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Influence of pH on Suppression of Fusarium Wilt of Carnation by *Pseudomonas fluorescens* WCS417r

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With one figure

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Abstract

The influence of the nutrient solution pH on suppression of fusarium wilt by *Pseudomonas fluorescens* WCS417r in carnation grown in rockwool was investigated.

Experiments were conducted with carnation cultivars Lena and Pallas, susceptible and moderately resistant to fusarium wilt, respectively. WCS417r significantly reduced fusarium wilt in the susceptible cv. Lena, that was root-inoculated with *Fusarium oxysporum* f.sp. *dianthi* (Fod), at pH 7.5, but not at pH 6.5 and 5.5. This corresponded with a higher *in vitro* siderophore production and antagonism of Fod by WCS417r at pH 7.5 than at pH 6.5 and 5.5. Fusarium wilt in the moderately resistant cv. Pallas, however, was also significantly reduced by treatment with WCS417r at pH 5.5. This corresponded with the low influence of pH on induced resistance by WCS417r in plants of cv. Pallas that were stem-inoculated with Fod.

The results indicate that the influence of pH on control of fusarium wilt of carnation by *Pseudomonas fluorescens* WCS417r differs between carnation cultivars that differ in their level of resistance against fusarium wilt. In susceptible cv. Lena, fusarium wilt is suppressed by antagonism by WCS417r, that is most effective at pH 7.5. In the moderately resistant cv. Pallas, fusarium wilt is suppressed by both antagonism and induced resistance by WCS417r. The latter is also effective at lower pH.

Zusammenfassung

Einfluß des pH-Wertes auf die Unterdrückung der Fusarium-Welke bei Nelken durch *Pseudomonas fluorescens* WCS417r

Untersucht wurde der Einfluß des Nährlösungs-pH-Wertes auf die Unterdrückung des Fusarium-Welke durch *Pseudomonas fluorescens* WCS417r bei Nelken, die in Steinwolle wuchsen.

Die Versuche wurden mit den Nelkensorten Lena und Pallas, anfällig bzw. mäßig resistent gegen die Fusarium-Welke, vorgenommen. Eine signifikante Reduzierung der Fusarium-Welke wurde mit WCS417r bei der Sorte Lena, die im Wurzelbereich mit *Fusarium oxysporum* f.sp. *dianthi* (Fod) inokuliert worden war, bei pH 7,5 aber nicht bei pH 6,5 und 5,5 erreicht. Diese Beobachtung korrelierte mit einer erhöhten *in vitro* Siderophorproduktion und Antagonismus von Fod durch WCS417r bei pH 7,5 als bei pH 6,5 bzw. 5,5. Die Fusarium-Welke wurde jedoch auch bei pH 5,5 durch WCS417r bei der mäßig resistenten Sorte Pallas signifikant reduziert. Dies korrelierte mit dem schwachen Einfluß des pH-Wertes auf die durch WCS417r induzierte Resistenz in Pflanzen der Sorte Pallas, die im Stengel mit Fod inokuliert worden war.

Die Ergebnisse deuten darauf hin, daß der Einfluß des pH-Wertes auf die Bekämpfung der Fusarium-Welke bei Nelken durch *Pseudomonas fluorescens* WCS417r sortenabhängig ist. Bei der anfälligen Sorten Lena wurde die Fusaium-Welke durch WCS417r-Antagonismus am besten bei pH 7,5 unterdrückt. Bei der mäßig resistenten Sorten Pallas wurde die Fusarium-Welke sowohl durch Antagonismus als auch durch induzierte Resistenz mit WCS417r unterdrückt. Die induzierte Resistenz war auch bei niedrigeren pH-Werten wirksam.

