ROOT ROT OF PEAS IN THE NETHERLANDS; FUNGAL PATHOGENS, INOCULUM POTENTIAL AND SOIL RECEPTIVITY

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Proefschrift

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Stellingen

1. De term "voetziekte" is ontoereikend en zou moeten worden vervangen door "voet- en wortelrot".

   Dit proefschrift

2. Het bestaan van *formae speciales*, als component van de populatie van een pathogene soort, maakt het gebruik van inoculum-dichtheid van die soort in de grond als schatter van infectie-druk onbetrouwbaar.

   Dit proefschrift

3. Van alle factoren, die van belang zijn voor de ontwikkeling van wortelrot, is de "inoculum potential of the soil" (IPS) de belangrijkste.

   Dit proefschrift

4. Bodemreceptiviteit bestaat, is pathogeen-specifiek, en is stabieler naarmate zij meer afhangt van abiotische bodemfactoren.

   Dit proefschrift

5. Bij de soort *Fusarium oxysporum* kan het begrip *forma specialis* worden beschouwd als een nuttig hulpmiddel voor de praktijk, maar aan de fylogenetische waarde ervan moet worden getwijfeld.

6. Onderzoek over de invloed van planteresiduen op omvang en virulentie van inoculum is van groot belang voor de beheersing van bodemgebonden schimmelziekten.

7. Voor een vollediger verklaring van de variatie tussen percelen in receptiviteit voor voet- en wortelrot is het noodzakelijk om ook onderzoek te doen aan de bodembewonende arthropoden en nematoden.

8. De eventuele toepassing van mengteelten in de vollegronds-groenteteelt wordt meer bepaald door de eisen die de markt stelt dan door de technische beperkingen.

9. De beste methode om de stikstofemissie bij de aardappelteelt te verminderen, is minder stikstof geven.

10. Bij het verwezenlijken van duurzame landbouw dient het lineaire model van kennisoverdracht te worden verlaten.

    N. Röling. 1994. Platforms for decision making about eco-systems. L.O. Fresco et al. (Ed.). The future of the land. J. Wiley & Sons, in progress.

11. Om landbouwkundige vernieuwingen te stimuleren is luisteren belangrijker dan praten.

    P. Engel, 1990. Two ears, one mouth... AT Source 18(4): 2-5.

12. Het regelmatig gecontroleerd branden van natuur-terreinen is niet in strijd met natuur-zorg maar een consequentie ervan.

13. Het is verstandiger lokale oplossingen voor universele problemen te zoeken dan universele oplossingen op lokale problemen toe te passen.

14. Integratie van minderheden door middel van gedwongen aanpassing leidt tot desintegratie.

15. Bijgeloof mag niet worden gelijkgesteld aan magisch realisme.

16. Indien flexibiliseren van onderzoek leidt tot korte dienstverbanden heeft dat een zelfde effect als vroegtijdig ontslag van oudere onderzoekers: kapitaal-vernietiging.

Stellingen behorend bij het proefschrift van P.J. Oyarzun:
"Root rot of peas in The Netherlands; fungal pathogens,
inoculum potential and soil receptivity".

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Author's abstract

Fungi associated to pea (*Pisum sativum* L.) root rot were studied. *Fusarium* and Oomycetes were most common. *Fusarium solani* f. sp. *pisi*, Fsp, was widely distributed and the most frequent fungus in roots of diseased crops. The pathogens *Thielaviopsis basicola*, Tb, and *Aphanomyces euteiches*, Ae, were new records for pea in The Netherlands. These pathogens showed physiological specialization on pea. *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella* were often found, mainly as blight diseases. For several root rot pathogens, seed infection was a source of primary inoculum. The disease pressure in soil, due to these pathogens, called inoculum potential of the soil (IPS), was determined by bioassay. The IPS of fields, determined before growing peas, was the best predictor of disease intensity in crops. The bioassay is now being used to advise pea growers. A field survey of about 200 pea fields showed that root rot could develop in crops on fields with marked differences in cropping history and agronomic qualities. Root rot severity in crops correlated with the cropping frequency of legumes, though this variable explained only a fraction of the variation in severity. The effect of soil-habitat on root rot pathogens, called soil receptivity (SR), was examined. In SR assays, inoculum is added to the soil. A computerized equipment was developed to automatically control the water potential and temperature during the SR experiments. In 1991, soil samples were taken from 50 commercial fields and 5 experimental plots, and SR to Fsp, Tb and Ae was assayed. About 20 samples showed increased IPS since earlier assessments, mainly due to soft rot. Pathogenic activity significantly depended on soil. Most soils were conducive to Ae, intermediate for Fsp and suppressive to Tb. In biocontrol experiments, isolates of non-pathogenic *F. oxysporum* and *Gliocladium roseum* reduced *Fusarium* root rot. The suppression in soils where Fsp was naturally present depended on the dose and the soil tested. The antagonism of *Actinomyces* spp. and fluorescent pseudomonads was independent of their origin from suppressive or conducive soils. Soil nutrients, soil physical properties and kind and size of microbial populations correlated with receptivity to Tb, Ae or Fsp. Soil sterilization rendered all soils conducive, suggesting that relations between abiotic properties and SR are mediated by microbial activities. The value of SR as a tool in integrated disease management is discussed.

Preface

In the time I started my work with soil-borne pathogens of peas and related diseases, the guidance by and fruitful discussions with P.H.M. Dekker, PAGV, was decisive in my wish to specialize in research on root rot disease of legumes.

I express my gratitude to Professor J.C. Zadoks who accepted the responsibility of being my supervisor, and with whom I had numerous stimulatory discussions. Without the stimulus, help and support of my co-supervisor Dr. M. Gerlagh this thesis would not have been prepared.

I am grateful to the colleagues in the project supervision commission, G. Dijst, C. Langerak, A.B. Bouwman, A.M. Ros, J. Lamers, A. van der Zweep and P.W.Th. Maas for their valuable discussions.

Several colleagues of the Extension Service participated actively in field surveys. Without their knowledge of the practice and heart for their work, the survey would have been a very difficult enterprise. To all of them and especially, to J. Basting who assisted me during four years, I express my gratitude. The bulk of work performed in preparing tests and testing soil receptivity was shared with Mrs. A.E. Hoogland.

To my IPO-colleagues of the technical departments with whom I spent a great deal of my time in developing a computerized equipment to determine inoculum potential of pathogens in soil, T. van der Zalm and H.W. Roelofsen, I extend my respect for their dedication.

Finally my deep thanks are due to my family for their patience, encouragement, and interest in my work.

The work presented in this thesis was financially supported by the Netherlands Grain Centre, for which I am grateful. I am also very indebted to the DLO-Research Institute for Plant Protection for offering the necessary infrastructure to write most of the papers presented in this thesis.

Pedro J. Oyarzun

Wageningen, January 1994.

Contents

Chapter 1

General introduction

The problem. In the Netherlands, two circumstances contributed to the comeback of the pea crop between 1978 and 1988 (in 1993 the acreage of pea was at the 1978 level again). 1. The 1978 decision of the European Community (EC) to subsidize the production of protein-rich crops within the EC countries. 2. The narrow crop rotation in Dutch agriculture, with primarily potato, sugarbeet and winter wheat. Increase and even maintenance of the productivity of these crops demanded a high input of agrochemicals and led to over-exploitation of the soil. In the seventies, undesirable side effects became apparent. The feeling that this high-input farming with a narrow rotation was not sustainable, led to the search of a 'fourth ' crop (Boeringa and Höbaus, 1984; Anonymous, 1986) and to experiments with new cropping systems (Zadoks, 1989).

