European Journal of Plant Pathology **101**: 35–49, 1995.

Soil receptivity to *Fusarium solani* f. sp. *pisi* and biological control of root rot of pea

M. Castejón-Muñoz[1] and P. J. Oyarzun[2]
[1] *Centro de Investigación y Desarrollo Agrario, 41200 Alcalá del Rio, Sevilla, Spain;* [2] *DLO Research Institute for Plant Protection (IPO-DLO), PO Box 9060, 6700 GW Wageningen, The Netherlands*

Accepted 22 August 1994

Key words: actinomycetes, *Aphanomyces euteiches*, fluorescent pseudomonads, *Gliocladium roseum*, soil micro-organisms

Abstract

Potential antagonists of *Fusarium solani* f. sp. *pisi* (Fsp) were selected from soil samples with varying degrees of receptivity to this pathogen. They were tested against Fsp isolate 48 (Fs48), in increasingly complex systems. Most species tested *in vitro* were able to antagonize Fs48. No relation could be established *in vitro* between the receptivity of the soil from which an isolate originated and its antagonism to Fs48. In soils naturally infested with pea root rot pathogens, which were stored humid at 4 °C for a period longer than a year, various isolates of *Fusarium*, *Gliocladium* and *Penicillium* spp. were able to reduce root rot. After sterilization of these soils, only *Gliocladium roseum* isolates, added at 10^5 conidia g^{-1} dry soil, significantly reduced disease severity and prevented root weight losses caused by Fs48 at 10^4 conidia g^{-1} dry soil. In soils in which the biota were activated by growing peas before the assays, doses of 10^6 and 10^7 of *G. roseum* were required to reduce root rot. In these soils, the antagonistic effects of fluorescent pseudomonad strains from soil of low receptivity to Fsp were variable. Some strains of fluorescent pseudomonads, from soil moderately receptive to Fsp and from highly infested soils, were also able to reduce root rot. Disease suppression by pseudomonad strains was more evident in the absence than in the presence of *Aphanomyces euteiches* in the root rot pathogen complex. The role of receptiveness of the soil with regard to potential antagonists is discussed.

Introduction

Fusarium solani (Mart.) Sacc. f. sp. *pisi* (Jones) Snyder & Hansen (Fsp) is a major pathogenic fungus of pea in the Netherlands, causing foot rot and dry root rot, further referred to as root rot. It is prevalent in the traditional pea growing area and shows a remarkable physiological specialization to its host. Although root rot in peas has been associated with a low lime content and bad soil structure [Labruyère and Riepma, 1954], it affects crops in fields with good agronomical properties nowadays as well [Oyarzun et al., 1993].

The effect of soil on the activity of soil-borne pathogens and on the resulting disease has become more apparent in recent years. This effect is called soil receptivity, SR [Alabouvette et al., 1982]. With respect to a particular disease, soils may be ranged from suppressive to conducive. Many soil characteristics have been reported to influence the level of SR [Rouxel, 1991] and low SR is often related to the activity of microbial populations [Louvet et al., 1976; Rouxel et al., 1977; Alabouvette et al., 1977; Sher and Baker, 1980: Alabouvette, 1990]. Attempts have been made to identify the micro-organisms involved and to exploit them in biocontrol measures [Baker, 1990].

Recently, conspicuous differences in SR to Fsp were found [Oyarzun et al., 1994a], which opened the possibility to investigate the antagonistic activity of microflora associated with soils of low receptivity – synonymous to suppressive – in

natural, highly infested soils considered to be highly receptive or conducive to Fsp.

Common soil fungi and bacteria, including actinomycetes, might have an antagonistic potential towards Fsp. Some species pertaining to these groups have already been documented to control root rot in peas or in other legumes. The fungus *Penicillium oxalicum*, dusted on pea seed showed a protective effect against the complex of root-infecting pathogens in laboratory trials [Kommedahl and Windels, 1978], but under field conditions its effect was variable [Windels, 1981; Windels and Kommedahl, 1982]. Soil infestation with *Penicillium* spp and *Trichoderma viride* controlled lentil root-rot caused by *F. roseum* [Lin and Cook, 1979]. Non-pathogenic *F. oxysporum* isolates were reported to control root rot caused by *F. solani* in bean [Lechappe et al., 1988], in red clover [Walz-Borgmeier, 1991] and in peas [Oyarzun et al., 1994b]. Parke [1991] and King and Parke [1993] reported control of Pythium damping-off and common root rot (*Aphanomyces euteiches*) in pea by *Pseudomonas cepacia* and *P. fluorescens* in glasshouse and field experiments.

Actinomycetes are well known to antagonize pathogens with chitinous cell walls like *Fusarium* spp. [Campbell, 1989]. Van der Spek [1968] reported the successful reduction of root rot severity in soils highly infested with *F. solani* by the use of *Actinomyces* spp.

The aims of this study were: (i) to study the microbial composition, particularly that known to be associated with antagonism, of field soils with low (SLR) and high (SHR) receptivity to *F. solani* f. sp. *pisi*; (ii) to select potential antagonists and (iii) to assess their effects on disease suppression in naturally infested soil where Fsp occurred.

Materials and methods

The pathogen. In experiments with *Fusarium solani* f. sp. *pisi* (Fsp), a monosporic culture of the virulent isolate Fs48 (provided by Dr. J. M. Kraft, Washington State, USA) was used. The culture was maintained either on Carnation Leaf Agar (CLA) for immediate use or by lyophilisation.

Soils. Soil samples with different abiotic and phytopathological characteristics were used (Table 1). A water retention curve was available for each (disturbed) soil. A diversity of soil types and

Table 1. Characterization of field soil samples for their receptivity to *Fusarium solani* f.sp. *pisi* (SR; SLR = low, SHR = high), composition of root rot complex, disease index (DI), frequency of pea and legume cropping in field history, some abiotic characteristics of soils and soil type

sample code	SR	Pathogens			Root-rot DI[b]		Frequency[c]		[d]				Soil[e] type
		Fs	Ae	Tb	B	F	Pea	Leg.	C/N	pH	Or.m %	Lime %	
CTF	SLR	+	–	+	0.5	–	0	0	26	7.6	2.3	7.7	hlo
ROC	SLR	+	–	–	0.9	1.5	2	4	13	7.4	1.7	2.8	hlo
MA1	SLR	+	–	–	0.6	1.9	2	2	14	7.3	3.7	4.6	hcl
CTE	SHR	+	+	–	5.0	4.0	10	10	23	7.3	2.3	7.7	hlo
HA1	SHR	+	+	–	4.4	2.5	2	2	13	7.5	3.5	4.9	hcl
JON	SHR	+	–	–	3.7	1.3	3	3	16	7.5	2.0	4.0	slo
BER	SHR	+	–	–	3.5	2.9	2	4	12	7.4	9.5	1.5	hcl
HER	SHR	+	–	+	4.0	4.0	3	4	14	7.2	3.4	8.3	hlo
HOE	SHR	+	+	–	4.0	1.4	1	5	12	7.5	2.1	4.0	llo
HIL	SHR	+	+	–	3.5	1.2	2	5	15	7.4	3.2	1.0	slo
DHO	SHR	+	+	–	4.1	0.7	2	3	15	7.4	2.0	7.8	hlo
HUL	SHR	+	+	–	4.0	1.7	4	5	14	7.3	2.5	5.4	lcl

[a] Fs = *Fusarium solani*, Ae = *Aphanomyces euteiches*, Tb = *Thielaviopsis basicola*; presence = +, absence = –.
[b] The root rot severity was determined as disease index (DI) in bioassays (B) and in a pea crop in the field (F) during the last pea cropping year (1986–1987).
[c] Frequency of pea and total legumes in the field during the last twenty years.
[d] C/N = carbon/nitrogen ratio; Or.m % = percentage organic matter; Lime % = percentage carbonates.
[e] Soil type: hcl = heavy clay; hlo = heavy loam; lcl = light clay; llo = light loam; slo = sandy loam.

pathogens producing a high level of root rot disease in peas was used to test antagonists. The presence of Fsp, the varying composition of the pea root rot pathogen complex and the difference in receptivity to Fsp were used as criteria for selecting the soils. A soil sample was considered to be highly receptive (SHR) if pea strongly responded to soil infestation with Fsp or if the sample had a high natural root rot inoculum potential. The soils were part of a sample set from commercial fields used to investigate soil receptivity to pea root rot pathogenic fungi [Oyarzun et al., 1994a].