Introduction

Fusarium wilt of carnation is suppressed by *Pseudomonas* strain WCS417r (Duijff et al., 1993; Van Peer et al., 1990, 1991). The pathogen, *Fusarium oxysporum* f.sp. *dianthi*, is antagonized by WCS417r by siderophore-mediated competition for iron and by a non-siderophore antifungal factor (Duijff et al., 1993). Apart from antagonism, fusarium wilt in carnation is suppressed by induction of resistance by WCS417r, as was shown if the roots were treated with WCS417r and the pathogen was inoculated directly into the stem (Duijff et al., 1993; Van Peer et al., 1991). The outer-membrane lipopolysaccharides of WCS417r were demonstrated to be involved in triggering induced resistance in carnation against fusarium wilt (Van Peer and Schippers, 1992). Resistance against Fod was consistently induced by WCS417r in the moderately resistant carnation cultivar Pallas, but only occasionally in the susceptible cultivar Lena (Duijff et al., 1993; Van Peer et al., 1991). Because of its multiple mechanisms of disease suppression, strain WCS417r is a promising strain for practical application in control of fusarium wilt of carnation. The success of this practical application will in part depend on the range of environmental conditions at which WCS417r effectively suppresses the wilt disease.

Providing favourable environmental conditions for activity of biocontrol agents can increase the success of biological control of soil-borne plant pathogens by selected antagonists (Baker, 1986; Harman and Lumsden, 1990). Among other environmental factors, soil pH plays an important role in plant-pathogen (Colhoun 1973; Schuerger and Mitchell, 1992) and antagonist-pathogen interactions (Park et al., 1988; Scher and Baker, 1980). Fusarium wilt diseases are most severe in slightly acid soils (Woltz and Jones, 1981). Raising the soil pH towards or above neutrality is used as a method for cultural control of fusarium wilt diseases (Engelhard et al., 1989). Biological control of fusarium wilt diseases by fluorescent pseudomonads was

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reduced or eliminated if soils were acidified below neutral pH (Park et al., 1988; Scher and Baker, 1980). The control of fusarium wilt diseases in those studies was ascribed to siderophore-mediated competition for iron. The lower suppressiveness of the soil at a lower pH was attributed to an increase of iron availability and consequently a decrease of antagonistic activity by the pseudomonads.

In the present study, we investigate the influence of the pH of the nutrient solution on control of fusarium wilt in carnation, grown in rockwool, by *Pseudomonas* strain WCS417r. FeEDDHA was used as an iron source to maintain iron in solution independent on the pH of the carnation nutrient solution (Lindsay and Schwab, 1982).

Material and Methods

Micro-organisms and preparation of bacterial and conidial suspensions

The rifampicin resistant *Pseudomonas* strain WCS417r (Lamers et al., 1988; Van Peer et al., 1990) and a Tn5 mutant of this strain defective in siderophore biosynthesis (sid$^-$): S680 (Duijff et al., 1993) were used in this study. Strain WCS417r was identified as *Pseudomonas fluorescens* with a probability of 99% with API 20 NE diagnostic strips (bioMérieux, s'-Hertogenbosch, The Netherlands) followed by tests for sorbitol assimilation and xylose acidification (Bergey, 1984). Inoculum of *Pseudomonas* was prepared from cultures grown for 2 days at 27°C on King's B agar (King et al., 1954) that were washed twice and suspended in sterile tap water.

The carnation wilt pathogen used is *Fusarium oxysporum* f.sp. *dianthi* (Fod) race 2 isolate WCS816 (Baayen and Elgersma, 1985). Inoculum of Fod was prepared from potato dextrose agar (Difco, Detroit, USA) cultures, grown for 7 days at 23°C, from which the conidia were harvested in sterile tap water and filtered through glasswool to remove mycelial fragments.

For root treatment of carnations the bacterial and conidial suspensions were diluted in carnation nutrient solution (described below) that was adjusted to the pH of the treatment (5.5, 6.5 or 7.5).

Plant nutrient solution and culture medium

A carnation nutrient solution (CNS) described by De Voogt (1986) and modified for the iron source, had the following composition: 4.75 mM $Ca(NO_3)_2.4H_2O$, 0.5 mM NH_4NO_3, 5 mM KNO_3, 1.75 mM KH_2PO_4, 0.25 mM K_2SO_4, 1.25 mM $MgSO_4$, 10 μM $MnSO_4$, 4 μM $ZnSO_4$, 6 μM $Na_2B_4O_7$, 0.5 μM $CuSO_4$, 0.5 μM $Na_2MoO_4.2H_2O$. The influence of pH on Fe(III)-availability was minimized by using 10 μM 80% ferrated ($FeCl_3$) ethylenediamine[di(o-hydroxyphenyl)acetic]acid (EDDHA) (Sigma, Bornem, Belgium) as a pH-independent iron source.

