis, Agriculture and Agrifoods Canada, Harrow, Ontario, Canada) was produced on the V-8 juice-vermiculite (medium grade, Vil Vermiculite Inc., Montreal, Canada) medium (V-8 juice : vermiculite: water = 2 : 1 : 2 v/w/v) (Martin, 1992). *P. aphanidermatum* inoculum (PAI) was incubated at 25 °C for ten days. The PAI contained 10^6 propagules g^{-1}, which consisted of oospores (71%),

sporangia (16%), mycelial segments (12%) and very few zoospores (<1%), as determined by a hemacytometer observation under the microscope.

To produce zoospores for the behaviour trial, the fungus was cultured on V8 agar plates at 30 °C for 48 h. The agar culture was cut into five mm-wide strips and half of the strips were moved to an empty petri plate. Both petri plates were flooded with 20 ml of sterile distilled water. After 0.5–1 h, the water was removed and replaced with the same amount of sterile water. This treatment leached out some nutrients or metabolites during the previous incubation. The plates were inoculated at 30 °C under fluorescent light (Sylvania, Gro-lux, USA; 15W) for 24 h, then at 20 °C for 4 h to stimulate zoospore release (Paulitz et al., 1992; Rahimiam and Banihashemi, 1979). Swimming zoospores were collected on a 0.45 μm filter and re-suspended with sterile distilled water at 10^5 zoospores ml^{-1}.

Plant growth and inoculation

Cucumber (*Cucumis sativus cv*. Corona) plants were grown from seeds in vermiculite in the greenhouse under 16-h photoperiod at 25/20 °C during day/night. After germination, cucumber plants were fertilized daily with a plant nutrient solution that contained 0.97 g l^{-1} of Peter's Hydro-sol fertilizer (5-11-26) and 0.64 g l^{-1} of $Ca(NO_3)_2$ (Zhou and Paulitz, 1993). The crowns of ten-day-old seedlings were split with a surgical blade and transplanted immediately into inverse V-shape PVC tubes. The ends of the PVC tubes were extended 16 cm further with a tube of transparent plastic film open at the ends. When the cucumber plant was placed into the tube, the PVC V-shaped pipe with plastic film was filled with vermiculite (medium grade). The PVC V-shaped pipe with the two plastic film tubes was separately buried in two 12.7-cm-diameter round plastic pots containing vermiculite. The cucumber roots could be observed in each side of the PVC tube to see if they grew uniformly in both sides before the upcoming treatments (Figure 1).

Seven days after the split, cucumber roots were bacterized with cell suspensions of *Pseudomonas* spp. (50 ml of 10^8 cells ml^{-1} solution per pot) drenched into one pot of the pair (bacteria side). For the root colonization experiments, the other pot (*Pythium* side) was inoculated with the solid PAI from the bottom of the pot, four days after bacterization. Vermiculite was removed from the open end of plastic film on one side,