Microbial populations in rhizosphere and roots of peas grown in soils with different receptivity levels. Fungi, bacteria and actinomycetes were enumerated from pea rhizosphere soils (rs) and in and on the roots (rhizoplane: rp), respectively. Soil samples with low (CTF, ROC, MA1) and high (CTE, HA1, JON, BER, HER, DHO, HUL, HIL, HOE) receptivity to Fsp [Oyarzun et al., 1994a] were examined.

Four minipots (4 × 4 × 12 cm) were filled with a known amount of the sieved soil samples. Four surface-sterilized pea seeds, cv Allround, were sown at 1.5 cm depth. After sowing, soil water content was carefully raised to water holding capacity. The pots were placed in a dark growth room at 22 °C until the plants had germinated. After emergence the pots were transferred to a climate room with an air temperature of 24/20 °C (day/night), 12 h light at 90 W m^{-2} and 80% RH. Three weeks after sowing, the root system had almost completely invaded the soil, which was then considered to be a rhizosphere soil. For enumeration in the rhizosphere, the soil was separated from the roots in Erlenmeyer flasks containing 400 ml of 0.1% sterile sodium pyrophosphate solution. Roots were then additionally washed in 100 ml 0.1% sodium pyrophosphate. This suspension was added to the former soil suspension and shaken on a rotary shaker for 10 min at 200 rpm. Tenfold serial dilutions of the suspensions were made with 0.1% sodium pyrophosphate and these were plated on culture media.

For enumeration in the rhizoplane, roots were dried on filter paper and weighed, sampled for dry matter determination and macerated in 0.1% sodium pyrophosphate (rate 1:10 w/v) in a Braun blender (maximum speed) for 2 min. Dry weight was determined after drying at 70 °C for 48 h.

Appropriate dilutions were plated on the following media. Isolation of *Fusarium* spp. was performed on Selective Fusarium Agar (SFA) containing 20.0 g D-(+)-glucose, 0.5 g KH_2PO_4, 2.0 g $NaNO_3$, 0.5 g $MgSO_4.7H_2O$, 20.0 g agar, 25 mg dichloran (Allisan, 50% a.i.), 100 mg streptomycin, 10 mg tetracyclin, in 1 l de-ionized water [Burgess and Liddell, 1983]. *Trichoderma* and *Gliocladium* spp were detected on Selective Solidified Medium (SGM) made of 39 g PDA, 50 mg chloramphenicol, 2 ml triton X-100, 500 mg sodium-propionate, 100 mg fongilan (50% furalaxyl) in 1 l de-ionized water. Tryptone Soya Agar (TSA) at 1/10 strength was used for the total bacterial counts [Lawley et al., 1983]. Fluorescent pseudomonads were detected on Gould's S1 medium [Gould et al., 1985] and colonies enumerated under near UV (365 nm). The Chitin Oatmeal Agar (COA) method [Miller et al., 1989] was used for the isolation and counts of actinomycetes. Gould's S1 and fungus media were incubated at 24 °C and scored after 7 days. Other media were incubated for 10 days at 20 °C.

Selection of candidate antagonists. From the rhizosphere and the rhizoplane, numerous fluorescent pseudomonads and actinomycete colonies were selected at random and purified by subculturing. Fungal colonies showing antibiosis were selected in addition to random selections. Fungal isolates originated from SLR only.

Various isolates of actinomycetes and pseudomonads, originating from soil samples with different levels of receptivity to Fsp, other than those characterized in Table 1, were additionally used for these experiments.

Actinomycetes were kept in COA. Fluorescent bacteria were transferred to 10% Tryptone Soya Broth (Oxoid) containing 10% glycerol and frozen to –80 °C until use.

In vitro *antagonism to* F. solani *f. sp.* pisi

Fungi. Twenty-one isolates from SLR samples were tested against Fs48 in Petri dishes. Fsp was grown for 2 weeks on Czapek Dox agar (CDA;

33.4 g Czapek Dox Medium (Oxoid) and 20 g agar in 1 l de-ionized water) at 25 °C under near UV (365 nm). Other fungal isolates were grown on PDA (39.0 g PDA (Oxoid) in 1 l de-ionized water) for 7 days at 25 °C. Mycelial disks of 5 mm were taken from the edge of vigorously growing colonies and placed on Malt Agar (MA) in two ways: (i) antagonist and pathogen next to each other in the middle of the Petri dish ('juxta position'), (ii) the two fungi 2.5 cm from each other ('dual culture'). All combinations were replicated four times. An angular interaction and inhibition of mycelial growth was calculated [Lechappe et al., 1988] after an incubation period of 8 days. Monosporic cultures of fungal isolates were sent to the Centraalbureau voor Schimmelcultures, CBS, at Baarn for final identification.

Bacteria. Twenty-three isolates of fluorescent pseudomonads were subcultured on King's B medium, KBM [King et al., 1954] and individually tested for their ability to inhibit the mycelial growth and spore germination of Fs48 on agar plates of KBM. Three equidistant agar disks (5 mm diameter) of the candidate antagonists were placed in a Petri dish containing KBM and incubated for 48 h at 25 °C before adding the pathogen. The addition of Fs48 was performed in two ways, (i) a mycelial disk of the pathogen grown on CDA was placed in the centre of the plate (2 cm from the antagonist disks) and (ii) about 0.5 ml of Fs48 spore suspension, adjusted to 5×10^4 spores ml^{-1}, was evenly sprayed onto the medium. The tests were replicated four times and incubated at 25 °C. After six days, the size of a zone of growth or germination inhibition was scored.

Actinomycetes. Thirty-one isolates were tested for their ability to inhibit spore germination of Fs48 on COA medium. The confrontation was made as described above for fluorescent pseudomonads.

Antagonism in soil. Due to insufficient inoculum, actinomycete isolates and some fungal species like *Mortierella*, were not further tested in soil. In all experiments, antagonists were evaluated for their ability to reduce root rot and their effect on plant weight.

Fungal antagonism in soil. Nine fungal isolates were selected for antagonism against naturally present Fsp or against Fs48 introduced into the soils after sterilization. Inoculum of the pathogen was obtained from Petri dishes containing CDA, incubated at 25 °C under black light for three weeks. A mixture of macro- and microconidia was suspended in sterilized de-ionized water, filtered through cheese cloth and adjusted to the proper concentration.

Experiment 1. Samples of three soils (CTE, HA1, JON), sterilized by γ-radiation (4 Mrad) and non-sterilized, were used. Moist soil samples used in this experiment were stored at 4 °C for a period of longer than one year.

Isolates used in this experiment were: *Gliocladium roseum* Gr1, Gr2, Gr3; *Penicillium aurantiogriseum* (Pa); *Penicillium* cf. *griseofulvum* (Pg); *Fusarium oxysporum* (Fo1, Fo2, Fo3) and *Fusarium culmorum* (Fc). Inoculum was prepared by growing *Gliocladium* and *Fusarium* on PDA for 18, respectively 10 days at 25 °C. *Penicillium* was grown on MA at 25 °C for 10 days. Conidia were suspended in sterilized de-ionized water, filtered through cheese cloth and adjusted to the desired concentration.