The culture medium that was used for *in vitro* experiments consisted of CNS, buffered with 0.1 M MES (2-[N-morpholino] ethane sulfonic acid) (Sigma) and supplemented with 0.059 μM thiamine (Merck, Darmstad, Germany), 0.0041 μM biotin (Merck), 1% (w/v) casamino acids (Difco) and 1% (w/v) sucrose (Merck) according to the RS-medium described by Buyer et al. (1989). FeEDDHA, $MnSO_4$, $ZnSO_4$, $CuSO_4$, $Na_2MoO_4.2H_2O$, biotin and thiamine were added filter-sterilized (Millipore, 0.2 μm). Casamino acids, sucrose and KH_2PO_4 were autoclaved separately. This medium is referred to as carnation nutrient solution medium (CNSM).

The pH of CNS and of CNSM was adjusted with H_2SO_4(0.1 M) or KOH (0.1 M).

Influence of pH on conidial germination of Fod

Erlenmeyer flasks (100 ml) filled with 30 ml of sterile CNSM, adjusted to pH 5.5, 6.5 and 7.5, were inoculated with 3 ml of a suspension of 8×10^5 conidia of Fod in sterile tap water. After incubation for 4 and 8 h at 22°C with mechanical agitation, percent germination was estimated microscopically from 100 conidia per flask. The germ-tube length was estimated from 20 germinated conidia per flask after incubation for 8 h, using a microscope in combination with a semi-automatic image analyser (Morphomat 10, Carl Zeiss BV. The Netherlands). Each treatment consisted of three replicate Erlenmeyer flasks. The experiment was repeated once with similar results.

Influence of pH on biomass and siderophore production by WCS417r

Erlenmeyer flasks (100 ml) filled with 30 ml of sterile CNSM, adjusted to pH 5.5, 6.5, and 7.5, were inoculated with 100 μl of a suspension of 10^8 cells of WCS417r ml^{-1} sterile tap water and incubated at 24°C under mechanical agitation. During the incubation period, samples were taken from the cultures and OD_{660} was measured. After incubation for 25 h, cultures were centrifuged (10 000 rpm, 10 min), the supernatants were adjusted to pH 7 and siderophore concentration was measured spectrophotometrically at 400 nm (Meyer and Abdallah, 1978). The pellets of the cultures were lyophilized and weighed. The siderophore production by WCS417r was quantified as the absorbance of the supernatant at 400 nm per g of biomass production per litre. Each treatment consisted of four replicate Erlenmeyer flasks. The experiment was repeated once with similar results.

Influence of pH on *in vitro* inhibition of Fod by WCS417r

Agar plates were prepared from CNS medium, adjusted to the appropriate pH, and 15 g of technical agar (Oxoid, Basingstoke, UK) L^{-1}. These agar plates were inoculated at three spots with about 1 μl of a suspension of strain WCS417r or S680 diluted in sterile tap water to a concentration of 10^8 cells ml^{-1}. After incubation for 48 h at 27°C, a suspension of 10^7 conidia of Fod ml^{-1} sterile tap water was atomized over the plates. After incubation for 48 h at 23°C, antagonism was quantified as the ratio of the width of the zone without fungal growth (mm), surrounding the *Pseudomonas* colony, and the diameter of the inhibiting *Pseudomonas* colony (mm). Each treatment consisted of three replicate agar plates. The experiment was repeated twice with similar results.

Influence of pH on control of fusarium wilt by WCS417r

Commercial cuttings of carnation (*Dianthus caryophyllus* L.) cultivars Lena and Pallas (Van Staaveren BV, Aalsmeer, The Netherlands), rooted in rockwool granulate plugs, were planted in 365 cm^3 rockwool cubes (Rockwool/Grodan BV, Roermond, The Netherlands) that were saturated with CNS. Cultivar Lena is susceptible and cv. Pallas is moderately resistant to race 2 of Fod (Niemann and Baayen, 1988). For pH treatments 5.5 and 6.5, rockwool cubes were saturated with nutrient solution adjusted to pH 5.0 and 6.0, respectively, to compensate for the initial pH increase caused by the rockwool material. For pH treatment 7.5, nutrient solution adjusted to pH 7.5 was used for saturating the rockwool cubes.

