Suppression of Fusarium Wilt of Carnation by *Pseudomonas putida* WCS358 at Different Levels of Disease Incidence and Iron Availability

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Treatment with *Pseudomonas putida* WCS358r, a rifampicin-resistant derivative of strain WCS358, significantly reduced fusarium wilt of carnation grown in rockwool if disease incidence was moderate, but not if disease incidence was high. Differences in disease incidence could intentionally be established by varying the inoculum density of the pathogen Fusarium oxysporum f. sp. dianthi (Fod). The effectiveness of disease suppression by WCS358r increased with decrease of inoculum density and consequently decrease of disease incidence. WCS358r and a Tn5 marked derivative of WCS358 (B243) reduced fusarium wilt of carnation most effectively if a low iron availability for the pathogen was established by adding unferrated or only partially ferrated ethylenediamine [dil(o-hydroxyphenylacetic) acid]. A Tn5 mutant of WCS358 defective in siderophore biosynthesis (JM218) did not reduce disease incidence. Siderophore production and inhibition of Fod by WCS358r in vitro decreased with increasing iron availability, supporting the more effective disease suppression by strains WCS358r and B243 at low iron availability. Siderophore-mediated competition for iron was shown to be the mechanism of suppression of fusarium wilt of carnation by *P. putida* WCS358. Its effectiveness was highest at a low iron availability and at a moderate disease incidence.

**Keywords:** biological control, disease incidence, Fusarium oxysporum f. sp. dianthi, iron availability, *Pseudomonas putida*, siderophores

**INTRODUCTION**

Under iron-limiting conditions, microorganisms produce siderophores, i.e. low molecular weight compounds that chelate ferrie iron and transport it into the microbial cell (Neilands, 1981). The pyoverdines or pseudobactins, siderophores produced by root-colonizing fluorescent pseudomonads, have received much attention because of their possible role in biological control of soil-borne plant pathogens by competition for iron (Loper & Buyer, 1991; O’Sullivan & O’Gara, 1992; Schippers, 1992).

*Pseudomonas putida* strain WCS358 produces a siderophore, pseudobactin 358 (PSB358), that
is highly specific for iron uptake by WCS358 (Bakker et al., 1993). Production of PSB358 is involved in the increase of potato tuber yield in high frequency potato cropping by strain WCS358 (Bakker et al., 1986). Duijff et al. (1993) suggested that siderophore-mediated competition for iron is the mechanism responsible for suppression of fusarium wilt of carnation, caused by Fusarium oxysporum f. sp. dianthi, by strain WCS358. However, WCS358 did not significantly reduce fusarium wilt of carnation if the saprophytic microbial activity of the soil was reduced by steaming and disease incidence was high (Duijff et al., 1991). Lemanceau et al. (1992) showed that PSB358 production by WCS358 suppressed fusarium wilt of carnation only if WCS358 was applied in combination with a saprophytic Fusarium oxysporum strain.

The studies of Duijff et al. (1991) and Lemanceau et al. (1992) suggest that siderophore production by WCS358 on its own is not effective enough to reduce fusarium wilt disease of carnation significantly. However, in these studies, disease incidence was high. Moreover, iron availability was not controlled and may not have supported effective siderophore-mediated competition for iron between WCS358 and the pathogen. In the present study, the effectiveness of strain WCS358 to suppress fusarium wilt of carnation is evaluated at different levels of disease incidence and at different levels of iron availability.

MATERIALS AND METHODS

Microorganisms and Preparation of Bacterial and Conidial Suspensions
A rifampicin-resistant derivative of P. putida WCS358: WCS358r (Geels & Schippers, 1983) and transposon Tn5 mutants of WCS358: JM218 and B243 were used. WCS358r does not differ from WCS358 with regard to rhizosphere competence, growth rate (Glandorf et al., 1992), siderophore production and in vitro antagonism of Fod (B.J. Duijff, unpublished results). JM218 is defective in siderophore biosynthesis (sid−) (Marugg et al., 1985). The Tn5 mutant B243 does not differ from its parental strain with regard to siderophore production and growth rate (Bakker et al., 1986).

The carnation wilt pathogen used is Fusarium oxysporum f. sp. dianthi (Fod) race 2 isolate WCS816 (Baayen, 1986).