The soil samples were infested with 10^5, 4×10^5 or 10^6 spores g^{-1} of dry soil for *Fusarium*, *Gliocladium* and *Penicillium* isolates, respectively. The conidial suspensions were atomized onto the soil. As a control, part of each soil sample, sterilized and non-sterilized, was treated with sterile water. The soil samples were incubated for 15 days in darkness at 22 °C. Then, Fs48 was added to the sterilized soil at 10^4 spores g^{-1} dry weight.

Two pea seeds, cv. Allround, were sown in minipots ($2 \times 4 \times 12$ cm) at 1.5 cm depth. After emergence, the pots were placed in tanks and randomized over five blocks in a split-split-plot design with soil conditions (sterilized/non-sterilized) as main plots and soils as subplots. The experiment lasted one month in a semi-automatic test device described earlier [Oyarzun et al., 1994b] under the same experimental conditions as described above.

Plants were harvested by cutting them off at soil level. All roots were cleaned with running tap water and assessed for symptoms of root rot (disease index) on a scale from 0 = no necroses to 5 = 100% necrotic roots [Oyarzun, 1991]. Fresh

weight was determined, a number of roots sampled to determine the dry weight and the rest used to study colonization.

To study the colonization of pea roots by the pathogen and its antagonists, a sample of three roots per treatment was surface-sterilized for 3 min in 0.5% sodium hypochlorite with 5% ethanol and rinsed three times in sterile water. The roots were placed in sterile de-ionized water (1:10 w/v) and blended for 2 min in a Braun blender (maximum speed). The suspension was filtered through cheese cloth. Serial dilutions were plated in triplicate on PDA for the enumeration of colony forming units (cfu).

Experiment 2. G. roseum isolates Gr1, Gr2 and Gr3, were further tested in natural soils. Prior to this experiment the soil biota were activated by growing peas in containers with 25 kg of soil for 45 days. The resulting condition of the soil is termed 'activated'. To account for the nutrients removed by the plants, the soil was slightly fertilized. The plants were uprooted and the soil was gently dried and sieved through a 4 mm mesh screen.

Spore suspensions of the antagonists were prepared and added to the soils as described previously. The isolates were introduced into the soils CTE, HA1, JON, BER and HER at 10^6 spores g^{-1} dry soil.

Experiment 3. Experiment two was repeated with soils HER, HIL and HOE, at 10^7 spores g^{-1} dry soil. Experimental conditions and treatments were as described before. The design was a split-plot with soils as mainplots and *Gliocladium* isolates as the subplots. Treatments were replicated four times and objects randomized in blocks.

Bacterial antagonism in soil. Fluorescent pseudomonads were tested for their ability to control root rot in naturally infested soil by seed-coating and by addition to soil.

The bacteria were grown on fresh KMB agar in Petri dishes, which were inoculated by flooding with 5 ml of a turbid suspension. Plates were incubated at 25 °C for 2 days and bacteria were washed from the agar surface with 5 ml sterile water. The bacterial cell number was determined by measuring the optical density of the suspension at 620–630 nm. Parallel to this measurement the suspension was used for a plate-count. A calibration curve of the density was calculated and the inoculum density was adjusted.

Seed coating. For seed coating, pea seeds were surface-sterilized, rinsed in sterilized water and dried overnight under a sterile air stream. A bacterial suspension of 2×10^9 cfu ml^{-1} was diluted with 1% methylcellulose (1:1 v/v) to a final inoculum density of 1×10^9. The resulting suspension was mixed with pea seeds (5 ml of the suspension per 10 g of seed). The control consisted of seed coated with methylcellulose only. Coated seeds were placed in a Petri dish and dried overnight under a sterile air stream. The number of colony forming units was determined by macerating 10 seeds in a mortar and pestle with 100 ml of phosphate buffer (pH 7.2) and plating 0.1 ml of appropriate dilutions of the homogenate on KMB. Coated seeds contained about 10^8 cfu per seed.

Three soils were used, HA1, HER and JON, representing Fsp + *Aphanomyces euteiches* (Ae), Fsp + *Thielaviopsis basicola* (Tb) and Fsp alone respectively. Sowing, statistical design and experimental conditions were as described for the experiment with *G. roseum* in natural soil.

Soil application. In this experiment, bacterial suspensions (10^8 cfu g^{-1} dry soil) were added to the soils HER, DHO and HUL by atomizing the suspensions. Pea seeds were sown immediately. Soils DHO and HUL were both naturally infested with Fsp and Ae. The experimental conditions were not changed. As a control, soil was treated with sterile water.

Statistical analysis. Analysis of variance was carried out with the statistical program Statistix [Anonymous, 1985]. Least significant differences (LSD) were calculated at a significance level of $P = 0.05$. Inhibition values of growth or germination at 0 and 100% were excluded from the analysis. If trended residuals were present and the range of percentages exceeded 40 an arcsin $\sqrt{x}$ transformation was used [Little and Hills, 1979]. Unless otherwise stated, means are averages of four replicates. Numbers of micro-organisms in soil or roots were compared by analysis of variance after $\log_{10}(x + 1)$ transformation.

Results

Microbial populations in rhizosphere and roots of peas grown in soils with different receptivity levels. The microbial populations in soils of low and high receptivity to Fsp showed some differences (Table 2 A, B). In the rhizoplane, the number of Fo was highest in ROC, one of the soils in the SLR group. The highest number of *Fusarium* spp and actinomycetes were present in the rhizoplane of plants in MA1. The number of total bacteria, including fluorescent pseudomonads and of *Gliocladium* and *Penicillium* were below the detection level of 10^{-4} in the SLR group. In contrast, the number of these species was sometimes high in the rhizoplane of plants in the group of SHR. *F. solani* was well represented in the rhizoplane in conducive soils but also in two of the three soils in the SLR group.

In the rhizosphere, the highest number of actinomycetes and of *Fusarium* spp. and *F. oxysporum*, respectively, was observed in CTF and ROC (Table 2B). Both soils belong to the SLR group. Bacteria as well as *Gliocladium* and *Penicillium* were clearly present in SLR. In contrast with the results in the rhizoplane, bacteria in the rhizosphere of SLR were the highest. In general, species of *Fusarium* and *Gliocladium* were well represented in the rhizosphere of SHR.

In vitro *antagonism to* F. solani *f. sp.* pisi

Fungi. Of the 21 fungal isolates tested for antagonism to Fs48, 11 inhibited the mycelial growth of the pathogen in dual culture or juxta position (Table 3). In dual culture, a pre-contact growth reduction of Fs48 was observed by the *Penicillium* isolates only. In this test, isolates of *G. roseum* produced discoloration and disturbance of the Fs48 colony. The growth inhibitory effects were

Table 2. Number of colonies[a] in the rhizoplane (A) and the rhizosphere (B) of 21-day-old pea plants, grown in soil of low and high receptivity to *Fusarium solani* f. sp. *pisi*. The populations were enumerated by the dilution plate method