One day after planting, plants were treated with *Pseudomonas* by pouring 10 ml of a suspension of 5×10^8 cells ml^{-1} CNS on the plugs of the rockwool cubes. Eleven days later, plants were treated with *Pseudomonas* once again by pouring 20 ml of a suspension of 5×107 cells ml^{-1} CNS on the rockwool plugs. Control plants were treated with CNS adjusted to the appropriate pH.

Two weeks after planting, plants were inoculated with Fod by pouring 10 ml of a suspension of 2×10^6 conidia of Fod ml^{-1} CNS on the rockwool plugs. To study the influence of pH on induced resistance by WCS417r, Fod and WCS417r were spatially separated, to exclude direct antagonistic interactions between WCS417r and the pathogen (Van Peer et al., 1991). For this, plants of cv. Pallas were stem-inoculated with 20 μl of 10^7 conidia of Fod ml^{-1} tap water, as described by Baayen and Elgersma (1985), 3 weeks after the first treatments of the cuttings with WCS417r. Four days before stem-inoculation, plants did not receive any water or nutrient solution and water content of the rockwool cubes decreased to about 30% of the total water-holding capacity of the rockwool cube. Directly after stem-inoculation, plants were watered again.

Plants were watered two times a week, once with tap water and once with CNS, both adjusted to pH 5.5, 6.5 or 7.5. During the experiments, pH and electric conductivity (EC) of the nutrient solution in the rockwool cubes was measured. When the pH of the nutrient solution

increased, plants were watered with tap water or CNS adjusted to a pH 0.5 to 1 unit lower than the appropriate pH. The nutrient gift was intensified or reduced if EC was below or above 2 ms cm^{-1}, respectively. The concentration of soluble iron in the nutrient solution was determined by addition of three drops of [3-(2-pyridil-5,6-bis(phenylsulfonic acid)-1,2,4 triazinedisodium] in ammonium thioglycolate ('Ferrospectral 14761', Merck) to a 5 ml sample and measured spectrophotometrically at 562 nm. Biweekly or when the concentration of soluble iron in the nutrient solution exceeded the desired iron concentration of 8 μM, FeEDDHA was left out of the CNS. Plants were grown in a glasshouse with a photoperiod of 16 h and a temperature of 22°C during the light period and 18°C during the dark period. For recording of disease incidence in cv. Lena, 6 replicates of 5 or 6 plants each were used and in cv. Pallas, 7 replicates of 5 or 6 plants each were used. The experiments were terminated 15 weeks after stem-inoculation, or 16 weeks after root-inoculation with Fod, when disease incidence no longer increased. Plants were considered to be diseased at wilt index 2 or higher, according to the disease rating scale for fusarium wilt symptoms in carnation used by Baayen and Niemann (1989). At the end of the experiment, disease rating was checked by observation of the stem for brown discolouration of the xylem vessels (Baayen and Elgersma, 1985). Isolation of *Fusarium oxysporum* from the stem tissue corresponded with the brown discolouration of the xylem vessels.

Three plants per treatment of cv. Pallas, that were stem-inoculated, were examined at the time of inoculation and at 5 and 12 weeks after inoculation with Fod for the presence of WCS417r in or on the stem of the plant. The entire carnation stem, starting from 2 cm below the site of inoculation with Fod, was examined. Stem surface colonization by the introduced bacteria was examined by pressing 5 cm stem segments into KB^+ agar (Geels and Schippers, 1983) supplemented with 150 ppm rifampicin (KB^+ rif). Internal colonization was examined by pressing slices of the surface-disinfected stem (flamed after dipping in 70% ethanol) into the KB^+ rif agar. Slices were cut at the point of inoculation with Fod, at 1 cm below, and at 3 and 5 cm above this point and also further upward at each 5 cm interval. Bacterial growth was checked after incubation of the agar plates for 72 h at 27°C.