Because of its fodder quality, pea profited from this situation. From 1978 to 1987, the area under pea increased from approximately 3000 to 30.000 ha. A major obstacle in growing pea was the high susceptibility of commercial cultivars to pathogenic fungi attacking the roots. The prospects of resistance breeding were meagre and the cultural practices inadequate to alleviate the disease pressure. Reduction of root rot to an economically acceptable level could only be achieved by avoidance of contaminated fields. The development of procedures to determine the level of root rot pressure in soil and the use of this information to forewarning the farmer on **short-term** risks of individual fields thus became focal points. Various other questions were formulated to understand the factors affecting root rot epidemiology, and therewith to enlarge the probability of obtaining a high and stable pea yield.

Soil inoculum potential, research approach 1. First, a test was developed to quantitatively assess the degree of soil infestation by pea root rot pathogens. The degree to which a pathogen expresses its inoculum potential (Garret, 1956) in soil was described by Mitchell (1979) as the inoculum potential of the soil (IPS), a term considered synonymous with the root rot potential of a soil as used by Sherwood and Hagedorn (1958). Since the concept of IPS involves abiotic and biotic effects of the soil on pathogen-host interactions, it also applies to diseases caused by a complex of pathogens. A greenhouse bioassay to estimate the level of disease pressure, called 'root rot potential of a field', was developed for *Aphanomyces euteiches* by Sherwood and Hagedorn (1958). In the USA it is still in use to advise the farmer. In Great Britain, the Processors and Growers Research Organisation, PGRO, and in France the Laboratory for Diagnoses of Soil Pathogenic Microflora, commercially offer a similar service.

Up-to-date knowledge about the pathogenic fungi involved in root rot of pea, their importance, and the factors affecting their epidemiology were hardly available in the Netherlands. Despite the extent of the older Dutch research on pea diseases, its multidisciplinarity, and its well structured organization, the results were never published. The current view was that pea root rot is a problem of marginal fields, badly drained heavy clay, poor in lime. 'Ascochyta ', in particular *Phoma medicaginis* var. *pinodella* was considered the major root rot pathogen. *Fusarium solani* was thought to enter the

plant through wounds caused by other agents or by normal plant growth. The identity of the Oomycetes causing severe attacks in the north of the Netherlands had not yet been revealed.

Habitat effect on root rot pathogens, research approach 2. A field survey was performed from 1985 to 1989 (Oyarzun, 1991). Conspicuous differences in root rot were observed between crops on fields with good agronomic properties and healthy crops were found on fields which should be considered marginal, an observation hardly explicable by traditional views on pea root rot. Root rot disease of pea appeared to have an important site-specific component. In the literature, outbreaks of root rot had been linked to soil types but not to cropping history (Temp and Hagedorn, 1967), or they were found to be uncorrelated with the latter (Burke et al., 1970).

Soil Receptivity. Burke (1965) reported the existence of 'resistant soil' and 'root rot soil'. Many publications appeared on this topic and on the putative underlying mechanisms. The view on the role of soil in soil-borne diseases changed. Soil is now considered as an ecosystem in which abiotic soil factors and populations of inhabitants actively affect the establishment, survival, growth and pathogenic activity of plant pathogens. This effect of the soil ecosystem was called 'soil receptivity', SR (Alabouvette et al., 1982). Soils of low receptivity, also known as 'suppressive' soils (Baker and Cook, 1974) attracted the mainstream of ecological research on soil-borne pathogens.

Characterization of SR and identification of the major factors responsible were expected to allow a better understanding of cultivation effects and to lead to **long term** management of pea root rot. SR to three major soil-borne pathogens of pea was investigated. To make SR into an operational concept, we assumed SR to condition variations in IPS. Thus a comparison of the dynamics of IPS in different soils should permit to make quantitative approximations. For each pathogen, IPS was manipulated by changing the amount of inoculum in the soil. We developed a computerized equipment which improved the reproducibility and efficiency of IPS determinations, by control of soil water potential and soil temperature. The samples were taken from fields with known low IPS. In 1991-1992, SR tests were performed on a limited set of soil samples.

Layout of this report. Chapter 2 describes the fungi involved in pea root rot, the symptoms they cause and their importance in the field. Chapter 3 contains the first report on the occurrence of *Aphanomyces euteiches* in the Netherlands. In chapter 4, factors affecting the epidemiology such as the frequency of pea and other legume hosts, are described. In chapter 5 we analyse fungus species occurring in commercial seed lots and discuss the role of seed infections in the dissemination of root rot. The method of testing IPS, its results and effects of root rot on pea yield are discussed in chapter 6. Some technical aspects of the computerized equipment used to assay SR with illustrations of its performance are described in chapter 7. Procedures to characterize SR, results of SR tests and use of SR data in ecological research are elaborated taking *F. solani* f. sp. *pisi* as case (Chapter 8). The procedure outlined is subsequently applied to two other root rot pathogens, *Thielaviopsis basicola* and *A. euteiches* (Chapter 9). The introduction of microbial antagonists into natural soils in order to reduce root rot, is examined in

chapters 10 and 11. The relation of the new SR variates, constructed in the preceding chapters, with several soil environmental factors is investigated in chapter 12. The report is concluded by a general discussion, chapter 13.

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Chapter 2

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Pathogenic fungi involved in root rot of peas in the Netherlands and their physiological specialization

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Abstract

Research on root rot pathogens of peas in the Netherlands has confirmed the prevalence of *Fusarium solani*, *F. oxysporum*, *Pythium* spp., *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella*. *Aphanomyces euteiches* and *Thielaviopsis basicola* were identified for the first time as pea pathogens in the Netherlands. Other pathogens such as *Rhizoctonia solani* and *Cylindrocarpon destructans* were also found on diseased parts of roots.

F. solani existed in different degrees of pathogenicity, and was sometimes highly specific to pea, dwarf bean or field bean, depending on the cropping history of the field. *A. euteiches* was specific to peas, whereas *T. basicola* showed some degree of physiological specialization.

Additional keywords: *Pisum sativum*, *Vicia faba*, *Phaseolus vulgaris*, foot rot, *Aphanomyces euteiches*, *Fusarium solani*, *Mycosphaerella pinodes*, *Phoma medicaginis* var. *pinodella*, *Thielaviopsis basicola*

Introduction

In the first half of the 1980s pea growing gained in importance in the EC countries due to a combination of a high demand for protein for feed and EC subsidies. This stimulated renewed interest in the effect of the frequency of pea crops in rotation on the occurrence of root rot. (In this paper root rot will be used to indicate lesions on and rotting of both roots and epicotyls.) Peas are traditionally known not to sustain frequent cropping, and a maximum of one pea crop in a 6-year rotation is considered appropriate. However, even with this low frequency, incidents in which crops suffer badly from root rot do occur. In the past, this has led to several efforts to identify damaging pathogens in the Netherlands. In 1927, Buisman studied the role of *Phycomycetes* in pea root rot. She found *Pythium irregulare* and *P. debaryanum* as causal organisms. Went (1934) reported on the role of *Fusarium solani*. Kerling (1949) studied *Mycosphaerella pinodes*, whereas Boerema et al. (1964) described the importance of *Phoma medicaginis* var. *pinodella* on pea and other legumes. These detailed studies, however, did not clarify the situation. In 1954 Labruyère and Riepma distinguished between root rot symptoms in Zeeland and Groningen, i.e. in the Southwest and Northeast of the Netherlands. The root rot in Zeeland, which was first attributed to *F. solani*, proved to be caused by bean (pea) leaf roll virus (Hubbeling, 1954); that in Groningen was primarily considered to be caused by bad soil structure, which would stress the roots and make them susceptible to pathogens.