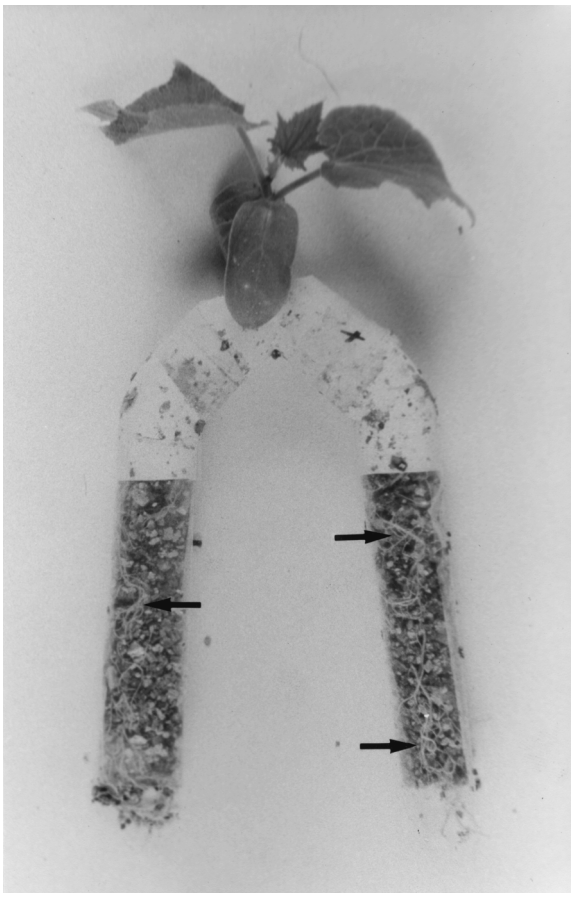

Figure 1. Cucumber split root system design. Cucumber seedlings were split and grown in the V-shaped PVC tube. Arrows indicate the roots.

and replaced with one gram of PAI. After treatment, each pot was covered with a plastic bag to prevent contamination. Water was daily provided to the plant from a hole made in each bag. Treatments with 0.1 M $MgSO_4$ and the same amount of autoclaved PAI served as controls in each experimental design. At the same time, the same treatments were conducted in non-split, intact roots (Table 1). For experiments on the influence of ISR on zoospore germination and colonization (Tables 2 and 3), the roots were not inoculated with PAI, but were exposed to zoospores of *P. aphanidermatum* after they were harvested from the tubes.

Influence of ISR on zoospore germination

To examine zoospore germination, the following experiment was performed. Split cucumber roots were harvested from both sides 4 days after bacterization and

Table 1. Design and treatments for *Pythium aphanidermatum* root colonization experiments. Split cucumbers were planted into a V-shaped PVC tube and intact cucumbers planted into a straight plastic tube of the same diameter

Split root experiment		Intact root experiment
Bacteria side[1]	Distant or opposite side[2]	
Strain 13	PAI	Strain 13 + PAI
Strain 63-28	PAI	Strain 63-28 + PAI
Buffer	PAI	Buffer + PAI
Buffer	Sterilized PAI	Sterilized PAI

[1] Bacterial cells were diluted with 0.1 M $MgSO_4$ to a concentration at 10^8CFU ml^{-1}. Fifty ml of the cell suspension was drenched onto cucumber roots and the same volume of 0.1 M $MgSO_4$ buffer was used as a control.
[2] Solid *P. aphanidermatum* inoculum (PAI) was produced on V-8 juice vermiculite medium (V-8 juice : vermiculite : water=2 : 1 : 2 v/w/v). *P. aphanidermatum* grew in the medium for about ten days at 25 °C. One gram of this fresh inoculum (10^6 propagules g^{-1}) was inoculated to cucumber root tips from the bottom of the plastic tubes.

Table 2. Germination of zoospores of *P. aphanidermatum* in water with *Pseudomonas* bacterized or distant induced cucumber roots. Zoospores were incubated with the root tissue for 3 h and observed after staining with trypan blue

Experimental design	Treatment	Zoos%[1]	Cyst%[1]	Germ%[1]
Split	Strain 13	20b[2]	27d	52c
	distant side	13ef	19e	67b
Split	Strain 63-28	18bc	28d	54c
	distant side	15de	21e	64b
Split	control	6g	11f	83a
Intact	Strain 13	17cd	35b	48d
Intact	Strain 63-28	12f	44a	45d
Intact	Control	3h	12f	85a
No roots	Control	47a	31c	22e
$LSD_{0.05}$		2.7	3.4	3.8

[1] Zoos – zoospores, Cyst – encysted zoospores, and Germ – germinated zoospores.
[2] The means in each column followed by the same letter are not significantly different as determined by LSD ($P \leq 0.05$). The experiment was conducted three times with three replicates per treatment.