The experiments were repeated once with similar results for disease suppression in cv. Lena and similar tendencies for disease suppression in cv. Pallas.

Root colonization

At 1, 3, 7 and 16 weeks after the second treatment of the roots with WCS417r, about 300 mg of roots per plant from three plants per treatment were sampled and vortexed for 30 s in glass test tubes containing 5 ml 0.1 M $MgSO_4$ and 1 g of glass beads (0.18 mm diameter). The suspensions were diluted and plated on KB^+ rif agar for estimating colony forming units (CFU) of strain WCS417r. Samples were also plated on KB^+ agar for estimating CFU of pseudomonads, on tryptic soy agar supplemented with 100 ppm cycloheximide for estimating CFU of aerobic bacteria and on Komada agar (Komada, 1975) supplied with 0.2% (v/v) solacol (Aagrunol BV, Haren, The Netherlands) (Gams and Van Laar, 1982) for estimating CFU of *Fusarium oxysporum*. Root colonization was expressed as the log value of CFU per gram of root fresh weight.

Data analysis

Data were analysed by analysis of variance, followed by mean separation with least significant difference (LSD) at P = 0.05 (Sokal and Rohlf, 1981). Alternatively, 95%-confidence intervals of the means were calculated (Sokal and Rohlf, 1981). Data of representative experiments are presented.

Results

Influence of pH on conidial germination of Fod

After incubation for 4 h, significantly fewer conidia of Fod germinated in CNS medium at pH 7.5 and 6.5 than at pH 5.5 (Table 1). After incubation for 8 h, conidial germination was significantly less at pH 7.5 than that at pH 6.5 and 5.5. The germ-tube growth of the germinated conidia was significantly reduced with each unit increase of pH of the CNS medium (Table 1).

Table 1
Germination and germ-tube growth of conidia of *Fusarium oxysporum* f.sp. *dianthi* WCS816 in CNS medium at different pH, after incubation for 4 or 8 h at 22°C

	Germinated conidia (%)		Germ-tube length (μm)
pH	t = 4 h	t = 8 h	t = 8 h
5.5	19 b[a]	84 b	31 c
6.5	10 a	84 b	22 b
7.5	5 a	38 a	13 a

[a] Values within a same column and with a different letter differ significantly (P = 0.05).

Table 2
Biomass and siderophore production by *Pseudomonas fluorescens* WCS417r in CNS medium at different pH, after incubation for 25 h at 24°C

pH	Biomass (g dry wt L^{-1})	Siderophores (OD_{400} g^{-1} dry wt L^{-1})
5.5	1.89 a[a]	0.3 a
6.5	2.78 b	0.4 a
7.5	2.89 b	0.9 b

[a] Values within a same column and with a different letter differ significantly (P = 0.05).

Influence of pH on biomass and siderophore production by WCS417r

Both *in vitro* biomass and siderophore production by WCS417r increased with increase of the pH of the CNS medium (Table 2).

Influence of pH on *in vitro* inhibition of Fod by WCS417r

At pH 5.5, growth of WCS47r and its sid^- mutant S680 on CNSM agar plates was poor and both strains did not inhibit Fod at this pH (Table 3). At pH 6.5 and 7.5, Fod was inhibited by both S680 and WCS417r. Inhibition of Fod by WCS417r significantly increased with increase of pH from 6.5 to 7.5. Inhibition of Fod by the sid^- mutant S680 did not differ between pH 6.5 and 7.5.

Table 3
Antagonism of *Fusarium oxysporum* f. sp. *dianthi* by *Pseudomonas fluorescens* WCS417r and its sid^- mutant S680 on CNSM agar plates at different pH. Antagonism is quantified as the ratio of width of the zone with no fungal growth (mm), surrounding the *Pseudomonas* colonies, and the diameter of the inhibiting *Pseudomonas* colony (mm)

	Antagonism (arbitrary units)	
pH	S680 (sid^-)	WCS417r
5.5	0	0
6.5	1.38 ± 0.12[a]	1.64 ± 0.08
7.5	1.42 ± 0.10	2.00 ± 0.16

[a] 95%-Confidence interval of the mean.