Suspensions of WCS358r and the Tn5 mutants of WCS358 were prepared from cultures grown for 48 h at 27°C on King’s medium B (KB) agar (King et al., 1954). Pseudomonas cells were harvested in 0.01 M-MgSO4, washed twice in 0.01 M-MgSO4, collected by centrifugation (20 min, 1247 × g), and then suspended in 0.01 M-MgSO4.

Conidial suspensions of Fod were prepared from potato dextrose agar cultures that were grown for 7 days at 23°C. Conidia were harvested in 0.01 M-MgSO4, and the suspension was filtered through glasswool to remove mycelial fragments.

Bacterial and conidial suspensions used for root treatment of carnations were diluted in carnation nutrient solution (CNS) with the appropriate (Fe) ethylenediamine [di(o-hydroxyphenylacetic) acid] (EDDHA) treatment.

Fusarium Inoculum Density and Iron Availability
Cutivars of carnation (Dianthus caryophyllus L.) cultivar Lena, rooted in rockwool granulate plugs, were obtained from van Staaveren BV (Aalsmeer, The Netherlands). Cultivar Lena is susceptible to race 2 of Fusarium oxysporum f. sp. dianthi (Niemann & Baayen, 1988). The cutivars were planted in 365 cm3 rockwool cubes (Rockwool/Grodan BV, Roermond, The Netherlands), saturated with CNS (De Voogt, 1986) that was adjusted to pH 7 and modified for the iron source. The nutrient solution had the following composition: 4.75 mM Ca(NO3)2·4H2O, 0.5 mM NH4NO3, 5 mM KNO3, 1.75 mM KH2PO4, 0.25 mM K2SO4, 1.25 mM MgSO4, 10 μM MnSO4, 4 μM ZnSO4, 6 μM Na2B4O7, 0.5 μM CuSO4, 0.5 μM Na2MoO4·2H2O. Iron was supplied as 10 μM 80% ferrated (FeCl3), EDDHA (Sigma, Bornem, Belgium). In the experiments to study the influence of iron availability, iron was supplied as 10 μM EDDHA ferrated for 0, 50 or 100%.

One day after planting, plants were treated with bacteria by pouring 10 ml of a suspension of
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5 × 10⁶ Pseudomonas cells per millilitre of CNS on the plugs of the rockwool cubes. Eleven days later, this was repeated with 20 ml of a suspension of 5 × 10⁷ Pseudomonas cells per millilitre. Control plants were treated with 0.01 m-MgSO₄ diluted in CNS with the appropriate (Fe)EDDHA treatment.

Two weeks after planting, plants were inoculated with Fod by pouring 10 ml of a suspension of 2 × 10⁶ conidia of Fod per millilitre of CNS on the rockwool plugs. In the experiments to study the influence of inoculum density on disease suppression by P. putida WCS358, plants were inoculated with 10 ml of a suspension of 2 × 10⁵, 2 × 10⁶ or 2 × 10⁷ conidia per millilitre of CNS.

Plants were watered twice a week, once with tap water and once with CNS, both adjusted to pH 7. The nutrient supply was intensified or reduced if the electric conductivity of the solution in the rockwool cubes was below or above 2 mS, respectively. About once per two weeks, 10 μM-(Fe)EDDHA was added to the CNS. The concentration of EDDHA in the nutrient solution of the rockwool cubes was measured spectrophotometrically. This was done by saturating the EDDHA in a nutrient solution sample with excess FeCl₃ and measuring the absorbance of the resultant FeEDDHA at 450 nm. If EDDHA concentrations exceeded 12 μM, the supply of (Fe)EDDHA was reduced. The plants treated with 100% ferrated EDDHA also received 2 μM-FeCl₃ with every supply of water or nutrient solution, to keep EDDHA saturated with iron and the iron availability high. During the experiments, soluble iron in the nutrient solution of the rockwool cubes was determined spectrophotometrically by the addition of three drops of the reagent Ferrospectral 14761 (3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4 triazinedisodium) in ammonium thioglycolate (Merck, Darmstadt, Germany) to a 5-ml sample of the nutrient solution and measuring the absorbance of the resulting complex at 565 nm.