Table 3. Encystment and germination of zoospores examined *in situ* on 5-mm root tips of cucumber, induced or bacterized by *Pseudomonas* spp. Zoospores were incubated with the roots in sterile water for 5 h at 22 °C

Experimental design	Treatment	Zoos/mm[1]	% Germ[3]
Split	Strain 13	2.8 ± 0.7cd[2]	87.0a
	Distant side	5.2 ± 2.4b	83.1a
Split	Strain 63-28	3.0 ± 0.9cd	79.2a
	Distant side	4.2 ± 1.4bc	84.2a
Split	Control	7.2 ± 2.0a	86.1a
Intact	Strain 13	2.4 ± 0.4cd	78.7a
Intact	Strain 63-28	1.7 ± 0.2cd	78.6a
Intact	Control	8.0 ± 1.8a	87.0a

[1] Data in each column represents the number of encysted or germinated zoospores ± SD per millimeter of root tip. Results were pooled from two trials, five replicates per treatment.
[2] The means in the same column followed by the same letter are not significantly according to LSD ($P \leq 0.05$).
[3] The number of germinating or penetrating zoospores/total number of zoospores attached to roots ×100.

50 mg of fresh root tissues of each sample was placed into 0.5 ml zoospore suspension (10^5 zoospores ml^{-1}) in a test tube. The tube was shaken on a vortex mixer for 30 s to immobilize zoospores and stimulate them to encyst. After three hours of incubation at room temperature (22 ± 1°C), 100 µl of the zoospore suspension was placed on a cavity slide, then two drops of 95% ethanol were added to kill the zoospores, cysts or germinating cysts. After staining with 0.05% trypan blue in lactophenol, all types of zoospore stages, including zoospores, encysted zoospores and germinating cysts, were examined under a light microscope (Olympus BH-2) at magnification of 200X. Forty to eighty zoospores were counted in each cavity slide. The experiments were conducted three times with the same treatments, and three replicates per treatment.

Influence of ISR on zoospore colonization of roots in situ

To examine the colonization of zoospores on cucumber roots, including attraction, attachment and encystment, the root tips were incubated with the zoospores by immersion in a suspension of 10^5 zoospores ml^{-1} on a cavity slide at 22 °C for 5 h. The root tips were stained with 0.05% trypan blue in lactophenol. Zoospore colonization, cyst germination and germ tube penetration *in situ* on a five-mm long region of elongation behind the root cap were examined under a light microscope (Olympus BH-2) at a magnification of 400×, with five root tip replications per treatment.The experiment was conducted twice.

Colonization of cucumber roots by P. aphanidermatum *in planta*

Roots were sampled from *Pythium*-inoculated pots at 3-day intervals after pathogen inoculation, up to 18 days. The plastic tube was broken by a sterile razor blade and the roots were taken out carefully from the PVC tube. Vermiculite remaining on the root system was washed away under running tap water and the roots were dried on Kimwipe tissues. To determine if *P. aphanidermatum* infected root tissue internally or externally, half of the root sample was further disinfested in 0.5% sodium hypochlorite (NaClO) solution for one minute. Preliminary tests showed that this treatment killed *P. aphanidermatum* on surface contaminated roots, but still allowed survival of the pathogen inside colonized roots. After disinfestation, roots were immediately rinsed in sterile distilled water three times, and dried again on Kimwipe tissues. The roots were cut into 2-cm segments from crown to root tip and five segments from each position on the root were separately placed on the *Pythium* selective medium plates. Finally, the root segments on the plate were examined for *Pythium* growth under a dissecting microscope after 48-h incubation at 25 °C. The experiment was conducted twice with the same design, five replicates per treatment.

Data collection and statistical analyses

All experiments were conducted two or three times with the same design. The data of zoospore behaviour influenced by the *Pseudomonas* spp. and zoospore attraction to induced roots were analyzed by Fisher's protected LSD test ($P \leq 0.05$).