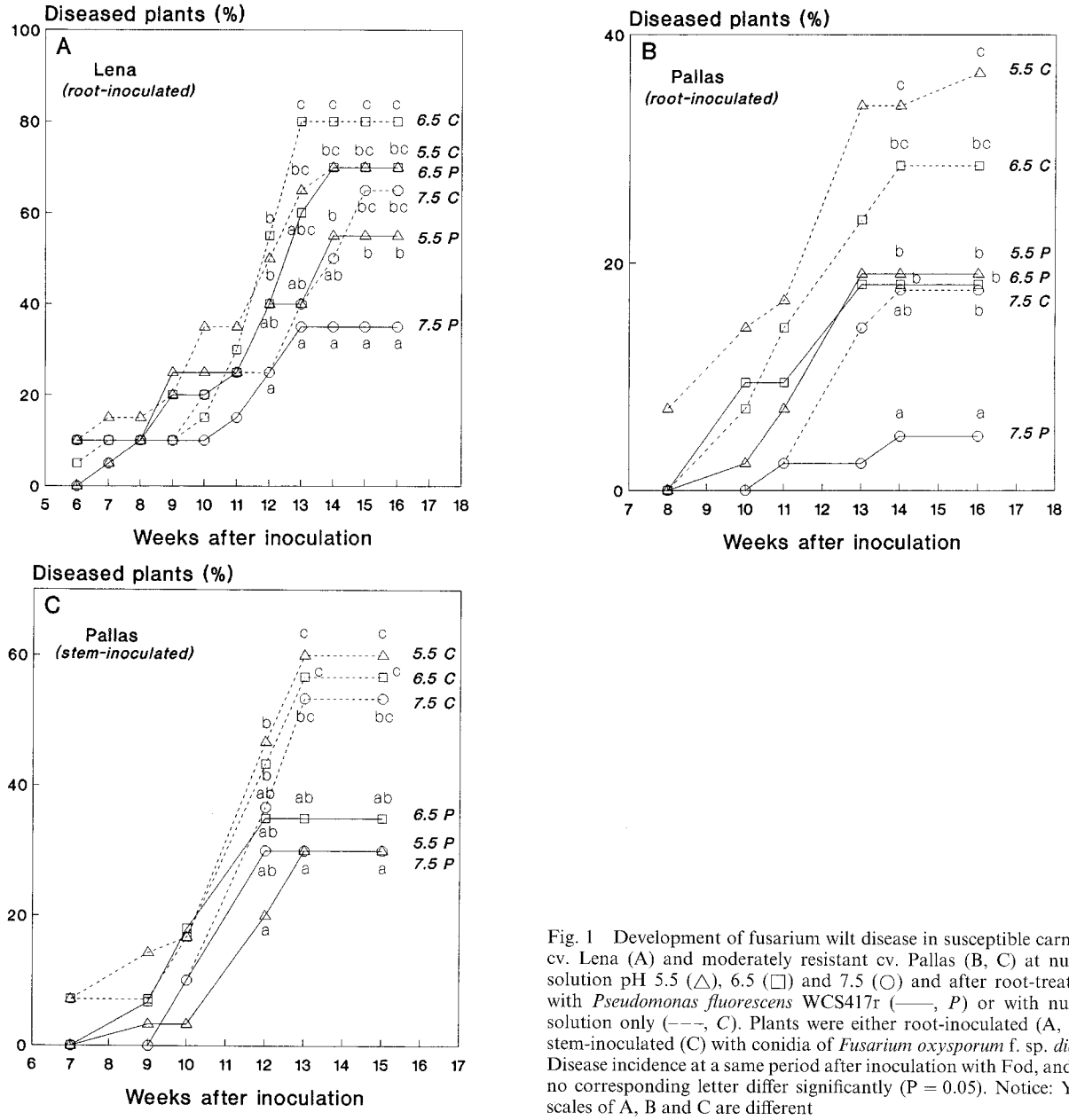

Fig. 1 Development of fusarium wilt disease in susceptible carnation cv. Lena (A) and moderately resistant cv. Pallas (B, C) at nutrient solution pH 5.5 (△), 6.5 (□) and 7.5 (○) and after root-treatment with *Pseudomonas fluorescens* WCS417r (——, *P*) or with nutrient solution only (– – –, *C*). Plants were either root-inoculated (A, B) or stem-inoculated (C) with conidia of *Fusarium oxysporum* f. sp. *dianthi*. Disease incidence at a same period after inoculation with Fod, and with no corresponding letter differ significantly (P = 0.05). Notice: Y-axis scales of A, B and C are different

Influence of pH on reduction of fusarium wilt of carnation by WCS417r

The pH of the nutrient solution in the rockwool cubes tended to rise. During the experiments, mean pH values of the nutrient solution with their standard deviations were: 6.01 ± 0.37 in the treatment adjusted to pH 5.5, 6.85 ± 0.22 in the treatment adjusted to pH 6.5 and 8.03 ± 0.23 in the treatment adjusted to pH 7.5.

Treatment of susceptible carnation cv. Lena with WCS417r reduced disease incidence significantly from 65% to 35% diseased plants at pH 7.5 at 15 and 16 weeks after inoculation of the rockwool cubes with Fod, but not at pH 5.5 and 6.5 (Fig. 1A).

Influence of pH on disease incidence in the moderately resistant cv. Pallas, was studied in plants that were root-inoculated with Fod and in plants that were stem-inoculated with Fod. Disease development in the control treatment of the root-inoculated plants decreased with increase of pH (Fig. 1B). At 14 weeks after inoculation with Fod, disease incidence was significantly reduced by WCS417r at pH 5.5, and at 16 weeks after inoculation at pH 5.5 and 7.5 (Fig. 1B).

In the stem-inoculated plants increase of pH tended to decrease disease incidence only slightly in the control treatment (Fig. 1C). Treatment with WCS417r significantly reduced disease incidence at 13 and 15 weeks after inoculation and was not influenced by pH (Fig. 1C).