Plants were grown in a glasshouse with a photoperiod of 16 h at 22°C, and at 18°C during the dark period. The number of diseased plants was recorded weekly. 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). Disease incidence was assessed from replicates of six plants each. The number of replicates per treatment was different in the different experiments: seven replicates were used in the experiments with one Fod inoculum density (I); four replicates were used in the experiment with three different Fod inoculum densities (II); ten replicates were used in the experiments with different iron availability and different Fod inoculum densities (III). Experiments I and III were performed twice. Experiment II was not repeated as such, but the influence of inoculum density was repeated in experiment III. The experiments were ended 14 to 16 weeks after inoculation with Fod, when disease incidence no longer increased.

Root Colonization
At regular intervals, approximately 300 mg of roots per plant were sampled from four plants per treatment. The root samples were shaken (Vortex) for 30 s in glass test-tubes containing 5 ml of 0.1 m-MgSO₄ and approximately 1 g of glass beads (0.18 mm diameter). The suspensions were diluted and plated on KB⁺ agar (Geels & Schippers, 1983) supplemented with 150 ppm rifampicin for estimating the number of colony forming units (CFUs) of strain WCS358r per gram of root fresh weight, or on KB⁺ supplemented with 200 ppm kanamycin sulphate and 200 ppm streptomycin sulphate for estimating the numbers of CFUs of Tn5 mutants JM218 and B243. Samples were also plated on KB⁺ agar for estimating the total Pseudomonas population, on tryptic soy agar supplemented with 100 ppm cycloheximide for estimating the total aerobic bacterial population, and on Komada agar (Komada, 1975) modified as described by Gams and van Laar (1982) for estimating the total number of CFUs of Fusarium oxysporum. After incubation for 48 h at 27°C the numbers of CFUs were determined. In some samples the numbers of CFUs of the introduced Pseudomonas strain or Fusarium oxysporum were below detection level. These data were transformed to CFU + 1 to enable statistical analysis (Sokal & Rohlf, 1981).
Iron Availability and Siderophore Production by WCS358r

The culture medium that was used for in vitro experiments consisted of CNS, buffered with 0.1 M-HEPES (N-[2-hydroxyethyl]piperezine-N'-[2-ethanesulfonic acid]) (Sigma) and supplemented with 0.059 μM-thiamine (Merck), 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). MnSO₄, ZnSO₄, CuSO₄, Na₂MoO₄·2H₂O, biotin and thiamine were added filter sterilized (Millipore, 0.2 μm). Casamino acids, sucrose and KH₂PO₄ were autoclaved separately. This medium, adjusted to pH 7, is referred to as carnation nutrient solution medium (CNSM).

Erlenmeyer flasks (100 ml) were filled with 25 ml of sterile CNSM. As an iron source, 10 μM filter-sterilized (Millipore, 0.2 μm) EDDHA, ferrated for 0, 50 or 100%, was added. The Erlenmeyer flasks were inoculated with about 5 × 10⁷ cells of WCS358r and incubated for 48 h at 25°C under mechanical agitation. Growth of the Pseudomonas cultures was measured spectrophotometrically at 660 nm at intervals during the incubation period.

After incubation for 48 h, when cultures of all treatments had reached the stationary phase, they were centrifuged (7796 × g, 10 min), supernatants were adjusted to pH 7 and siderophore concentration was determined spectrophotometrically at 400 nm (Meyer & Abdallah, 1978). The cell pellets of the cultures were lyophilized and weighed. Siderophore production by WCS358r was expressed per gram of biomass production. Each treatment consisted of three replicate Erlenmeyer flasks. The experiment was repeated once with similar results.

Iron Availability and Inhibition of Fod by WCS358r

Agar plates were prepared from CNSM that was buffered with 0.1 M-HEPES (pH 7) and supplemented with 10 μM-EDDHA, ferrated for 0, 50 or 100%, and with 15 g of technical agar (Oxoid, Basingstoke, UK) per litre. The agar plates were spot-inoculated (three spots per plate) with 1 μl of a suspension of 10⁸ cells per millilitre of WCS358r, B243 or JM218. After incubation for 48 h at 27°C, a suspension of 10⁶ conidia of Fod per millilitre was atomized over the plates. After incubation for 32 h at 23°C, antagonism was quantified as the ratio of 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 four replicate agar plates. The experiment was repeated twice with similar results.