A simple linear regression analysis was used to characterize the relationship between the length of root colonization (cm) by *P. aphanidermatum* and sampling time (day). The SAS PROC GLM procedure (Version 6.12, SAS Institute Inc., USA) was used for all regression analyses. Data from repeated experiments were combined with the initial experiment if regression parameters were not significantly different ($P \geq 0.05$) using a *t* test (Gomez and Gomez, 1984).

Results

Influence of ISR on zoospore germination

When a suspension of zoospores was incubated with cucumber root tissues for three hours at room temperature (22 °C), all stages of zoospore development could be observed under a light microscope (Figure 2). Cucumber roots significantly stimulated zoospore germination, compared to treatments without roots (Table 2). Nevertheless, the percentage of zoospore encystment or germination varied among the root treatments. When the zoospores suspension was incubated in the presence of roots bacterized with *Pseudomonas* strain 13 or 63-28, the germination rate decreased by 37% and 35% respectively, compared to split control without bacterization. Similarly, germination of zoospores in the suspension with the distant split roots induced by the two *Pseudomonas* spp. also decreased by 19% and 23% compared to split control without bacterial induction, respectively. In intact roots that were bacterized, germination was reduced by 43% and 47%, respectively. No significant difference existed between split and intact controls on the percentage of encystment and germination. Therefore, the results indicated that the exudates released from cucumber roots which were bacterized or induced with the *Pseudomonas* spp. influenced zoospore germination.

Zoospore colonization in situ

When cucumber roots were immersed in water with zoospores, some zoospores migrated to the roots, attached and colonized the surface. The average number of encysted or germinated zoospores per millimeter of root tip varied among the treatments from 1.7 ± 0.2 to 8.0 ± 1.8. The number of encysted or germinated zoospores on distant induced roots was significantly decreased compared with non-induced

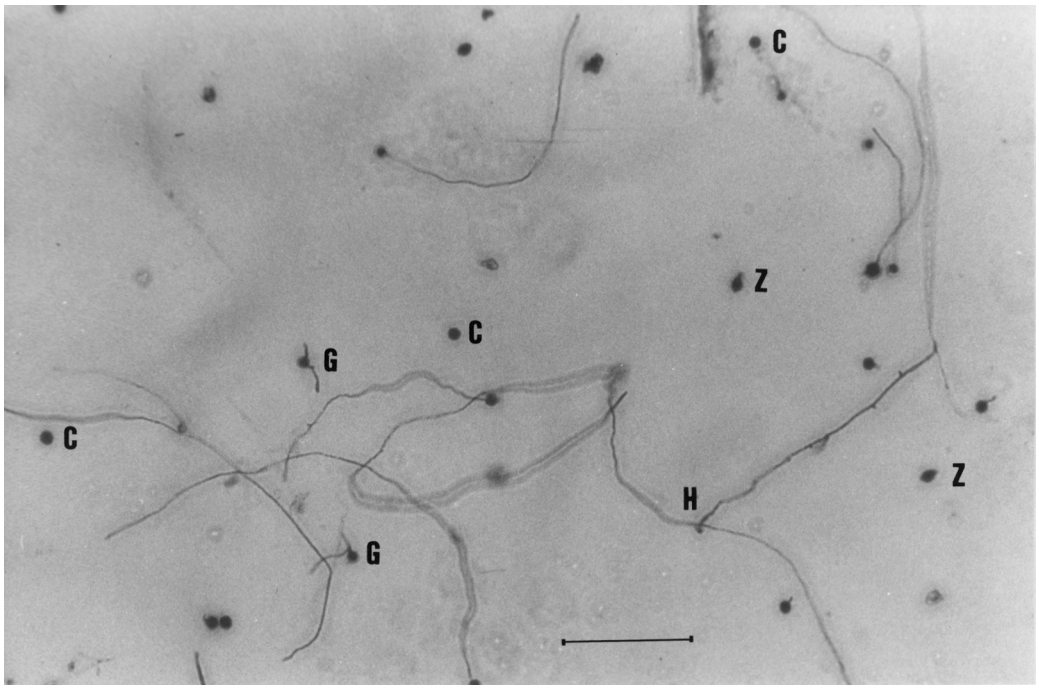

Figure 2. Various developing stages of zoospores , incubated with cucumber root tissues in distilled water for three hours at 22 ± 1 °C. The stages include encysted zoospore, termed cyst (C), germinating encysted zoospore (G), hypha (H); and motile zoospore without a cell wall (Z). Scale bar represents 100 μm.