In 1961, a project was started to elucidate the cause of the difference in pea yields

between the North and the South of the Netherlands. Results were published in a poorly accessible form (Anonymous, 1966; Riepma, 1967). Root rot was not always associated with bad soils, and was sometimes encountered on excellent soils. *F. solani*, the most frequent pathogenic species in diseased roots, was still considered to be a weak pathogen. No new species were reported.

In 1985, with so many factors still unclear, another project was started to elucidate the nature of the root rot pathogens and to estimate the current incidence of root rot on peas in the Netherlands. Particular attention was paid to the relations of cropping frequency of peas and other legumes and root rot incidence. Literature on pea root rot pathogens is available from all major pea growing areas (e.g. Zogg, 1964; Burke and Hagedorn, 1968; Burke and Kraft, 1974; Shipton, 1977). The most frequent pathogens include *F. solani*, *Fusarium oxysporum*, *Aphanomyces euteiches*, *P. medicaginis* var. *pinodella*, *M. pinodes*, *Thielaviopsis basicola* and *Pythium* spp. This wide array of pathogens may result in complicated interactions. Each pathogen has its own specific biology, some of them showing physiological specialization at species or even cultivar level (Sundheim, 1972; Grau et al., 1991). Additional variables are the interactions of these pathogens with other elements of the soil microflora, climate and soil type (Lloyd and Lockwood, 1963; Alconero and Hagedorn, 1967; Hagedorn, 1986).

The root rot pathogens of legumes in the temperate zone, peas (*Pisum sativum* L.), dwarf beans (*Phaseolus vulgaris* L.) and broad beans (*Vicia faba* L.), are largely the same at species level. *M. pinodes*, however, is more or less limited to peas. Most researchers (Burke and Kraft, 1974; Kraft and Burke, 1974; Davis and Shehata, 1985; Kraft, 1986) agree that the important root pathogens show physiological specialization. Thus, the notorious root rot pathogen *F. solani* is recognized as f.sp. *pisi* for peas, f.sp. *phaseoli* for phaseoulus beans and f.sp. *fabae* for faba beans. Clarson (1978), however, concluded from inoculation trials that the distinction in formae speciales is questionable. Peas, beans and other legumes were equally susceptible to these 'formae'. Comparable results were reported by Yang and Hagedorn (1968) and Messiaen and Cassini (1968).

Physiological specialization is important in relation to the effect of different legumes in the rotation. It is also important in screening of cultivars for resistance. The provision of accurate data on the pathogens involved in root rot, including their physiological specialization, contributes to a better management of pea production as it depends on safe rotations, if possible supported by the use of less susceptible cultivars.

The present paper deals with pathogenic fungi causing root rot of peas in the Netherlands and their physiological specialization. In an other paper we will discuss the relation of crop rotations to root rot of peas.

Materials and methods

Sampling soil for screening of pathogens. From 1985 to 1987 soil samples were obtained from fields in the traditional pea production areas in the North and South of the Netherlands.

Most soils were clay loams or loams. All investigated fields had been cropped with a legume at least once in the past decade. Cropping history and crop data were recorded. One hectare of the most homogeneous part of each field was identified and sampled. Fifty soil cores of 20–25 cm depth were taken in a W-pattern. Soil cores were mixed together,

reduced to an aggregate size of maximum 0.8 cm and stored in the dark at 4 °C.

The typical pathogenic microflora of peas, dwarf beans and broad beans was studied in soil samples originating from experimental plots where these legumes had been grown continuously for 10 years (since 1979). Soil on which peas had been grown continuously since 1979 is indicated as CCP. The corresponding code for dwarf beans is CCB, and for field bean CCF.

Whether other legumes stimulate the increase in root rot pathogens of peas was investigated by isolating fungi from roots of peas grown in soil which had been cropped every second year since 1983 with garden pea, dwarf french bean or broad bean in the rotation: sugar beet – legume – potato – legume – spring wheat – legume. For each plot the legume crop remained the same in all years.

The cropping history of fields. In the field, the pea crop may consist of vining pea for the canning industry or dry pea for seed production or animal feed. No field with a history of field bean cropping was involved in the survey. Cropping effects of field bean on pathogenic fungi were studied in soils of experimental plots. Dwarf bean was traditionally grown in the South of the Netherlands. A cropping plan in the South consists for almost 3/4 of sugar beet, potato and wheat and in the North primarily of wheat and sugar beet.

Isolation of pathogens. Pathogens were isolated from diseased field plants, or from plants growing in pots filled with soil samples originating from the sources indicated above. In the latter case, plants were uprooted when they had reached the green flower bud stage, and the roots washed free of adhering soil. A subsample of five plants with root rot symptoms was selected for isolation purposes. Isolates were taken from dark brown to black lesions or from rotten roots (soft rot) only. Pieces of plant tissue were superficially sterilized in 1% NaOCl for 1 min., rinsed with sterile water and plated on media such as water agar, cherry agar, Czapek-Dox agar or PDA. After 5 days of incubation at 20 °C cultures were transferred for further identification. The presence or absence of each species was recorded per soil sample, with 100 samples screened, and expressed as percentage occurrence. Microscopical observation of diseased tissue, immediately or after incubation in sterile water, was used to complement the plating methods.

In 1988, soil samples inducing soft rot were specifically screened for the presence of *A. euteiches*. Details are described elsewhere (Oyarzun and van Loon, 1989).

Pathogenicity and physiological specialization. F. solani. Because of the dominant presence and the unclear role of *F. solani* in root rot of peas, pathogenicity tests were restricted to this pathogen.

Seventy-five isolates originating from different fields, as described above, were screened. To perform pathogenicity tests, monosporous isolates of *F. solani* were produced. Peas were grown in 2 × 4 × 12 cm plastic tubes with sand, to which a spore suspension of *F. solani* was added to generate 50 000 spores per g of soil. The tubes were incubated at 25 °C with 12 h per day of 30 000 lux (90 W m^{-2}) . Three weeks after inoculation a root disease index was scored on a 0–5 scale (0 = healthy, white roots and epicotyl; 5 = roots fully discoloured over a length of at least 5 cm).

Physiological specialization of *F. solani* was studied with highly virulent monosporous isolates obtained from CCP, CCB and CCF plots, and from commercial fields. For com-

parison isolate F48 was added which was kindly provided by J.M. Kraft (Washington State, USA). In two experiments, seedlings were inoculated by dipping their roots in suspensions of 10^6 macroconidia per ml, and subsequently incubated in a liquid nutrient medium. These experiments were performed with a 2-month interval. The inoculum for the second experiment originated from re-isolation of the pathogen from the corresponding first experiment.