Data Analysis

Data were analyzed by analysis of variance, followed by mean separation with least significant difference (LSD) at P = 0.05 (Sokal & Rohlf, 1981).

RESULTS

Influence of Disease Incidence and Iron Availability on Disease Suppression

Treatment of carnation with WCS358r, with 80% ferrated EDDHA as an iron source, significantly reduced fusarium wilt if the disease incidence in the control treatment was moderate (52%) (Table 1). If disease incidence was high (74%), WCS358r had no effect. Treatment with sid⁻ mutant JM218 did not affect disease incidence.

To further test the effectivity of strain WCS358r at different levels of disease incidence, plants were inoculated with different inoculum densities of the pathogen. Disease incidence significantly increased with increase of inoculum density of Fod (Table 2). The relative disease reduction by WCS358r increased with decrease of inoculum density. However, in this experiment, disease suppression by WCS358r was not significant.

In a third experimental set-up, the suppression of fusarium wilt by WCS358 was investigated at two pathogen inoculum densities and at different levels of iron availability (Figure 1). The sid⁻ mutant JM218 again had no effect on disease incidence. WCS358r and Tn5 derivative B243 reduced disease incidence significantly, but only at low iron availability (0 and 50% ferrated EDDHA). The average of the relative disease reduction caused by WCS358r and B243 in the
three iron treatments increased from 14.5% at high inoculum density (2 × 10⁷ conidia of Fod per plant) to 32% at the low inoculum density (2 × 10⁶ conidia of Fod per plant).

Iron availability in the third experimental set-up changed during the time course of the experiment. Iron availability in the treatment with 100% ferrated EDDHA, however, was always significantly higher than the iron availability in the treatments with 0 or 50% ferrated EDDHA (Table 3).

**Root Colonization**

Root colonization by the sid⁻ mutant JM218 was not impaired compared with that by the siderophore-producing strains (Table 4). At 2 and 4 weeks after planting, the numbers of CFUs of the Tn5 derivatives were significantly higher than that of strain WCS358r. Iron availability did not significantly influence root colonization by the introduced *Pseudomonas* strains. The population density of *Fusarium oxysporum* (most probably Fod) on carnation roots increased with increase of inoculum density of Fod (Table 5). It was not influenced by iron availability or treatment with pseudomonads (data not shown).

### TABLE 1. Incidence of fusarium wilt in carnation cv. Lena after treatment with *P. putida* WCS358r or a Tn5 mutant of WCS358 defective in siderophore biosynthesis (JM218), 14 weeks after inoculation of the rockwool cubes with 2 × 10⁷ conidia of *Fusarium oxysporum* f. sp. dianthi per plant; iron was supplied as 80% ferrated EDDHA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diseased plants (%)</th>
<th>Exp. a</th>
<th>Exp. b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52b</td>
<td>74a</td>
<td></td>
</tr>
<tr>
<td>JM218 (sid⁻)</td>
<td>54b</td>
<td>72a</td>
<td></td>
</tr>
<tr>
<td>WCS358r</td>
<td>30a</td>
<td>71a</td>
<td></td>
</tr>
</tbody>
</table>

*Data are based on seven replicates of six plants each.
*Values within a same column and with a different letter differ significantly (*P = 0.05*).

### TABLE 2. Incidence of fusarium wilt in carnation cv. Lena after treatment with *P. putida* WCS358r, 14 weeks after inoculation of the rockwool cubes with different densities of conidia of *Fusarium oxysporum* f. sp. dianthi (Fod); iron was supplied as 80% ferrated EDDHA

<table>
<thead>
<tr>
<th>Conidia Fod per plant</th>
<th>Disease plants (%)</th>
<th>Relative disease reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>WCS358r</td>
</tr>
<tr>
<td>2 × 10⁶</td>
<td>33ab</td>
<td>17a</td>
</tr>
<tr>
<td>2 × 10⁷</td>
<td>58bc</td>
<td>42ab</td>
</tr>
<tr>
<td>2 × 10⁸</td>
<td>83c</td>
<td>83c</td>
</tr>
</tbody>
</table>

*Data are based on four replicates of six plants each.
*Values with no corresponding letter differ significantly (*P = 0.05*).
Influence of Iron Availability on Siderophore Production and Antagonism of Fod by WCS358r

Siderophore production by WCS358r in CNSM decreased and biomass production increased with increasing iron availability (Table 6). Inhibition of Fod on CNSM agar plates by WCS358r decreased with increasing iron availability (Table 6). Tn5 mutant B243 inhibited Fod equally to WCS358r. The sid+ mutant JM218 did not inhibit Fod (data not shown).