(A)	Soil sample										
	low receptivity (SLR)			high receptivity (SHR)							
Isolates	CTF	ROC	MA1	CTE	HA1	JON	BER	HER	DHO	HUL	HIL	HOE
F. solani	91 abcd	50 bcde	*	18 bcde	9 ef	166 a	6 def	50 abc	50 abc	68 ab	11 cde	*
F. oxysporum	60 b	690 a	28 b	nd	nd	nd	11 bc	8 bc	25 b	nd	5 bc	nd
Other *Fusarium*	nd	5 cd	188 a	nd	nd	nd	2 cd	nd	8 bc	14 b	2 cd	nd
Gliocladium sp	nd	nd	nd	nd	nd	18 b	nd	50 a	37 a	7 b	nd	nd
Penicillium sp	nd	nd	nd	nd	nd	3 ab	15 a	nd	nd	nd	2 ab	nd
Total bacteria	*	*	*	*	1 de	5 c	24 b	56 a	54 a	27 b	20 b	2 d
Total pseudomonads	2 de	*	1 de	*	2 ef	4 d	21 b	*	150 a	11 c	8 c	*
Fluorescent psd.	*	*	*	*	*	1 bc	21 a	*	1 bc	1 b	*	*
Actinomycetes	35 ab	5 ef	48 a	11 cde	10 f	7 def	5 ef	14 abc	16 ab	13 cde	3 fg	1 g
(B)												
F. solani	7 c	6 c	7 c	8 bc	21 ab	25 a	5 c	7 c	6 c	10 abc	1 d	1 d
F. oxysporum	3 ab	5 a	3 ab	1 bc	1 bc	1 bc	1 bc	nd	1 bc	2 ab	1 bc	nd
Other *Fusarium*	nd	8 a	4 abc	3 bcd	6 ab	1 de	1 de	nd	3 bcd	2 cde	2 cde	2 cde
Gliocladium sp	4 abc	2 cd	nd	2 cd	4 abc	4 abc	2 bcd	nd	11 abc	13 a	9 ab	4 bcd
Penicillium sp	nd	1 c	15 a	2 cd	6 b	2 bc	nd	nd	nd c	1 c	2 bc	4 b
Total bacteria	63 a	6 c	20 b	3 d	2 e	1 f	4 cd	5 c	5 cd	2 e	*	*
Total pseudomonads	20 a	*	*	3 b	1 bc	2 b	2 b	7 b	12 a	22 a	*	*
Fluorescent psd.	3 b	*	*	1 bc	*	*	2 b	7 b	1 bc	7 a	*	*
Actinomycetes	21 a	*	2 c	4 b	*	3 b	1 c	4 b	*	1 d	1 d	*

[a] Number of colonies of: fungi 10^4, pseudomonads 10^6, bacteria 10^8 and actinomycetes 10^8 g^{-1} of root dry weight.
nd: not detected at the selected dilution. Data were statistically analyzed by ANOVA after $\log_{10}(x + 1)$ transformation.
Row values followed by the same letter are not significantly different ($P \leq 0.05$, based on LSD). The * represent averages less than 0.5 colonies at the selected dilution, which were ignored.

Table 3. In vitro inhibition (% of control) of mycelial growth of *Fusarium solani* f. sp. *pisi* (Fs48) in dual culture and in juxtaposition by various fungi isolated from rhizosphere (rs) or rhizoplane (rp) of pea in soils of low receptivity to Fsp. Observations were made 6 days after incubation on MA

Candidate antagonists	Source of isolates	Test	
		dual culture	juxtaposition
Penicillium aurantiogriseum	CTF rs[a]	21 b	98 a
P. griseofulvum	ROC rs	20 b	0
Mortierella sp	ROC rs	0	50 c
Mortierella sp	MA1 rs	0	62 bc
Fusarium oxysporum	CTF rs	0	44 c
F. oxysporum	ROC rp	0	25 d
F. oxysporum	MA1 rs	0	19 d
F. culmorum	MA1 rp	32 a	70 b
Gliocladium roseum	CTF rs	3 c	0
G. roseum	ROC rs	3 c	0
G. roseum	ROC rp	3 c	0

[a] Soil code.
For each test, values followed by the same letter(s) are not significantly different ($P \leq 0.05$, basd on LSD). 0 values was ignored.

slight. No effects were found in juxta position. In juxtaposition only, the *F. oxysporum* isolates were able to reduce the growth of Fs48.

Bacteria. Nineteen of the 23 fluorescent pseudomonad isolates significantly inhibited mycelial growth of Fo48 (Fig. 1). When the spore suspension was sprayed, no germination of Fs48 occurred in 14 cases, whereas in all other cases a zone of spore inhibition of variable size occurred.

Actinomycetes. Thirty of the 31 isolates significantly reduced the spore germination of Fs48 ($P \leq 0.05$; data not shown).

Fs48 was inhibited by the different actinomycetes and fluorescent pseudomonad isolates, irrespective of the receptivity of the soil from which they were isolated.

Fungal antagonism in soil

Experiment 1. Infestation of the sterilized soils CTE, HA1 and JON with Fs48 led to severe root rot disease similar to that caused by Fsp and other naturally occurring root-rot pathogens in non-sterilized soils (Table 4). The disease severity was significantly reduced by all isolates tested after introduction into the non-sterilized soils.

In sterilized soils, the disease reduction was always significantly less than in non-sterilized soil. The three isolates of *F. oxysporum* were able to reduce the root-rot severity in the sterilized soil HA1 significantly. Fo1 and Fo2 were effective in JON soil and only Fo2 reduced root necrosis in CTE. These disease reductions were not reflected in the plant dry matter weight (Table 4). Plants grown under these treatments showed poorly developed roots. *F. culmorum* caused rotting of most seeds and is not represented in the tables.

With the exception of *P. aurantiogriseum* in soil HA1, no reduction of root-rot caused by Fs48 was achieved by *Penicillium* isolates. *Penicillium* spp did not cause an increase in dry matter.

The most consistent disease reduction was obtained with isolates belonging to *G. roseum*, with a positive effect ($P \leq 0.05$) on total plant dry weight (Table 4) and root dry weight ($P \leq 0.05$; data not shown).

From plants grown in soils infested with *G. roseum* and Fs48, neither of these fungi could be isolated from inside the roots, but Fs48 was frequently recovered from plants grown in soil treated with the other species (data not shown).

Experiment 2. Gliocladium roseum. In this experiment *G. roseum* isolates, Gr1, Gr2 and Gr3 were introduced into naturally infested, 'activated' soils (CTE, HA1, JON, HER and BER) at 10^6 spores g^{-1} dry soil. Under such conditions, the suppressive effect of the *G. roseum* isolates was negligible. Only in soil JON was significantly less root rot found than the control (Fig. 2). The dry weight of plants correspondingly increased ($P \leq 0.05$). For the three *G. roseum* isolates, the plant dry weight did not differ from the control grown in sterilized soil.

Experiment 3. At an inoculum density of 10^7 spores g^{-1} dry soil, the isolates of *G. roseum* significantly antagonized the indigenous root rot pathogens, but their efficacy depended on the soil ($P \leq 0.05$) as illustrated in Fig. 3. In addition, the isolates increased the dry weight of peas in all three tested soil ($P \leq 0.05$; data not shown). Figure 4 illustrates the root rot alleviation by *G. roseum* isolates.