To further examine whether there was physiological specialization this type of experiments was repeated with several isolates obtained from pea and with Kraft's F48. In these experiments, sterilized sandy loam was inoculated with conidial suspensions to reach 5000 cfu per g of soil. Nine monospore isolates, including the very virulent F48, were examined on two cultivars per crop: peas 'Colette' and 'Allround', phaseolus beans 'Salerna' and 'Narda' and faba beans 'Compacta' and 'Alfred', representing the horticultural and agricultural form of the crop, respectively. For technical reasons the experiment was performed in two runs with six and three isolates, respectively. The first was designed as a split-plot, in four replications, with cultivars in main plots and the isolate in the sub-plot. The second was designed as a split-split-plot, in four replications, with the cultivar form of the host species as subplot and the isolate as sub-sub-plot. Incubation was at 24 °C with 12 h of 30 000 lux (90 W m^{-2}).

A. euteiches. Three isolates of the pathogen were cultured in cornmeal/sand. Test plants were inoculated by mixing the inoculum with a sterilized sandy loam soil and adding zoospores to the seedlings, as described elsewhere (Oyarzun et al., 1990). Temperature was set at 24 °C; 12 h light per day of 30 000 lux (90 W m^{-2}) was supplied. Soft root rot was assessed 3 weeks after sowing. The experiment was designed as a split-plot, in four replications, with host species as plot and cultivar as the sub-plot.

T. basicola. Plants were grown in plastic tubes, 3 × 3 × 20 cm on a mixture of equal volumes of sterilized sand, potting soil and clay soil. Isolates of *T. basicola* were obtained from roots on a selective medium (Papavizas, 1964). A spore suspension consisting of a mixture of three isolates, maintained on malt agar, 10^6 endoconidia per ml, was added to seedlings near the cotyledons, to reach 4000 conidia per g of soil. Incubation was at 22 °C and 12 h per day of 30 000 lux (90 W m^{-2}). Roots were assessed two weeks after sowing. The experiments were designed as a split-plot as with *A. euteiches*, in eight replicates.

Results

Root rot pathogens from soil samples. This study allowed the linking of certain disease symptoms to the presence of specific pathogens. From dry, brown, dark brown to blackish lesions on roots and epicotyls of peas grown on soil samples from farmers' fields from all over the country, *F. solani* and *F. oxysporum* were most frequently isolated, both on 62% of the fields (Fig. 1). *F. oxysporum,* best known as the causal organism of wilting, was often isolated from lesions as the sole organism. Other *Fusarium* spp., notably *F. culmorum, F. avenaceum* and *F. graminearum*, were found in 41% of the samples. In Fig. 1, '*Ascochyta*', which is often used to indicate *P. medicaginis* var. *pinodella, M. pinodes*, and *Ascochyta pisi*, refers to the first two organisms only. They were found at a frequency of 18%. General pathogens such as *R. solani* and *C. destructans* were also recorded at

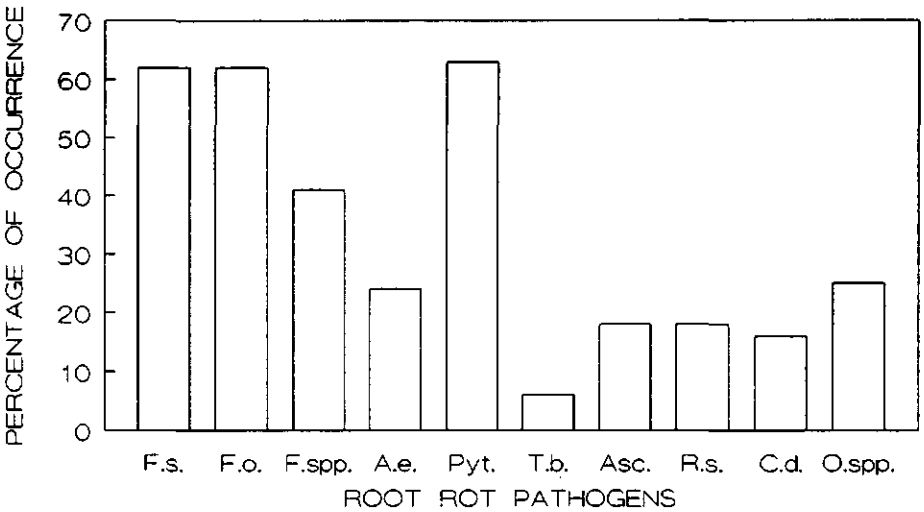

Fig. 1. Frequency of occurrence of fungal species in diseased roots and epicotyls of peas. The figures are the averaged results of isolations of fungi by several methods from samples of 100 different origins (F.s. = *Fusarium solani*; F.o. = *F. oxysporum*; F.spp. = other *Fusarium* spp.; A.e. = *Aphanomyces euteiches*; Pyt. = *Pythium* spp.; T.b. = *Thielaviopsis basicola*; Asc. = '*Ascochyta*'; R.s. = *Rhizoctonia solani*; C.d. = *Cylindrocarpon destructans*; O. spp. = other spp.)

18 and 16%, respectively. Other species/genera isolated added up to 25%.

From soft, light cream to golden brown root lesions, *A. euteiches* was identified for the first time in the Netherlands in 1988 (Oyarzun and Van Loon, 1989). The frequency was 24%. This pathogen was the most damaging of all, death of pea seedlings following extended soft rot. The frequency of *Pythium* spp. was about equal to that of the two dominant *Fusarium* spp., 63%.

A typically black root rot, not extending beyond the level of cotyledon attachment, was found in test plants growing on samples from several fields situated all over the country. The causal organism proved to be *T. basicola*. It was found on 6% of the samples.

In the 1:2 rotations of vegetable legumes with other field crops (Table 1), *M. pinodes* and *P. medicaginis* var. *pinodella* were only found in the rotations with peas. *Verticillium* spp. were found on pea roots in soils without a history of pea growing. *F. solani*, *Fusarium* spp. (among which *F. oxysporum*) and *R. solani* were found on pea roots independently of the history of legume cropping of the soil.

F. solani was frequently found in roots, cotyledons, epicotyls and vascular bundles of pea plants grown on CCP, CCB or CCF soil (Table 2). *F. oxysporum* was isolated from cotyledons especially. *R. solani* and *F. solani* were frequently isolated from dwarf beans on CCB soil. From broad beans on CCF soil *F. solani* was rarely isolated, but *C. destructans* was frequent. On CCP soil *P. medicaginis var. pinodella* was frequently isolated from underground parts of both peas and broad beans. Its presence on broad beans, but not on peas on CCF soil is remarkable. In this experiment, *T. basicola* was only found on field bean and dwarf bean on CCF and CCB soil respectively. Several tests were performed to investigate the presence of *A. euteiches* in CCF or CCB soils, without positive results. *A. euteiches* proved to be specific to the pea field. In general, *Pythium* spp. showed up in all

Table 1. Semi-quantitative representation of fungi isolated from diseased underground tissue of peas grown in soil samples taken after growing vegetable legumes. Each legume species had been cropped for the second time in a 1:2 alternated rotation with potato, sugar beet and spring wheat.

Fungal species	Preceding legume		
	Vining pea	Broad bean	Dwarf french bean
Mycosphaerella pinodes	++	–	–
Phoma medicaginis	++	–	–
Fusarium solani	++	++	++
Fusarium spp.	++	++	++
Rhizoctonia solani	++	++	+
Cylindrocarpon destructans	–	–	+
Verticillium spp. – + +			

– = absent; + = present; ++ = common.