TABLE 3. Soluble iron in nutrient solutions of carnations grown in rockwool, influenced by 10 μM EDDHA, ferrated for 0, 50 or 100%, and at different weeks after planting

<table>
<thead>
<tr>
<th>Ferrated EDDHA (%)</th>
<th>Soluble iron (μM): weeks after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>5.3a</td>
</tr>
<tr>
<td>50</td>
<td>8.7b</td>
</tr>
<tr>
<td>100</td>
<td>11.3c</td>
</tr>
</tbody>
</table>

*aValues within a same column and with no corresponding letter differ significantly (P = 0.05).
TABLE 4. Root colonization of carnation cv. Lena growth in rockwool by *P. putida* WCS358r or Tn5 mutants of WCS358 at different weeks after planting and treatment with the pseudomonads: Tn5 mutant JM218 is defective in siderophore biosynthesis; Tn5 mutant B243 is not impaired in siderophore biosynthesis; iron availability in the nutrient solution was varied by adding 10 μM-EDDHA ferrated for 0, 50 or 100%.

<table>
<thead>
<tr>
<th>Ferrated EDDHA (%)</th>
<th>Log (CFU + l/g root fresh wt): weeks after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>JM218( Sid^- )</td>
</tr>
<tr>
<td></td>
<td>B243</td>
</tr>
<tr>
<td></td>
<td>WCS358r</td>
</tr>
<tr>
<td>50</td>
<td>JM218</td>
</tr>
<tr>
<td></td>
<td>B243</td>
</tr>
<tr>
<td></td>
<td>WCS358r</td>
</tr>
<tr>
<td>100</td>
<td>JM218</td>
</tr>
<tr>
<td></td>
<td>B243</td>
</tr>
<tr>
<td></td>
<td>WCS358r</td>
</tr>
</tbody>
</table>

*Values within a same column and with no corresponding letter differ significantly (*P* = 0.05).

TABLE 5. Root colonization of carnation cv. Lena grown in rockwool by *Fusarium oxysporum*, after inoculation with different densities of conidia of *Fusarium oxysporum f. sp. dianthi* (Fod); plants were inoculated 2 weeks after planting.

<table>
<thead>
<tr>
<th>Conidia Fod per plant</th>
<th>Log (CFU + l/g root fresh wt): weeks after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>2 × 10^6</td>
<td>3.29a</td>
</tr>
<tr>
<td>2 × 10^7</td>
<td>3.77b</td>
</tr>
</tbody>
</table>

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

TABLE 6. Influence of iron availability on siderophore and biomass production by *P. putida* WCS358r in CNS medium and inhibition of *Fusarium oxysporum f. sp. dianthi* by WCS358r on CNS agar plates (antagonism); iron supplied as 0, 50 or 100% ferrated EDDHA (10 μM).

<table>
<thead>
<tr>
<th>Ferrated EDDHA (%)</th>
<th>Siderophores (μmol g^-1 biomass dry wt)</th>
<th>Biomass (g dry wt l^-1)</th>
<th>Antagonism (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>602c*</td>
<td>1.36a</td>
<td>1.56b</td>
</tr>
<tr>
<td>50</td>
<td>145b</td>
<td>2.05b</td>
<td>1.40b</td>
</tr>
<tr>
<td>100</td>
<td>22a</td>
<td>2.54c</td>
<td>0.53a</td>
</tr>
</tbody>
</table>

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

*P. putida* WCS358r significantly reduced fusarium wilt of carnation grown in rockwool if disease incidence was moderate, but not if disease incidence was high (Table 1). This was earlier demonstrated for carnation grown in soil (Duijff *et al.*, 1991). The differences in disease incidence between the two experiments in Table 1 probably are caused by seasonal influences. The experiment with the low disease incidence was performed in the winter, whereas the experiment with the higher disease incidence was carried out during the summer months when greenhouse temperature was often above 22°C. A high soil and air temperature are favourable for the development of fusarium wilt diseases (Bosland *et al.*, 1988; Harling *et al.*, 1988).