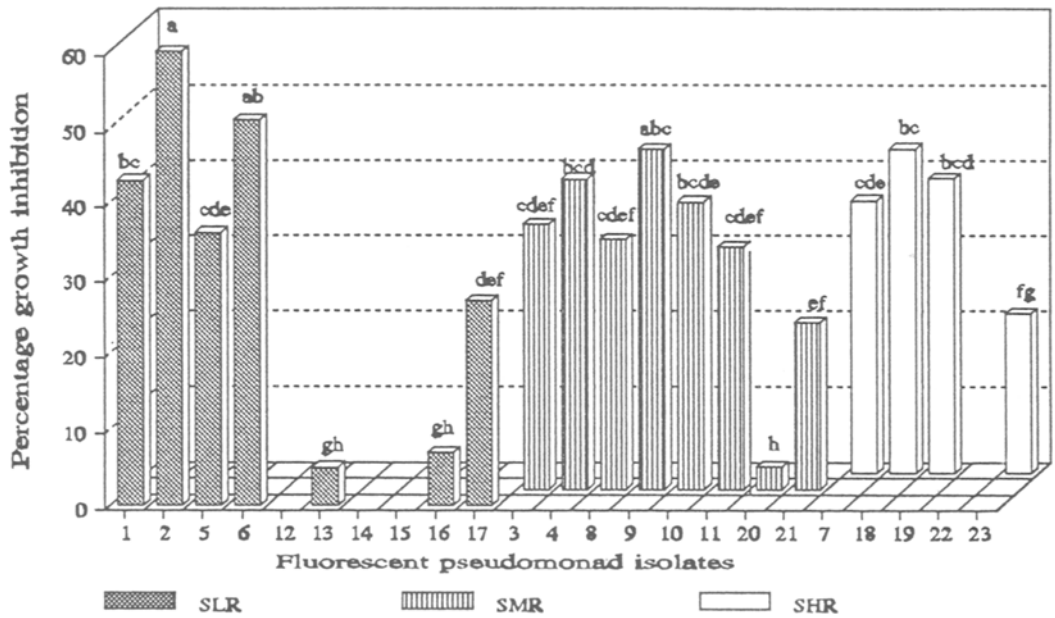

Fig. 1. In vitro inhibition of mycelial growth (as percentage of the control) of *Fusarium solani* f.sp. *pisi*, Fs48, by fluorescent pseudomonad isolates originating from soils with low (SLR), moderate (SMR) and high (SHR) receptivity to Fs48. Significant differences are indicated by different letters above columns ($P \leq 0.05$, based on LSD).

Table 4. Antagonistic effects of fungal isolates on (A) the root rot severity (DI, 0 = healthy, 5 = 100% necrotic roots) and (B) plant dry weight (g) of pea grown in sterilized (st) soils artificially infested with *Fusarium solani* f.sp. *pisi* (Fs48 10^4 spores.g^{-1} dry soil) and in non-sterilized soils (n.st) highly infested with root-rot pathogens

			Antagonists[a]								
	Sample code	Soil condition	Gr1	Gr2	Gr3	Pa	Pg	Fo1	Fo2	Fo3	Ref
A	CTE	st	1.66	1.94	2.92	4.02	4.14	4.05	3.62	4.20	4.44
		n.st	0.16	0.16	0.20	0.26	1.58	0.32	0.30	0.50	4.34
	JON	st	1.68	2.24	3.06	4.39	4.63	3.76	3.88	4.02	4.38
		n.st	0.34	0.42	0.36	0.42	0.70	0.42	0.66	1.08	4.14
	HA1	st	0.38	0.62	0.46	3.12	4.00	3.57	2.92	2.68	4.40
		n.st	0.14	0.32	0.24	0.14	0.20	0.68	0.38	1.52	4.26
B	CTE	st	1.05	0.98	1.10	0.65	0.69	0.60	0.62	0.58	0.71
		n.st	0.93	0.79	0.80	0.72	0.73	0.72	0.77	0.65	0.76
	JON	st	1.06	0.93	0.93	0.40	0.54	0.53	0.79	0.71	0.69
		n.st	0.91	0.97	0.98	0.82	0.84	0.86	0.76	0.65	0.77
	HA1	st	1.06	0.93	1.00	0.68	0.67	0.47	0.70	0.70	0.67
		n.st	1.06	0.86	0.89	0.83	0.83	0.54	0.80	0.55	0.51

LSD^1: 0.55; LSD^2: 0.15

[a] Antagonist species are *G. roseum* = Gr1, Gr2, Gr3; *Penicillium aurantiogriseum* (Pa), *Penicillium griseofulvum* (Pg), *Fusarium oxysporum* = Fo1, Fo2, Fo3. Ref st = sterile soil with Fs48; Ref n.st, non sterilized soil containing Fsp and other root rot pathogens. LSD^1 valid for all comparsons of DI; LSD^2 valid for all comparisons of dry weight ($P \leq 0.05$).

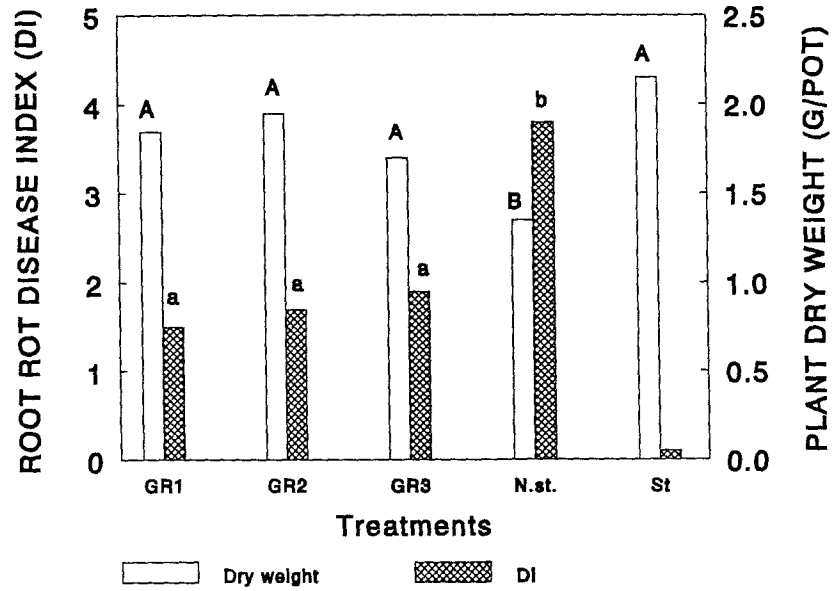

Fig. 2. Antagonistic effect of three *Gliocladium roseum* isolates (Gr1, Gr2, Gr3) on the root rot severity (DI, 0 = healthy, 5 = 100% necrotic roots) and plant dry weight of pea. The isolates were applied to a highly infested soil (JON) at 10^6 spores g^{-1} dry soil. N.st. = control, non-sterilized soil without *Gliocladium*; st = control sterilized soil without *Gliocladium*. Significant differences per variable (open or hatched columns) are indicated by different letters ($P \leq 0.05$, based on LSD).

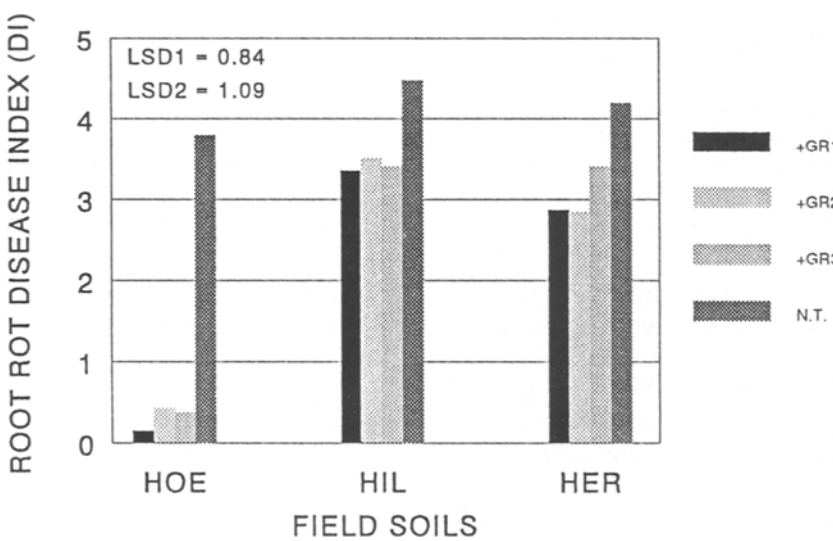

Fig. 3. Effect of three *Gliocladium roseum* isolates (Gr1, Gr2, Gr3) on root rot disease index (DI, 0 = healthy, 5 = 100% necrotic roots). The isolates were applied to three highly infested soils (HOE, HIL and HER) at 10^7 spores g^{-1} dry soil. NT = control, without *Gliocladium*. LSD1 for comparison within a soil, LSD2 for other comparisons ($P \leq 0.05$).