Table 2. Semi-quantitative representation of isolates of fungi frequently obtained from diseased underground tissue of peas, dwarf beans and field beans grown as test plants in soil originating from fields with a history of continuous cropping to peas (CCP), broad beans (CCF) and dwarf beans (CCB).

Fungal	CCP			CCF			CCB		
	Pea	Field bean	Dwarf bean	Pea	Field bean	Dwarf bean	Pea	Field bean	Dwarf bean
Fusarium solani	+++	+++	++	++	+	0	+++	+	+++
Fusarium oxysporum	+++	++	+	+++	++	+++	+	+	++
Fusarium spp.	0	+	0	+	0	0	++	+	0
Phoma medicaginis	++	++	0	0	++	0	0	0	0
Pythium spp.	++	+	0	+	+	++	+	0	+
Rhizoctonia spp.	++	0	0	0	0	++	0	0	+++
Cylindrocarpon destructans	0	++	0	0	+++	0	0	0	+
Aphanomyces euteiches	+++	–	–	–	0[a]	–	–	–	0[a]
Thielaviopsis basicola	0	–	–	–	+++	–	–	–	+++

+ = one isolate; ++ = two isolates; +++ = three to five isolates of five investigated plants; 0 = no isolate obtained; – = not tested.

[a] Bio-assay with pea for the presence of *A. euteiches* was also negative, whereas it was positive for CCP soil.

combinations. Overall, *Fusarium* was the most frequent fungus isolated. Several fungi were always isolated from any one plant.

Pathogenicity of F. solani. The results of the screening for pathogenicity of 75 isolates of *F. solani* on peas are presented in Fig. 2. All isolates were pathogenic to pea and more than 50% of the isolates proved to be moderately to highly virulent.

Physiological specialization. F. solani. Results of the first two experiments in hydroculture with isolates obtained from crops grown continuously on the same field are sum-

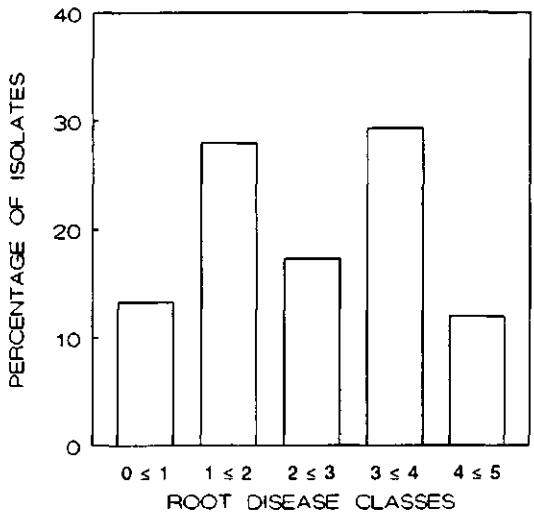

Fig. 2. Frequency distribution of 75 *Fusarium solani* isolates over different root disease index classes (0–1: not or hardly virulent; 4–5: highly virulent) tested on peas.

marized in Table 3. Variance analysis indicated a significant interaction between the *Fusarium* isolates and the crop species ($P < 0.001$). In the first experiment, the *F. solani* isolate from pea was the most virulent one, especially on pea itself. This isolate caused slight to moderate root rot of field bean and dwarf bean respectively. The isolate from field bean on CCF soil only slightly attacked pea, but caused a remarkably heavy root rot of dwarf bean compared with the moderate root rot caused by the isolate on field bean itself. The infection of pea was restricted to the cotelydon attachment area.

The CCB isolate was unable to attack pea considerably. Its effect on field bean and dwarf bean was slight. Inoculation of dwarf bean both with CCP and CCF isolates gave a higher root rot index than with its 'own' CCB isolate.

The repetition of the experiment led to a comparable result, except for the much higher

Table 3. Experiment 1, mean root disease index (0–5) for three legume species following cross-inoculation with isolates of *Fusarium solani* obtained from these three crops grown continuously on the same field. Experiment 2 is a repetition in which inoculation was performed with strains re-isolated from the crop indicated in the first column. Both experiments performed in hydroculture.

Crop	*F. solani* isolated from					
	Pea on CCP		Field bean on CCF		Dwarf bean on CCB	
	Expt 1	Expt2	Expt 1	Expt 2	Expt 1	Expt 2
Pea	3.28	3.07	0.90	3.50	0.15	0.59
Field bean	1.50	1.65	1.85	3.76	1.00	1.24
Dwarf bean	2.00	1.42	2.53	2.56	1.33	1.70

Interaction crop × isolate: $P < 0.001$. For all comparisons in Expt 1: LSD = 0.40 ($P < 0.05$). For all comparisons in Expt 2: LSD = 0.80 ($P < 0.05$).

Table 4. Mean root disease index (0–5) on two cultivars of each of the host species pea, faba bean and phaseolus bean, inoculated with isolates of *Fusarium solani* obtained from pea roots.

Isolate	*Pisum sativum*			*Vicia faba*			*Phaseolus vulgaris*		
	Col.	All.	Mean	Com.	Alf.	Mean	Sal.	Nar.	Mean
F48	5.00	5.00	5.00	5.00	3.18	4.09	1.10	2.13	1.62
CT 2.1	0.38	1.63	1.01	1.13	0.85	0.99	0.15	0.03	0.09
–14	2.28	3.40	2.84	2.15	1.45	1.80	0.13	0.98	0.56
–07	2.95	3.83	3.39	1.38	1.30	1.34	0.23	0.33	0.28
–05	1.35	3.23	2.29	0.48	1.33	0.91	0.15	0.68	0.42
–02	3.55	3.73	3.64	1.05	0.13	0.59	0.13	1.23	0.68
–46	0.63	1.80	1.21	1.05	1.18	1.11	0.23	0.18	0.20
–19	1.13	1.93	1.53	2.28	1.20	1.74	0.28	0.65	0.46
–04	2.68	4.00	3.34	1.38	1.38	1.38	0.95	1.75	1.35

Cultivars: Col. = Colette; All. = Allround; Com. = Compacta; Alf. = Alfred; Sal. = Salerna; Nar. = Narda.
For the first six isolates, interaction cultivar 3 isolate: $P < 0.001$. For all comparisons of single assessments: LSD = 0.96 ($P = 0.05$), except when comparing values within columns, LSD = 0.98; for the last three isolates, interaction host species 3 isolate: $P < 0.001$. LSD = 0.72 ($P = 0.05$), except when comparing means within columns: LSD = 0.53. (N.B. data obtained in separate experiments.)

Table 5. Mean root disease index (0–5) for different host species inoculated with *Thielaviopsis basicola* and *Aphanomyces euteiches* originating from pea.

Pathogen	*Pisum sativum*		*Vicia faba*		*Phaseolus vulgaris*	
	Mar/Col[a]	Finale	Compacta	Alfred	Salerna	Narda
T. basicola	3.5a	3.1b	1.0d	1.3d	2.9c	3.0bc
A. euteiches	3.7a	3.4b	0.0	0.0	0.0	0.0

[a] For each crop two cultivars were tested separately; for pea 'Marzia' was used in the experiment with *A. euteiches* and 'Colette' with *T. basicola*.
Figures in the same line followed by the same letters are not statistically different (Duncan's test; $P = 0.05$).

virulence of the CCF isolate (re-isolated from plants in the first experiment) on peas and field beans.

The results of the experiments with several isolates of *F. solani* of different origin, including F48, are summarized in Table 4. The isolates were tested on two cultivars per crop. Differences between crops were more important than between cultivars within crops. Most isolates scored highest on pea.