From the Fod stem-inoculated plants, that were treated with WCS417r, WCS417r was re-isolated only once from the stem tissue at 1 cm below the point of inoculation at 5 weeks after inoculation with Fod.

Root colonization

Root colonization by WCS417r or Fod was influenced neither by the pH of the nutrient solution, nor by the cultivar of the host-plant. The average number of colony forming units (CFU) per gram of root fresh weight of WCS417r decreased in time

from log 5.8 at 1 week, to log 4.2 at 7 weeks and to log 2.9 at 16 weeks after the second root treatment with WCS417r. Expressed as percentages of the total *Pseudomonas* population, the average number of CFU of WCS417r decreased from about 30% at 1 week to 1.4% after 7 and to 0.06% at 16 weeks after the second treatment with WCS417r.

The average number of CFU of *Fusarium oxysporum* was not affected by treatment with WCS417r and decreased in time from about log 4.0 at 3 days to log 3.2 at 15 weeks after inoculation with the conidial suspension of Fod.

Discussion

Conidial germination and growth of the germ tubes of *Fusarium oxysporum* f.sp. *dianthi* (Fod) was inhibited with increase of pH of the culture medium (Table 1). A reduction in the availability of essential nutrient elements like phosphorus, magnesium, iron, manganese, molybdenum and zinc could have reduced the development of Fod at higher pH (Woltz and Jones, 1981; Engelhard et al., 1989). In the present study, however, it is unlikely that iron availability is involved in the reduction of conidial germination and germ-tube growth of Fod, because iron availability was buffered by FeEDDHA. Chelation of iron and maintenance of iron in solution by EDDHA is not influenced by pH between pH 4 and 10 (Lindsay and Schwab, 1982). This was confirmed for 10 μM 80% ferrated EDDHA in carnation nutrient solution at pH values between 4.5 and 8.5 (B. J. Duijff, unpublished results).