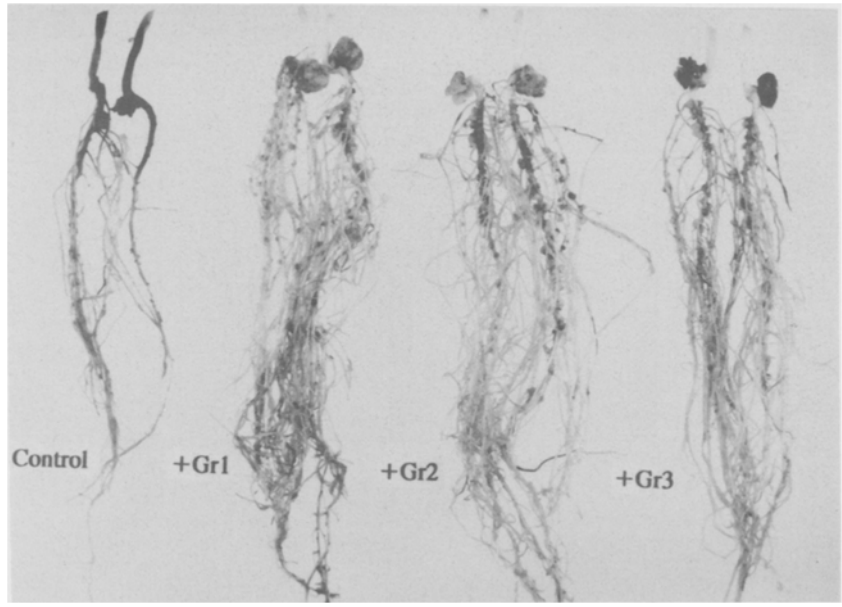

Fig. 4. Root rot symptoms of pea plants growing in a highly infested soil (G17 = HOE) without (left plant) and with incorporation of *Gliocladium* isolates (+Gr1, +Gr2, +Gr3) to the soil.

Bacterial antagonism in soil

Seed coating. When applied as a seed coating, none of the 23 bacterial isolates reduced the disease severity in the soils HER and BER. However, in soil JON, treatment with isolates 1, 2 and 6 (from SLR), 7 (from SHR) and 8 and 11 (from a soil with moderate receptiveness, SMR) led to a significantly lower root rot severity (Fig. 5a). Other isolates showed no antagonism (data not shown). The isolates 2, 6, 7, 11 increased shoot and root dry weight, while isolate 8 only increased the root dry weight ($P \leq 0.05$).

Soil application. In this experiment the soils HER, DHO and HUL were treated with a bacterial suspension. In the soils HER and HUL none of the tested isolates significantly reduced disease severity, but in soil DHO some isolates were effective. Some of these were different from those effective with seed coating in JON. The isolates 1, 2, 7 did not antagonize successfully, but the isolates 3, 12 and 16 in addition to 6, 8 and 11 showed good control (Fig. 5b). The isolates 8 and 12 significantly increased plant dry weight, whereas the isolates 6 and 16 increased the stem weight only.

Discussion

Microbial populations in soils with different receptivity. This study showed quantitative and qualitative differences between the microflora of soils of low (SLR) and high receptivity (SHR) to Fsp.

The high number of Fs in the rhizoplane of two SLR was remarkable. In SLR, Fs did not or hardly provoked root rot. Therefore, Fs was saprophytic, even antagonistic or if pathogenic, inhibited in its ability to cause disease. *F. oxysporum* was most abundant in SLR samples, in particular in the rhizoplane of ROC (Table 2A). This is consistent with observations made in *Fusarium* wilt suppressive soils [Alabouvette et al., 1982].

The high number of bacteria in the rhizosphere of SLR was in contrast with their virtual absence in the rhizoplane. The opposite occurred in SHR. The high number of bacteria in the rhizoplane of SHR may be associated with saprophytic growth on diseased roots.

The highest number of *Penicillium* and actinomycetes was found in the pea rhizosphere of SLR, but their abundance was soil dependent. In the pea rhizoplane of SLR, actinomycete populations were consistently high, in contrast to the virtual absence of fluorescent pseudomonads and *Gliocladium*

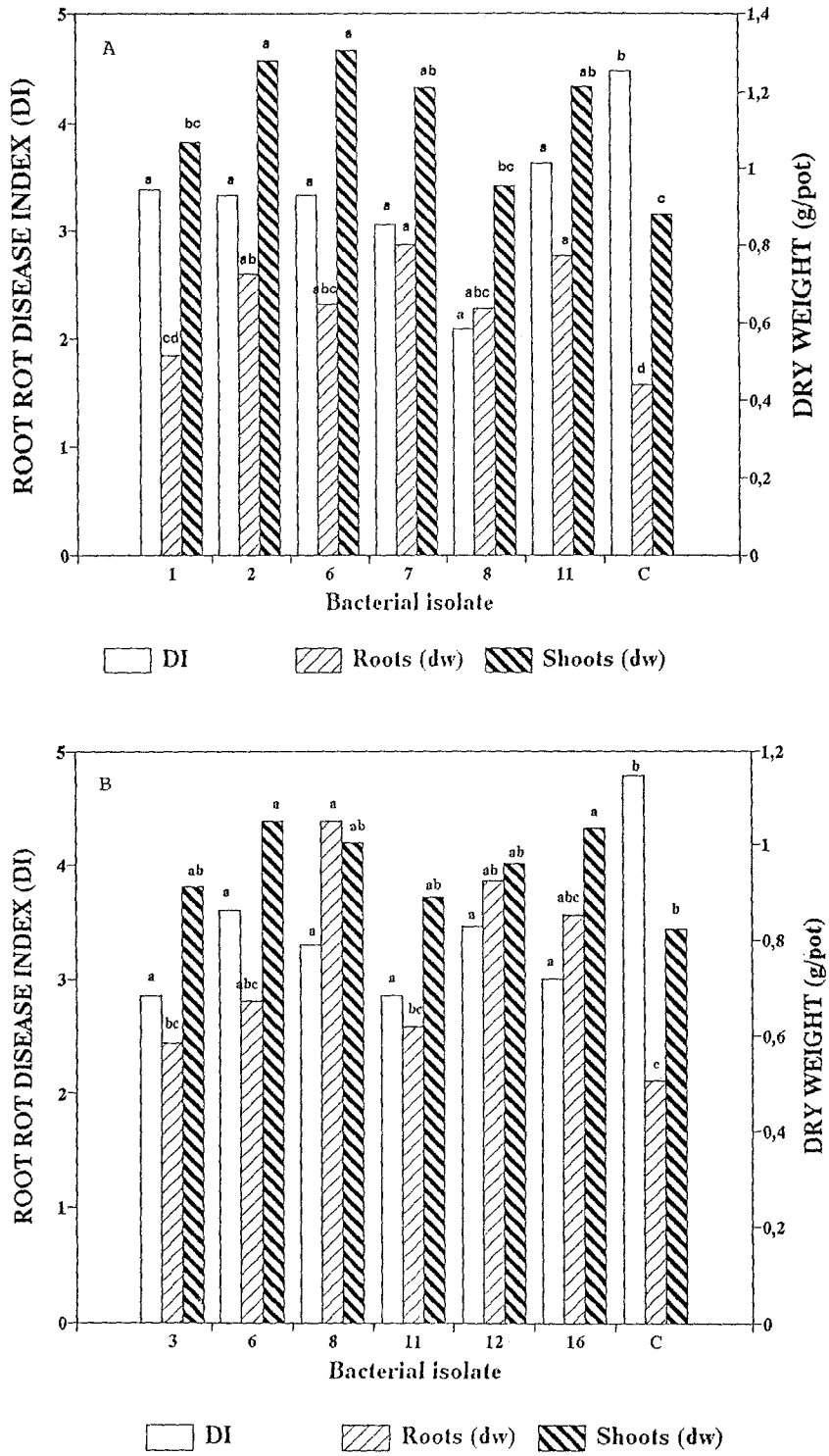

Fig. 5. (A) Antagonistic effects of seed coated fluorescent pseudomonad isolates on the disease index (DI) and dry weight of roots and shoots of pea grown in a highly infested soil (JON). (B) Antagonistic effects of fluorescent pseudomonad isolates, added to the soil, on the disease index (DI) and dry weight of roots and shoots of pea grown in a highly infested soil (DHO). Per variable (column pattern) significant differences are indicated by different letters ($P \leq 0.05$, based on LSD).

spp. In fact, the last two species were often present in SHR, indicating that they were not able to inhibit the pathogenic activity of Fsp and related root rot pathogens.