T. basicola and A. euteiches. The results of inoculation of peas, phaseolus beans and faba beans with *T. basicola* and *A. euteiches*, both originating from pea, are shown in Table 5. *T. basicola* had a high pathogenicity to pea and phaseolus bean, whereas *A. euteiches* was specific to pea.

Discussion

The most frequently occurring pea pathogens, as determined in this study, are not different from those found in other pea growing countries. Two pathogens, *A. euteiches* (Oyarzun and Van Loon, 1989) and *T. basicola*, were new records on peas for the Netherlands.

In most instances the pathogens isolated from a specific crop, or from crops grown in soil on which only one and the same legume had been grown for many years, showed a high but not an absolute degree of specificity. This result can be interpreted as a form of physiological specialization. Such a physiological specialization of a quantitative nature has been well documented for many soil-borne pathogens. *F. solani* and *A. euteiches* are good examples. Messiaen and Cassini (1968) distinguished ff.sp. of *F. solani*, but stated that the separation of e.g. f.sp. *phaseoli* and f.sp. *pisi* was rather vague. In general they identified isolates from pea as f.sp. *pisi* and those from phaseolus bean as f.sp. *phaseoli*, based on the comparatively heavy attack of the original host. Some isolates were identified as *pisi* + *phaseoli*, attacking both hosts about equally. In our experiments *F. solani* could be isolated from pea, dwarf bean or field bean, grown in any rotation. Different degrees of physiological specialization were found. There was a strong tendency for the most severe attack to be on the original host in cross-inoculation trials, but host overlap was common (Table 3). Zadoks and Van Leur (1983), analyzing these situations, called them 'small interaction phenomenon'.

The high degree of pathogenicity of many isolates (Fig. 2) contradicts the traditional view of *F. solani* as a weak pathogen.

A mixture of three isolates of *A. euteiches* only attacked pea, and thus these isolates had a strict f. sp. *pisi* status. However, this is not typical for *A. euteiches* in general. Pfender and Hagedorn (1982) isolated *A. euteiches* f.sp. *phaseoli* from dwarf bean roots and distinguished it from *A. euteiches* f.sp. *pisi* by comparing their pathogenicity on peas and beans. *A. euteiches* f.sp. *pisi* attacked peas and beans, killing peas and provoking a slight root rot on dwarf beans, whereas *A. euteiches* f.sp. *phaseoli* caused moderate to severe root rot of beans, but did not harm peas. Holub et al. (1991) described how *A. euteiches* generally attacked lucerne, but showed quantitative differential reactions with pea. According to Grau et al. (1991) isolates from pea have always the broadest spectrum, but this is clearly not the case for our isolates. We failed to demonstrate the presence of *A. euteiches* in soils after 10 years with continuous cropping of dwarf beans or field beans. This result seems in line with the statement of Reinking (1942) that *A. euteiches* does not occur in soil never cropped with pea.

Table 2 shows *T. basicola* to occur massively on field bean in CCF and on dwarf bean in CCB soil, whereas pea on CCP soil remained free from this pathogen. Table 5, however, confirms that cross-pathogenicity does occur. We have no explanation for this discrepancy. The absence of *T. basicola* from CCP soil seems to be exceptional, since *T. basicola* is so highly virulent on both pea and bean, whereas it is hardly pathogenic on field bean. The pathogen is reported to show some form of specialization (Lloyd and Lockwood, 1963).

P. medicaginis var. *pinodella* was found predominantly on roots of field bean and pea in CCP soil and on field bean only in CCF soil. This might indicate a certain degree of physiological specilization, in which an assumed f.sp. *pisi* parasitizes both pea and field

bean, whereas a f.sp. *viciae* only attacks field bean. Dwarf bean remained free from attack. Results of studies on physiological specialization in soil-borne pathogens are often difficult to interpret. This may be caused by a modification of pathogenic conduct of the ff.sp. on other plant species by environmental factors, such as the attack of roots by incompatible ff.sp. of *F. solani* under anaerobic conditions (Burke and Kraft, 1974; Miller et al., 1980; Kendra and Hadwiger, 1987; Smucker and Erickson, 1987; Allmaras et al., 1988). The pathogenicity of some of the pathogens to one or two crops only, might mean that other legumes in a crop rotation with pea need not be considered as severely as pea itself. This will apply for *A. euteiches*, the most devastating of the pea pathogens dealt with in the present paper, and highly specific to peas. The less pathogenic fungi *P. medicaginis* var. *pinodella* and *M. pinodes* are also specific to pea. Tables 1 and 2 show differences in dominant pathogens as a function of narrow rotations with just a single legume species, but at the species level of the pathogens the differences are limited. The data suggest the presence of formae speciales corresponding to the crop. Fungi such as the versatile, omnipresent and damaging *F. solani* seem to adapt gradually to any legume host. This can be envisaged either as real adaptation or as selection of rare genotypes which are favoured by a specific host. In a subsequent publication we will elaborate on the consequences of the present results on pea rotations and root disease.

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Neth. J. Pl. Path. 95 (1989) 259-264 SHORT COMMUNICATION

Aphanomyces euteiches as a component of the complex of foot and root pathogens of peas in Dutch soils

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Abstract

The occurrence of *Aphanomyces euteiches* Drechs. in Dutch soils is reported for the first time. Isolates of the pathogen were obtained from peas (*Pisum sativum* L.). A bioassay was used that baited the pathogen from soil into the cortex of stem and root of seedlings of a highly susceptible pea cultivar. The pathogen could subsequently be isolated on a semi-selective medium. Screening of soil samples from 13 fields known to be infested with fungi causing foot and root rot demonstrated the presence of *A. euteiches* in 10 cases. In a second screening on soil samples from 43 fields, the pathogen was present in 16 cases. A positive correlation was found between the disease severity caused by *A. euteiches* in the seedling bioassay and the disease severity caused by the complex of foot and root pathogens in the same soils as evidenced by a mature plant bioassay. It is considered probable that *A. euteiches* has since long been a common component of the foot and root rot complex in Dutch soils but has not been detected previously due to inadequate sampling and isolation techniques.

Additional keywords: Pisum sativum L., bioassay.

Aphanomyces euteiches Drechs. is considered a specialized pathogen of Leguminosae. It is one of the most destructive pathogens in the complex of foot and root rot pathogens of peas, *Pisum sativum* L. (Papavizas and Ayers, 1974; Pfender, 1984). It causes a soft rot of the entire root system that is known in the USA as 'common root rot' of peas. The economic loss caused by common root rot can be severe. In the United States, for green peas average yield losses in fields infested with common root rot have been estimated at 25-30% (Papavizas and Ayers, 1974).

Due to its economic importance *A. euteiches* is one of the best studied fungal diseases of the pea. The literature on its taxonomy and ecology has been extensively reviewed by Papavizas and Ayers (1974).

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A. euteiches is a cosmopolite and it has been reported from several European countries including Denmark (Gram et al., 1929), France (Labrousse, 1934), Great Britain (Beaumont, 1951), Sweden (Olofsson, 1967) and Norway (Sundheim and Wiggen, 1972). In the etiology of foot and root rot of peas in the Netherlands, *A. euteiches* has not been mentioned so far (Buisman, 1927; Riepma, 1952; Labruyère and Van der Spek, 1962).