Although *in vitro* conidial germination of Fod was significantly influenced by pH, the disease development in the control treatment of Fod root-inoculated plants was only significantly influenced by pH in the moderately resistant cv. Pallas (Fig. 1B), and not in the susceptible cv. Lena (Fig. 1A). Obviously, increase of pH on its own only reduced disease incidence effectively if disease development was also slowed down by the resistance of the host-plant.

Treatment with WCS417r, significantly suppressed fusarium wilt in cv. Lena at pH 7.5 and only tended to reduce disease incidence at pH 5.5 and 6.5 (Fig. 1A). This corresponds with the highest *in vitro* siderophore production by WCS417r (Table 2) and the highest antagonistic activity of WCS417r against Fod at pH 7.5 (Table 3). The antagonistic activity of the sid^- mutant of WCS417r, that inhibits Fod *in vitro* by a non-siderophore antifungal factor (Duijff et al., 1993), was not influenced by an increase of pH from 6.5 to 7.5 (Table 3). The less effective disease reduction of fusarium wilt by WCS417r in susceptible cv. Lena at pH 6.5 and 5.5 thus can be explained with a reduced siderophore production by WCS417r. Moreover, next to a decrease of siderophore production, the binding affinity for ferric iron of the hydroxamate/catechol siderophores produced by *Pseudomonas* also decreases with decrease of pH (Meyer and Abdallah, 1978). The affinity for ferric iron of the hydroxamate siderophores produced by *Fusarium*, however, is less influenced by decrease of pH (Buyer and Sikora, 1990; Winkelmann, 1992). Therefore, a decrease of pH will benefit Fod in the siderophore-mediated competition between WCS417r and Fod.

Suppression of fusarium wilt by WCS417r in root-inoculated moderately resistant cv. Pallas was not influenced by pH of the nutrient solution (Fig. 1B). This can be explained with the occurrence of pH-independent induced resistance by WCS417r against Fod in cv. Pallas (Fig. 1C). Induction of resistance by WCS417r against fusarium wilt in stem-inoculated carnation has been demonstrated consistently in the moderately resistant cv. Pallas, and only occasionally in the susceptible cv. Lena (Duijff et al., 1993; Van Peer et al., 1991). The occurrence of induced resistance by WCS417r in root-inoculated cv. Pallas was demonstrated with the suppression of fusarium wilt in cv. Pallas by WCS417r in a split-root system (E. Hoffland, unpublished results).

In the carnation nutrient solution, 3.3% of total nitrogen is added as ammonium and the remaining nitrogen as nitrate (De Voogt, 1986). Although with such a nitrogen composition pH should be buffered by the H^+ and HCO_3^- efflux due to uptake of ammonium and nitrate by the plant, respectively (Nye, 1981; Ruys, 1989), pH of the nutrient solution tended to rise about 0.5 pH unit. Probably, this increase of pH could have been prevented with a larger amount of ammonium in the total nitrogen supply. Recently, Gahoonia and Nielsen (1992) reported that rhizosphere pH of rape was kept constant if 6% of total nitrogen was supplied as ammonium.

The pH of the nutrient solution did not influence root colonization by WCS417r. Also Wüthrich et al. (1991) did not find any difference in colonization by *P. fluorescens* CHA0 of roots of gnotobiotic grown wheat plants if pH of the rhizosphere was decreased from about pH 7.5 to 6.4. Probably in these pH ranges, pH of the substrate or rhizosphere plays a limited and not detectable role in root colonization of *Pseudomonas* sp. that are introduced into the rhizosphere in high numbers. Yuen and Schroth (1986) showed that *P. fluorescens* strain E6 colonized roots of zinnias grown in soil with pH 4.9 or 5.9 in lower numbers than roots of zinnias grown in soil with pH 6.8 or 7.2. However, these soils also differed in other characteristics like texture and water holding capacity.

It is concluded that if antagonism is the mode of action in disease suppression by WCS417r, as for the susceptible cv. Lena, reduction of fusarium wilt in carnation by WCS417r is most effective at slightly alkaline conditions. If induced resistance is involved in disease suppression by WCS417r, as it is in the moderately resistant cv. Pallas, suppression of fusarium wilt in carnation by WCS417r is effective both at slightly alkaline and slightly acid conditions in the root zone.

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