In vitro *antagonism to* F. solani *f. sp.* pisi. All fungal isolates selected from SLR affected the growth of Fs48 on agar, but in different ways. *F. culmorum* overgrew it, especially in juxtaposition tests where competition for nutrients starts immediately after putting both fungi on the artificial substrate. The pre-contact growth reduction of Fs48 by *Penicillium* spp. suggests antibiotics are involved. With *G. roseum* isolates in dual culture, the discoloured mycelial inhibition zone in Fs48 might be due to enzymes or toxins which work over a short distance [Barnet and Lilly, 1962] and cause the death of the pathogen. The most relevant effect of *G. roseum* was caused by its ability to locally interfere with the colonies of Fs48 by post-contact antagonism, as was suggested for this species by Deacon and Berry [1992]. *F. oxysporum* isolates were able to reduce the growth of Fs48 in juxtaposition only. This indicates a better saprophytic ability of Fo on the artificial substrate.

The lack of relationship between the receptivity of the soils from which fluorescent pseudomonads and actinomycetes were isolated and their antagonistic ability in the *in vitro* tests, suggests that in the SHR some factor(s) impede or invalidate their antagonism. The relatively large number of isolates of these species showing antagonistic activity stresses their potential importance in biological control.

Fungal antagonism in soil. In the first experiment, the disease suppression by fungal antagonists was more successful in naturally infested soils with a complex of root rot pathogens than in sterilized soil which was infested with Fs48 only. The strong reduction of root rot in naturally infested soils was remarkable. Probably the freshly cultured antagonists had a higher energy status than the indigenous pathogenic microflora of the soil samples, favouring the antagonist when plant energy was becoming available. However, no change in the receptivity has been observed in samples stored for a year [Alabouvette et al., 1979].

The antagonistic activity of *G. roseum* isolates in sterilized and in naturally infested soil was clear. The absence of Fs48 from pea roots after surface sterilization may be a result of the ability of *G. roseum* to inactivate Fs48. Berry and Deacon [1992] pointed out that this antagonistic behaviour of *G. roseum* would enable it to reduce competition for the underlying substrate and suit its role as a secondary colonizer of organic substrates. Although the effect of the three isolates in increasing plant dry weight was similar on the tested soils ($P \leq 0.05$), their effect on root rot severity was soil dependent.

Despite a significantly reduced disease severity of plants grown in naturally infested soil treated with *Penicillium* and *F. oxysporum*, these species did not enhance plant dry matter weight. The root system was less dense, but this was not accompanied by necrosis. *F. culmorum*, which in *vitro* tests completely inhibited Fs48, severely attacked the pea seeds in the soil. The few plants which 'escaped', grew vigorously and were healthy.

In the experiment where the natural microflora was activated by previous pea cropping, soft rot became dominant in soils CTE and HA1 and *G. roseum* isolates did not suppress root rot as in the first experiment, despite the higher density of the antagonist used. *G. roseum* isolates were still able to control root rot in one of the three soils, JON.

In the next experiment, a successful control was obtained by increasing the density (10^7 spores g^{-1} dry soil) of the three *G. roseum* isolates in the activated soils, HER, HIL and HOE. In HER, the pathogen *T. basicola* causing black rot occurred besides Fsp, whereas the other two soils were also infested with *A. euteiches*. This density effect suggested that a 'no escape' condition is required for an effective control of the pathogenic microflora, including soft rot causing pathogens. Oyarzun et al. [1994b] reported that selective control of one individual pathogen might create an ecological vacuum, which possibly stimulates the activity of other pathogens. The observed antagonistic effects of *G. roseum* suggest that a high dose may help to overcome 'disease trading'.

Bacterial antagonism in soil. The control of root rot by fluorescent pseudomonads depended on the soil used. The ability of pseudomonad isolates to suppress root rot did not correlate with the receptivity of the soil from which they originated.

Factors such as soil water availability, soluble

Ca, pH and soil type have important implications for soil receptivity and the role of pseudomonads as antagonists [Rovira et al., 1992]. Under our experimental conditions, soil water was maintained at a high level and pH of the soils ranged from neutral to alkaline, conditions favourable to bacteria. The differences between isolates in reducing disease when used as seed or soil treatment, indicate that place and energy supply for the antagonist and competitive ability in respect to the other microflora also determine their effectiveness. Isolate 8, however, was highly effective in controlling root rot in pea both as soil application and as seed coating.

When the bacteria were coated onto seed, isolates of fluorescent pseudomonads successfully controlled root rot in soil JON, where Fsp was typically occurring singly. In HER, with a dominant population of *T. basicola*, no disease suppression was observed by seed or by soil applications. This is in contrast to the frequent reports of good control of *T. basicola* by *Pseudomonas* [Défago et al., 1990; Parke, 1991; Reddy and Patrick, 1992; Rovira et al., 1992]. In soil applications, antagonism was also observed in soil DHO which had a remarkably high carbonate content (7.8%) and a high initial population of *Pseudomonas* spp. The latter suggests that a high dose might be necessary to obtain an effect. Tu [1992] found a strong development of a saprophytic bacterial population after liming the soil and consequently pea root rot intensity was reduced.

Concluding remarks. We are aware that screening antagonists *in vitro* does not necessarily predict their performance in natural conditions. Biocontrol agents are living entities with particular needs and ecological requirements. In our study, various of the selected antagonists were effective in more complicated conditions. Several species were very antagonistic *in vitro*, particularly species of actinomycetes and *Mortierella*. Insufficient inoculum production of these species impeded further experiments.

Our experiments confirmed that the dose of the antagonists and the opportunities for competition influenced their effectiveness. The soil clearly affected the ability of isolates to suppress root rot. These findings support the opinion of Moore et al. [1983], that the development of disease suppression depends on the relative receptiveness of the soil to the microbiological entities.

The lack of relation between the antagonistic activity of *Pseudomonas* isolates and the receptivity of the soil from which they originate seems to invalidate the preferred use of SLR to obtain antagonists. Antagonists can also be obtained from highly receptive soils. Ineffective isolates obtained from SLR are not or not singly responsible for low receptivity. Therefore, to successfully control soilborne pathogens in pea, it is necessary, after selection of antagonists in the laboratory, to develop the application and management of biocontrol systems on a case by case basis, taking into account the natural soil receptivity to pathogens and antagonists.

Acknowledgements

The authors thank The Agricultural Research Institute (INIA) in Spain for providing a postdoctoral research grant and the Netherlands Grain Centre for financial support. We are grateful to Mrs Esther Hoogland for valuable technical assistance. We wish to thank Prof J.C. Zadoks, Dr. M. Gerlagh and Dr. J. Postma for critically reading the manuscript.