In the course of a research focussed on a bioassay to predict the risk for foot rot in dry-harvested peas, the symptoms described by Papavizas and Ayers (1974) were repeatedly observed in test plants of cv. Finale (Oyarzun, 1989). Using routine media like potato dextrose agar and water agar, mainly *Pythium* spp. were isolated from these plants. In June 1988, a set of pea varieties was screened for their suitability as test plants for the bioassay. The cultivar Marzia displayed a clear softrot. When sections from the stem base were exposed to tap water, zoosporangia developed and zoospores extruded within 36 hours. Inside the cortical tissue oospores with a morphology reminiscent of those of *A. euteiches* were observed.

The availability of a susceptible commercial variety, a bioassay that is described hereafter (J.M. Kraft, pers. comm.) and the experience with a suitable isolation technique, led us to reexamine soil samples from grower's fields that had previously been assayed for their foot rot potential in a mature plant bioassay. Thus, we aimed to establish as to how far *A. euteiches* was involved in the complex of foot and root pathogens.

Two series of soil samples were investigated for their infestation with *A. euteiches* in two separate bioassays. The first series consisted of 13 samples that had been found to produce soft rot in mature plant bioassays. Ten of these had been collected directly from grower's fields in March 1987, two in March 1986 and one was taken from an experimental field in the Eastern Flevoland Polder. On the latter field, only peas had been grown continuously from 1979 to 1988. On this soil, the characteristic symptoms of soft rot had developed in cv. Marzia in the varietal test. Samples had been collected by pooling 50 subsamples of 0.3 litre taken from the upper 20 cm soil layer at random from a one hectare surface of each field. The pooled samples were crumbled, thoroughly homogenized and sieved (8 mm). They were then stored in plastic bags at 5 °C in the dark. The bioassay was performed in September 1988.

The second series consisted of 43 samples taken from grower's fields in December 1988. Collection, processing and storage were identical to what has been described for the first series. These were bioassayed for their infestation with *A. euteiches* in February 1989. A bioassay with baiting plants for *A. euteiches* was employed that provides an indication on inoculum potential. Seed of peas cv. Marzia was superficially disinfested at room temperature by a 15 min soak in a 1% hypochlorite solution and subsequent washings in distilled water. It was pregerminated in petri-dishes lined with moist filter paper during 5 days at 20 °C. Healthy seedlings with tap roots of 4 cm length were placed on two layers of moistened filterpaper in petri-dishes of 15 cm diameter, three seedlings per dish. The seedlings were covered with soil (ca. 15 ml per petri dish). The dishes were incubated at 26 °C and 100% rh in the dark during 7 days. After this period, the seedlings were assessed for disease symptoms and tissue was excised to isolate fungi from it. Isolation and purification was performed on a semi-selective dilute corn agar medium (Pfender, 1984).

Soft rot symptoms developed in the seedlings within 7 days in 10 out of the 13 samples (77%) assayed in the first bioassay and in 16 out of 43 samples (37%) in the second

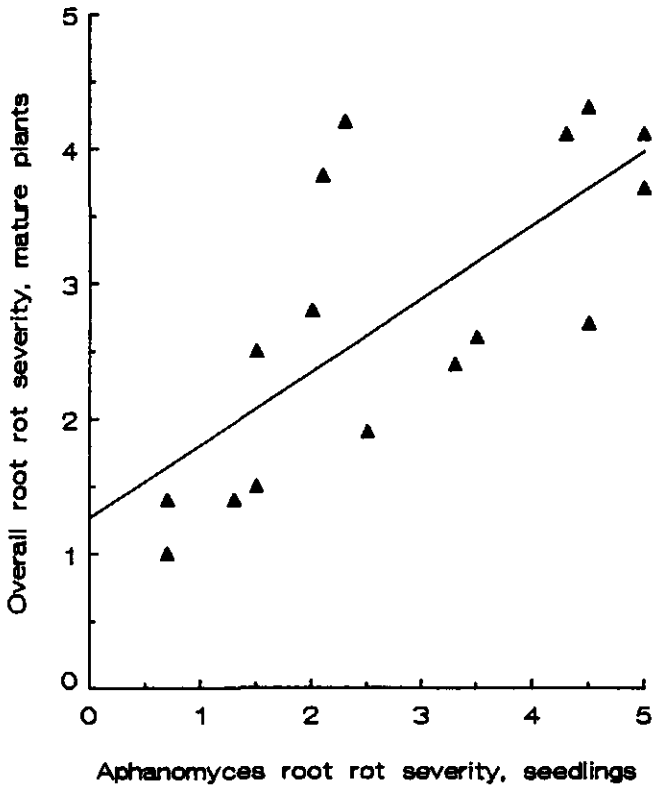

Fig. 1. Disease index assigned in the second bioassay on the inoculum potential of *A. euteiches* root rot in 16 soil samples (mean of three plants per sample; test plants were seedlings of pea cultivar Marzia) plotted against the disease index of overall root rot in a mature plant bioassay (mean of 30 plants of the cultivar Finale) in the greenhouse. Index scale from 0 (root system and epicotyl healthy) to 5 (root and epicotyl severely rotted, leading to death of plant). Regression line: $y = 0.58x + 1.31$.

one (Fig. 1). In the first bioassay, in 7 of the 10 cases, severe rotting of the cortex causing a translucent yellow-goldish discoloration of the tissue was observed. This rotting caused the death of the seedlings soon afterwards. This included both samples collected in 1986 and the one from the field with continuous pea cropping. In the other three cases, symptoms of soft rot were moderate but clearly visible. For comparison, the mean foot rot indices found for the same soils in a previous mature plant bioassay (Oyarzun, 1989), carried out in January 1989, are given. The latter bioassay involved the testing of soil from grower's fields, that had been collected, prepared and stored in a way identical to the description given earlier. The soil was then potted in 3 litre containers and such an amount of water was added as to reach field capacity, which had been determined previously for each soil. Moisture content of the soil was kept at field capacity by regular weighing of the pots and addition of the necessary amount of water. Twelve seeds of cv. Finale, taken from a lot tested to have less than 1% diseased seeds, were sown in each container. Each soil was represented by four replicate containers. The

bioassay was performed in a greenhouse at 18-24 °C under natural lighting. At the onset of flowering, 4-5 weeks after sowing, the soil was carefully washed from the roots of the test plants and the severity of foot and root rot disease was assessed visually. Severity was expressed in a disease index which ranged from 0 where no symptoms of foot and root rot were observed to 5 where the root system showed a severe rot leading to the death of the plant. For the seven samples inducing severe rotting, the mean mature plant disease index was 3.4, for the three samples showing moderate rotting 2.3 and for the three remaining samples apparently free from *A. euteiches* this was 2.2. Thus, it seems that the indicated level of *A. euteiches* is positively correlated with the higher foot rot indices found previously for these soils in the mature plant bioassay.

Previous large scale isolations from diseased plants from this bioassay consistently gave abundant numbers of four pathogenic fungal species traditionally known to be causal agents of foot and root rot in peas (Riepma, 1952; Oyarzun, 1989) but till now in no case *A. euteiches* had been identified.

In all 10 cases that showed soft rot symptoms on a macroscopical level, large numbers of oogonia and oospores were found in the cortex of tap root and epicotyl upon microscopical examination. Oospores measured 20-25 μm in diameter. Isolations on corn meal agar consistently gave highly similar, sparsely growing arachnoid colonies that abundantly produced oospores upon ageing. The identity of four of these colonies was confirmed as *Aphanomyces euteiches* Drechsler by the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands.