controls (Table 3). *Pseudomonas* strains reduced the attractiveness of cucumber roots when they were either induced or bacterized by *Pseudomonas* spp. In comparison to the intact control, zoospore encystment on intact cucumber roots bacterized with 13 or 63-28 was decreased by 70% and 78%, respectively. Similarly, when split cucumber roots were systemically induced by the two strains, the density of attached zoospores on the distant induced roots was also decreased by 28% and 42%, respectively. There was no significant difference in zoospore encystment between the split and intact controls (Table 3). From 78% to 87% of the encysted zoospores germinated on or penetrated host tissue in all treatments, with no significant difference among the treatments. This result indicated that *Pseudomonas* spp. could reduce the attractiveness of roots to the motile zoospores, but could not reduce germination or penetration of encysted zoospores which had attached to the host.

Pythium colonization of roots in planta

By regression analysis, there was a significant correlation between pathogen spread distance Y (cm) and time X (day). A curve significantly fit a linear model, $Y = \alpha + \beta X$, where α is the intercept of the line on the Y axis, while β is the slope coefficient. In the non-bacterized intact roots, *Pythium aphanidermatum* reached the crown in 12 days. The coefficient β, representing the rate of disease development, was significantly reduced by bacterization and induction compared to non-treated controls from 3–12 days (Table 4). After day 12, the disease development rate (β) was similar between the distant induced root and the bacterized root, compared to their respective control treatments (Figures 3 and 4). Thus, *Pseudomonas* spp. mainly affected disease development in the early stage. Moreover, a t test also indicated that average α or β of the regression on surface disinfested roots was significantly different from that of non-disinfested roots ($P \leq 0.0014$). Infection was always detected earlier in the non-disinfested roots at the same height. Therefore, the results suggest that *P. aphanidermatum* initially spreads on or in the outer cortex of the cucumber root.

Statistical results indicated that each of the regression parameters was not significantly different between the first experiment and the repeat experiment by t test

Table 4. Parameters of linear regression model, $Y = \alpha + \beta X$, for *Pythium aphanidermatum* recovery from inoculated cucumber roots, where Y = distance (cm), X = time (days) and α and β represent regression coefficients. External and internal colonization by *Pythium aphanidermatum* was obtained from non-disinfested and surface-disinfested roots, respectively. The data of two trials for this analysis were from 3–12 days after inoculation

Treatment	α[1]	β[2]	R-square[3]
External root colonization by *P. aphanidermatum*			
Split roots, distant induced side			
Strain 13	0.147a[4]	0.493b	0.986**
Strain 63-28	0.253a	0.448b	0.985**
Control	0.027a	0.974a	0.998**
Intact roots			
Strain 13	0.204a	1.148b	0.950**
Strain 63-28	−0.133a	1.211b	0.949**
Control	0.240a	1.456a	0.984**
Internal root colonization by *P. aphanidermatum*			
Split roots, distant induced side			
Strain 13	−0.533a	0.363b	0.942**
Strain 63-28	−0.640a	0.267b	0.766*
Control	−1.040b	0.830a	0.954**
Intact roots			
Strain 13	−0.907ab	0.996b	0.951**
Strain 63-28	−1.093b	1.062b	0.960**
Control	−0.613a	1.422a	0.969**

[1] α (cm) is the Y-intercept coefficient of the model.
[2] β (cm/day) is the slope coefficient of the model.
[3] R^2 is the coefficient of determination which indicated a linear relationship between X (time) and Y (distance). Values followed by ** or * indicate a significant correlation at the 5% or 10% level, respectively.
[4] α or β value within either root types or treatment in the same column followed by the same the letter is not statistically different ($P \leq 0.05$) according to t test.