References

Alabouvette C (1990) Biological control of *Fusarium* wilt pathogens in suppressive soils. In: Hornby D (ed) Biological control of soil-borne plant pathogens (pp. 27–43). CAB International, Wallingford, pp 479

Alabouvette C, Couteaudier Y and Louvet J (1982) Comparaison de la réceptivité de différents sols et substrats de culture aux fusarioses vasculaires. Agronomie 2: 1–6

Alabouvette C, Rouxel F and Louvet J (1977) Effets du rayonnement gamma sur la microflore d'un sol et sa résistance à la fusariose vasculaire du melon. Annales de Phytopathologie 9: 467–471

Alabouvette C, Rouxel F and Louvet J (1979) Characteristics of *Fusarium* wilt-suppressive soils and prospects for their utilization in biological control. In: Schippers B and Gams W (eds) Soil-borne plant pathogens (pp. 165–182). Academic Press, New York

Anonymous (1985) Statistix. An interactive statistical analysis program for microcomputers. NH Analytical Software. St. Paul, USA

Baker R (1990) An overview of current and future strategies

and models for biological control. In Hornby D (ed) Biological control of soil-borne plant pathogens (pp. 375–388). C.A.B. International, Wallingford, pp 479

Barnett HL and Lilly VG (1962) A destructive mycoparasite, *Gliocladium roseum*. Mycologia 54: 72–77

Berry LA and Deacon JW (1992) Video-analysis of *Gliocladium roseum* in relation to mechanisms of antagonism of plant pathogens. In: Jensen DF, Hockenhull J and Fokkema NJ (eds) New approaches in biological control of soil-borne diseases (pp 64–66). Proc. EFPP/IOBC Symposium, Copenhagen, Denmark, pp 222

Burgess LW and Liddell CM (1983) Laboratory Manual for *Fusarium* Research. The University of Sydney, Sydney, Australia. 162 pp

Campbell R (1989) Biological Control of Microbial Plant Pathogens. Cambridge University Press, Cambridge, UK. 218 pp

Deacon JW and Berry LA (1992) Mode of action of mycoparasites in relation to biocontrol of soilborne plant pathogens. In: Tjamos EC, Papavizas GC and Cook RJ (eds) Biological control of plant diseases, progress and challenges for the future (pp 157–168). NATO ASI, Life Science 230, Plenum Press, New York/London, pp 446

Défago G, Berling CH, Burger U, Hass D, Kahr G, Keel D, Voisard C, Wirthner P and Wüthrich B (1990) Suppression of black root-rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens*: potential applications and mechanisms. In: Hornby D (ed) Biological control of soil-borne plant pathogens (pp 93–108). C.A.B. International, Wallingford, pp 479

Gould WD, Hagedorn MK, Bardinelli TR and Zablotowicz RM (1985) New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. Applied Environmental Microbiology 49: 28–32

King EB and Parke JL (1993) Biocontrol of *Aphanomyces* root rot and *Pythium* damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars.

King EO, Ward MK and Raney DE (1954) Two simple methods for determination of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 44: 301–304

Kommedahl T and Windels CE (1978) Evaluation of biological seed treatment for controlling root diseases of pea. Phytopathology 68: 1087–1095

Labruyère RE and Riepma P (1954) De teelt van erwten in verband met bodem, klimaat en plantenziekten. Landbouwvoorlichting 11: 481–497

Lawley RA, Campbell R and Newman EI (1983) Composition of the bacterial flora of the rhizosphere of three grassland plants grown separately and in mixtures. Soil Biology and Biochemistry 15: 605–607

Lechappe J, Rouxel F and Samson MT (1988) Role de *Fusarium oxysporum* dans l'équilibre du complexe parasitaire du pied du haricot associant *Fusarium solani* f. sp. *phaseoli, Thielaviopsis basicola* et *Fusarium oxysporum*. EPPO Bulletin 18: 111–117

Lin YS and Cook RJ (1979) Suppression of *Fusarium roseum* 'Avenaceum' by soil microorganisms. Phytopathology 69: 384–388.

Little TM and Hills FJ (1979) Metodos estadísticos para la investigación en agricultura. Editorial Trillas, Mexico, 270 pp

Louvet J, Rouxel F and Alabouvette C (1976) Mise en évidence de la nature microbiologique de la résistance d'un sol au développement de la fusariose vasculaire du melon. Annales de Phytopathologie 8: 425–436

Miller HJ, Henken G and Van Veen JA (1989) Variation and composition of bacterial populations in rhizospheres of maize, wheat and grass cultivars. Canadian Journal of Microbiolology 35: 656–660

Moore LW, Baker KF and Cooksey DA (1983) Strategies for detecting and characterizing systems for biological control of soilborne plant pathogens. Plant Disease 67: 1058–1064.

Oyarzun PJ (1991) Biotoets voetziekten in erwten. Rapport 120, Proefstation voor de Akkerbouw en de Groenteteeelt in de Vollegrond (PAGV). Lelystad, 155 pp

Oyarzun PJ, Dijst G and Maas PWTh (1994a) Determination and analysis of soil receptivity to *F. solani* f. sp. *pisi* causing dry root rot of peas. Phytopathology (in press)

Oyarzun PJ, Gerlagh M and Hoogland AE (1993) Pathogenic fungi involved in root rot of peas in the Netherlands and their physiological specialization. Netherlands Journal of Plant Pathology 99: 23–33

Oyarzun PJ, Postma J, Luttikholt AJG and Hoogland AE (1994b) Biological control of foot and root rot in pea caused by *Fusarium solani* with nonpathogenic *Fusarium oxysporum* isolates. Canadian Journal of Botany (in press)

Parke JL (1991) Efficacy of *Pseudomonas cepacia* AMMD and *Pseudomonas fluorescens* PRA25 in biocontrol of *Pythium* damping-off and *Aphanomyces* root rot of pea. In Keel C, Koller B and Défago G (eds) Plant growth-promoting rhizobacteria-progress and prospects (pp 30–33). IOBC/WPRS Bulletin XIV/8, pp 418

Reddy MS and Patrick ZA (1992) Colonization of tobacco seedling roots by fluorescent pseudomonads suppressive to black root rot caused by *Thielaviopsis basicola*. Crop Protection 11: 148–154

Rouxel F (1991) Natural suppressiveness of soils to plant diseases. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schippers B and Tempel A (eds) Biotic interactions and soil-borne diseases (pp 287–296). Elsevier, Amsterdam, Oxford/New York/Tokio, pp 427

Rouxel F, Alabouvette C and Louvet J (1977) Incidence de traitements thermiques sur la résistance microbiologique d'un sol à la fusariose vasculaire du melon. Annales de Phytopathologie 9: 183–192

Rovira A, Ryder M and Harris A (1992) Biological control of root rot disease with pseudomonads. In: Tjamos EC, Papavizas GC and Cook RJ (eds) Biological control of plant diseases, progress and challenges for the future (pp 175–184). NATO ASI, Life Science 230. Plenum Press, New York/London, pp 446

Sher M and Baker R (1980) Mechanism of biological control in a *Fusarium* suppressive soil. Phytopathology 70: 412–417

Tu JC (1992) Management of root rot diseases of peas, beans and tomatoes. Canadian Journal of Plant Pathology 14: 92–99

Van der Spek (1968) Voet- en wortelrot van erwten, pp 41–42 in Jaarverslag IPO 1967, Wageningen

Walz-Borgmeier A (1991) Untersuchungen zur biologischen Bekämpfung von *Fusarium*-welken an Rotklee (*Trifolium pratense* L.) durch antagonistische und apathogene *Fusarium oxysporum* Schlecht. Stämme. PhD Thesis State University Utrecht, 83 pp

Windels CE (1981) Growth of *Penicillium oxalicum* as a biological seed treatment on pea seed in soil. Phytopathology 71: 929–933

Windels CE and Kommedahl T (1982) Pea cultivar effect on seed treatment with *Penicillium oxalicum* in the field. Phytopathology 72: 541–543