Different levels of disease incidence could be obtained by varying the inoculum density of Fod. Disease incidence decreased with decreasing inoculum density of Fod (Table 2), as was shown before by Hood and Stewart (1957). Although disease suppression by WCS358r in this experiment was not significant, the relative disease reduction by WCS358r increased if inoculum density of Fod was reduced (Table 2). Also, at varying levels of iron availability, a reduction of the inoculum density significantly reduced disease incidence and increased the relative disease reduction by WCS358r or B243 about two-fold (Figure 1). At a high inoculum density, inhibition of Fod by WCS358 is probably not effective enough to suppress significantly infection of the roots of carnation by Fod. Moreover, an increase of inoculum density increases the chance that conidia of Fod enter rooted carnation cuttings by passive transport through wounds of the roots via mass flow (Baayen & de Maat, 1987). This process is most likely not reduced by siderophore-mediated competition for iron by WCS358.

Mutants of *Pseudomonas* spp. defective in siderophore biosynthesis (sid−) have been shown to be useful tools to assess siderophore production by pseudomonads in the rhizosphere and the potential role of siderophores in biocontrol (Bakker *et al.*, 1986; Becker & Cook, 1988; Loper, 1988; Höfte *et al.*, 1991). In the present study, the sid− Tn5 mutant JM218 did not suppress fusarium wilt, whereas the siderophore-producing strains WCS358r and B243 significantly suppressed disease incidence (Table 1, Figure 1). Root colonization by the sid− mutant was not impaired compared with that of the siderophore-producing strains (Table 4). These results demonstrate that siderophore production by WCS358 is involved in the suppression of fusarium wilt of carnation. Strain B243, a Tn5 mutant of WCS358 that is not impaired in siderophore production (Bakker *et al.*, 1986), was as effective in the suppression of fusarium wilt as WCS358r (Figure 1). This suggests that the Tn5 insertion into the genomic DNA of WCS358 as such did not influence the disease-reducing capacity of WCS358.

Siderophore-mediated competition for iron seems to be the main mechanism responsible for inhibition of Fod by WCS358 (Duijff *et al.*, 1993). Therefore, effectiveness of suppression of fusarium wilt of carnation by WCS358 was expected to be sensitive to iron availability. Unferrated, partially or completely ferrated EDDHA was used to create different iron availabilities in the nutrient solution (Table 3). The Fe⁺⁺⁺-chelate stability constant of EDDHA (log K = 33.9) (Chaney, 1988) is higher than that of the hydroxamate siderophores, like fusaririne, produced by *Fusarium* spp. (Emery, 1965; Winkelmann, 1992). Accordingly, EDDHA competes with the fusaririne for ferric iron (Scher & Baker, 1982; Van Peer *et al.*, 1990). In the present study, however, the level of Fe chelation of EDDHA did not influence disease incidence in the control treatments (Figure 1). Disease suppression by WCS358r and B243 was more effective with 0 or 50% ferrated EDDHA than with 100% ferrated EDDHA (Figure 1). The higher siderophore production and consequently stronger inhibition of Fod in *vitro* by WCS358r at 0 or 50% ferrated EDDHA compared with that at 100% ferrated EDDHA (Table 6) correspond with the results of disease suppression. These results provide further evidence that competition for iron is involved in the siderophore-mediated suppression of fusarium wilt of carnation by WCS358.

Ten micromolars of unferrated EDDHA chelated about 5 μmol iron from the rockwool material (Table 3). Probably, this decreased the difference in iron availability between the treatments with 0% and 50% ferrated EDDHA too much to result in differences of disease suppression by strain WCS358 at these two iron availabilities.
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The present study demonstrates that \textit{P. putida} strain WCS358 significantly suppresses fusarium wilt of carnation if rhizosphere conditions (low iron availability) are favourable for the acting mechanism of disease suppression, i.e. siderophore-mediated competition for iron. If disease incidence is high, siderophore-mediated competition for iron by WCS358 is inadequate to suppress significantly fusarium wilt in carnation.

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