(α, $P > 0.357$; β, $P > 0.675$). External and internal colonization were separately examined on non-surface-disinfested roots (Figure 3) and surface-disinfested roots (Figure 4) respectively. The two *Pseudomonas* strains, 13 and 63-28, systemically delayed pathogen spread and development on both the outside and inside of the *Pseudomonas*-induced roots for 4–6 days by comparison with the non-induced control (Figures 3A, 4A). Similarly, pathogen infection and development were also delayed on intact roots which were previously bacterized with the two *Pseudomonas* strains (Figures 3B, 4B). These results indicated that the two strains not only suppressed *P. aphanidermatum* on bacterized roots directly, but also systemically induced resistance against the infection and development of the pathogen on the distant cucumber roots. *Pythium aphanidermatum* was not detected on non-inoculated cucumber roots in any experiment.

Discussion

In the current study, an experiment was designed to look at both ISR with a split root system (pathogen and biocontrol agent not in contact) and the influence of direct antagonism on *P. aphanidermatum* zoospore behaviour or pathogen spread with bacterized, intact roots (pathogen in contact with biocontrol agent). With the split and intact root design, we could compare the two possible mechanisms on the same plant and the relative contribution of induced systemic resistance and antibiosis or local induced resistance.

The number of zoospores encysted or germinated on roots bacterized or induced with *Pseudomonas* spp. were significantly reduced as compared to controls; while the germination of the attached and encysted zoospores did not vary between them (Table 3). Therefore, it appears possible that ISR may have more of an

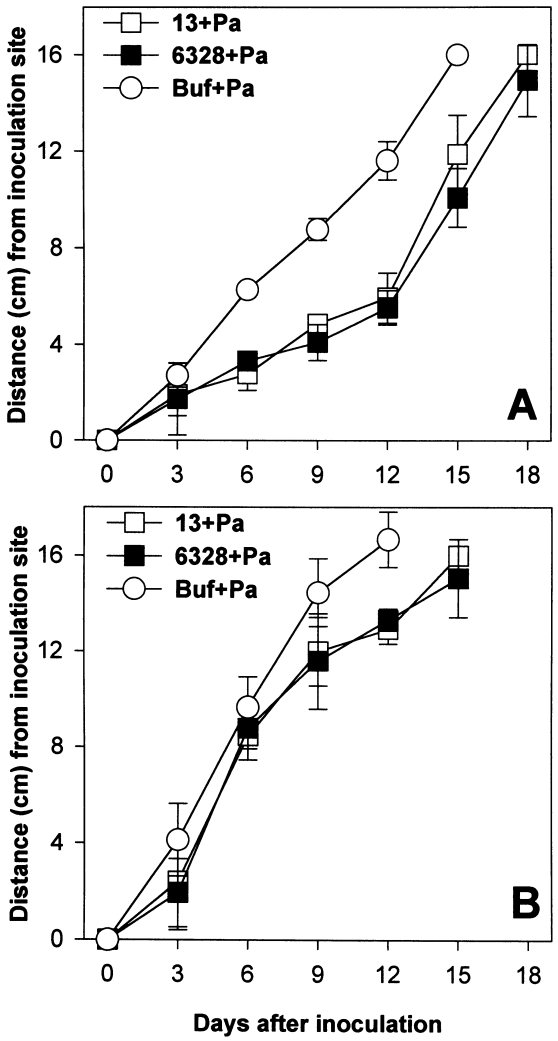

Figure 3. External colonization by *Pythium aphanidermatum* in cucumber roots bacterized or distantly induced by two *Pseudomonas* spp. Roots were harvested every three days after the cucumber was inoculated with one gram of solid *P. aphanidermatum* inoculum (PAI). The *Pythium*-inoculated roots were only washed in sterile water. The rootlets were cut into 2-cm long segments from crown to root tip before they were placed on the *Pythium* selective medium plate. Plates were examined under a dissecting microscope after 24-h incubation at 25°C. A. Split roots induced by *Pseudomonas* spp., distant induced side. B. Intact roots bacterized with the *Pseudomonas* spp.

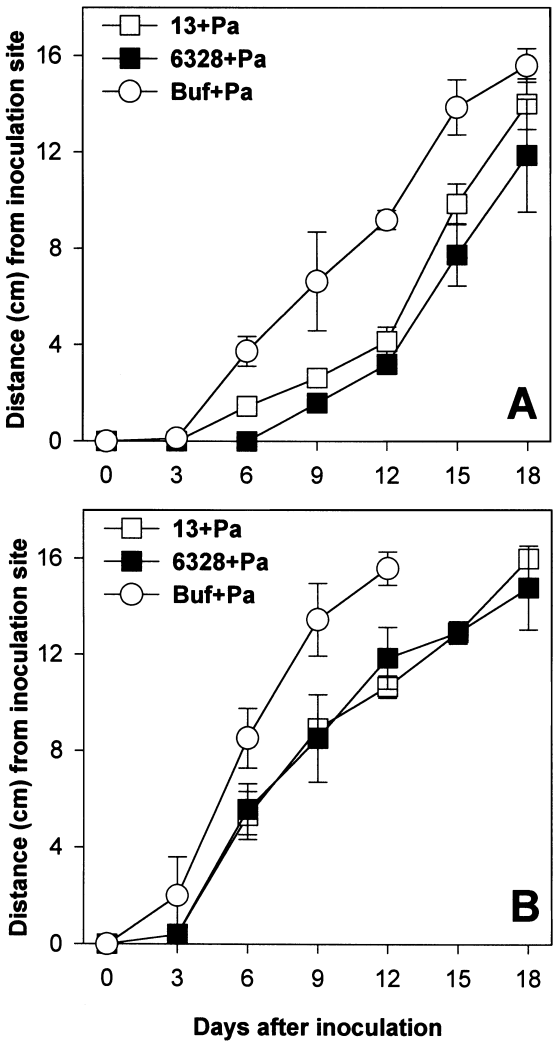

Figure 4. Internal colonization by *Pythium aphanidermatum* in cucumber roots induced or bacterized by PGPR. Sampled roots were washed in distilled water and further disinfested in 0.5% sodium hypochlorite (NaClO) solution for one minute. The pathogen was detected on *Pythium* selective media. A. Split roots induced by *Pseudomonas* spp., distant induced side B. Intact roots bacterized with the PGPR.

effect on zoospore attraction or attachment than on germination of encysted zoospores or penetration of the host tissues.

Certain components in root exudates or extracts may stimulate or inhibit plant pathogens in rhizosphere (Curl and Truelove, 1986). They could stimulate or inhibit encystment of swimming zoospore and germination of encysted zoospores (Donaldson and Deacon, 1993a). Undoubtedly, they also could attract or repel the swimming zoospores. In this study, zoospores of *P. aphanidermatum* were preferably attracted to cucumber roots in all treatments because of their chemotaxis. This behaviour could be indirectly manipulated by the *Pseudomonas* spp. bacterized on the cucumber roots. The metabolic processes involved in

Pseudomonas-induced ISR could change the components of root exudates or extracts.

Induced chemical defenses could be considered as one of the main mechanisms induced by the *Pseudomonas* spp., based on work with classical systemic acquired resistance (Sticher et al., 1997). *Pseudomonas* strains 13 and 63-28 can systemically stimulate some defense enzyme activities in cucumber roots, such as peroxidase, and phenylalanine ammonia-lyase (PAL) (Chen et al., 1996, 1997). The PAL activity is associated with biosynthesis of toxic metabolites such as phytoalexins and lignin in plant defense responses (Lamb et al., 1989). These plant physiological processes may also alter the quantity and quality of exudates, which would further influence the zoospore behaviour. For example, the PAL accumulation in induced roots will reduce the amount of phenylalanine, one of aromatic amino acids which could stimulate zoospore cysts germination and germ tube growth (Yung, 1970).

Our results revealed that the influence *of Pseudomonas* spp. on zoospore behaviour was stronger on bacterized roots (pathogen in contact with *Pseudomonas* spp.) than that on distant induced roots (pathogen not in contact with *Pseudomonas* spp.). These effects could be partially explained by bacterial metabolites, antibiotics and/or other localized mechanisms, which could restrain zoospore orientation or germination of encysted zoospores *in situ* or *in vitro*. Recently, a new kind of antibiotic produced by strain 63-28 was reported by Gamard et al. (1997). This butyrolactone has activity against *Pythium ultimum*. The difference in zoospore suppression between the distant induced and bacterized roots may be due to the antibiotic produced by the bacteria in bacterized roots. Ultrastructural evidence for antibiosis acting in the cortex of pea roots against *P. ultimum* was shown by Benhamou et al. (1996). However, local induced resistance may also be a factor on bacterized roots.

Pythium aphanidermatum could be recovered from all cucumber roots which were previously inoculated at the root tips. *Pythium aphanidermatum* grew up both the inside and outer parts of the root, starting at inoculation site, but the pathogen on the external parts of the root grew faster. In the external parts of the root, *P. aphanidermatum* requires about 12 days to grow from root tip to crown on control roots; while on distant induced roots it takes about 15 days or more (Figure 3B). Besides *P. aphanidermatum* infection by zoospores, the germ tubes arising from sporangia, oospores or mycelia in the PAI could also penetrate root tissues. When the PAI was inoculated to 20-day-old plants, a rot symptom was detected on the crown in 12 days; while when a zoospore suspension (10^5 zoospores per pot) was inoculated to a similar aged plant, the symptom was found in about 7 days under the same environmental conditions (unpublished data). This may also be due to a delay in oospore germination. Zoospores germinate much faster than oospores, which often have a dormancy for several weeks (Webster, 1980). Therefore, we speculated that zoospores could infect cucumber roots more efficiently than other types of *P. aphanidermatum* inoculum in greenhouse, especially under wet conditions favorable for the swimming zoospores to germinate and infect cucumber roots (Paulitz, 1997). However, any technique that can immobilize zoospores and restrict encysted germination could be an effective means to control greenhouse cucumber root disease caused by the pathogen.

Pseudomonas strains 13 and 63-28 could inhibit *P. aphanidermatum* infection of bacterized cucumber roots and systemically reduce the rate of spread in the distant induced roots. In the *Pythium* recovery trial, the slope coefficient β, representing rate of spread, was significantly different between *Pseudomonas*-treated and non-treated roots (Table 4). This difference happened in both distant induced roots and bacterized roots. The mechanism of the ISR could have a significant effect on spread of *P. aphanidermatum*. There was also a difference in the rate of spread between intact and split roots, with the slope coefficient higher on intact control roots than on split control roots. During the study, we found that the intact root system had more plentiful rootlets than the split root system in early stages, even though they were growing in the same size plastic tube. It could be that the pathogen spread faster between the dense root tissues. Another difference between split and intact treatments was due to a wound formed on split roots, which may generate a signal to induce systemic resistance against plant disease as Hammerschmidt (1993) summarized. But this effect in the experiments could be measured by comparing results of the split or intact controls.

Both antagonism and ISR are very important mechanisms in biological control of plant pathogens by *Pseudomonas* spp. The antagonists could directly suppress pathogens with metabolites or antibiotics in the rhizosphere or cortex of the roots, a pre-infection protection. In addition, induced systemic resistance may also act pre-infection by influencing zoospore behaviour and

post-infection by delaying the growth of the pathogen to the crown and stem.

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