Results of the second bioassay were essentially similar. Disease severity was assessed in more detail than in the first one, using the same scale as that applied in the mature plant bioassay on the corresponding samples. Fig. 1 shows that there is a positive relationship between both indices (Spearman's rank correlation coefficient $\varrho = 0.72$, $P < 0.01$ ($n = 16$)). This suggests that the disease severity found in the mature plant bioassay is in part dependent upon the severity of soft rot in seedlings caused by *A. euteiches*.

In the present paper we report for the first time on the presence of *A. euteiches* Drechs. in Dutch soils. The bioassay used may easily be adapted to establish differences in inoculum potential. The assay was rapid and simple to perform. In this study, a limited number of samples was assayed. The evidence supplied for the possible importance of *A. euteiches* as a root pathogen in peas needs substantiation and deserves extended research. In preliminary inoculation experiments, the purified pathogen showed a high degree of virulence (M. Gerlagh, pers. comm.). In 1987, 27.5% of the 51 samples screened induced soft rot. In 70% of these cases, *A. euteiches* was shown to be present, after 18 months of storage.

The failure of detecting *A. euteiches* in previous mycological research on species associated with foot and root rot of peas in the Netherlands may be explained by the use of isolation procedures that apparently were inadequate for this organism. The failure in isolating *A. euteiches* on commonly used agar media have been documented (Papavizas and Ayers, 1974; Pfender et al., 1984). One factor that may have played a role is the use of mature plants, i.e. in the flowering or pod stage, to obtain pathogen isolates. In the mature plant phase, the invasion of several species of secondary pathogens evidently interferes with the demonstration of *A. euteiches*.

Although the detection of *A. euteiches* reported here contributes to insight into the causes of the present incidence of foot and root rot of peas in Dutch soils, we feel that

it is not a new threat to the pea crop. In previous Dutch research on foot rot in peas, soft rot was repeatedly noticed where unidentified phycomycetous species were involved (Riepma, 1952; Labruyère and Van der Spek, 1962). It is well possible that *A. euteiches* has been present already from the start of large scale pea growing on, as in other parts of Europe and North America.

There are no effective curative control measures against *A. euteiches*. Cultural practice should aim at prevention of inoculum build-up to damaging levels, by observing an adequate interval between two pea or, which may be even more safe, two legume crops. An additional strategy to prevent yield loss caused by *A. euteiches* is the growing of pea varieties resistant to this pathogen (Shehata et al., 1983; Kraft, 1988; Lewis and Gritton, 1988). Breeding pea varieties for dry harvest with resistance to foot and root rot (Van Loon et al., 1988) should pay due attention to *A. euteiches*.

Acknowledgements

We wish to thank Dr J.M. Kraft for useful advices concerning the bioassay. We also thank Dr M. Gerlagh for commenting on a draft of the manuscript. The technical assistance of Mrs N. van Dijk and Mrs R. Blanco is gratefully acknowledged.

Samenvatting

Aphanomyces euteiches: een component in het voetziekte-complex van erwten in Nederlandse gronden

De aanwezigheid van *Aphanomyces euteiches* Drechs. in Nederlandse gronden is voor het eerst aangetoond. Isolaten van het pathogeen werden verkregen van erwten (*Pisum sativum* L.). De pathogene schimmel werd in petrischalen uit grond in het schorsweefsel van wortel en stengel van een zeer vatbaar erwteras gelokt. Met behulp van een semiselectief medium konden vervolgens isolaten van de schimmel worden verkregen. Toetsing van grondmonsters afkomstig van 13 percelen, waarvan bekend was dat ze besmet waren met schimmels die voetziekten in erwten veroorzaken, toonde de aanwezigheid van *A. euteiches* aan in 10 gevallen. In een tweede biotoets op grondmonsters van 43 percelen bleken 16 monsters het pathogeen te herbergen. Er werd een positieve correlatie gevonden tussen de ernst van de aantasting door *A. euteiches* van kiemplanten en de aantasting van volwassen planten in een biotoets in de kas. Het is waarschijnlijk dat de schimmel reeds lang in Nederlandse akkers voorkomt, maar door inadequate bemonsterings- en isolatietechnieken over het hoofd is gezien.

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Chapter 4

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Relation between cropping frequency of peas and other legumes and foot and root rot in peas

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Abstract

The relation between the frequency of legume crops in a rotation and the root rot severity in pea was examined in a field survey. Additionally, greenhouse experiments were performed with soil samples from legume rotation trials or from farmers' fields. The frequency of pea crops in current rotations proved to be much less than the recommended value of one in six years. The correlation between pea root rot and the number of years that pea or other legumes were not grown on the field under consideration (called crop interval) was weak. Root rot severity correlated better with the frequency of peas or legumes in general over a period of 18 years, but the frequency still explained only a minor fraction of the variation in disease index. Some experimental data pointed to the occurrence of a highly specific pathogen microflora with continuous cropping of only one legume species, but this phenomenon probably does not occur in farmers' fields. In field samples, root disease index for pea correlated well with that for field bean. The survival of resting structures of pathogens such as *Aphanomyces euteiches* probably explains why the frequency of legume cropping has a higher impact than crop interval on root disease incidence. Pea-free periods and legume frequencies have a poor predictive value for crop management purposes.

Additional keywords: soil pathogens, dwarf bean, *Phaseolus vulgaris* L., field bean, *Vicia faba* L., *Pisum sativum* L., *Aphanomyces euteiches*

Introduction

Due to their susceptibility to the attack by soil-borne pathogenic fungi, peas are known as a self-intolerant crop (Schreuder, 1949). In a recent paper (Oyarzun et al., 1993) we described the pathogenic fungi involved in pea foot and root rot (in this paper simply indicated as root rot). We also discussed aspects of physiological specialization of some of these fungi.

The changed situation in the 1980s, with growing acreage of pea, new cultivars and new cultural practices, asked for attention to the place of pea and related crops in the rotation with regard to the development of root rot diseases in pea. In the Netherlands, one pea crop in a 6-year (1:6) rotation is considered desirable agronomically (Achterstraat and Bouman, 1972; Timmer et al., 1989), but this view certainly needs further clarification. Studies relating root rot diseases in pea to crop rotation are scarce. Salt and Delaney (1985) cite Jones and Linford (1925), who stated that severe root rot develops after four consecutive cycles of pea growing. A comparable phenomenon was observed by Huiskamp (1987). These statements imply that rotations of more than 1:6 would not immediately lead to severe root rot development. However, the rate of increase of disease pressure in soil is not exclusively a function of cropping frequency. Normally, seed qual-

ity (Van Loon and Oyarzun, 1988), crop variety, soil type (Temp and Hagedorn, 1964) and soil structure (Smucker and Erickson, 1987) interfere with the level and activity of pathogenic fungus populations in soils. Further, pathogens greatly differ in production of types and number of survival structures and thus in the number of host-free years they can bridge (Wallen et al., 1967; Kraft et al., 1974). Probability of disease outbreaks by *Phoma medicaginis* var. *pinodella* or *Mycosphaerella pinodes* on pea after an interval of 5 years are considered nil by among others Sherf and Macnab (1986) and Sheridan (1973). Wallen et al. (1967) demonstrated the presence of pathogenic strains of *M. pinodes* in soils even 20 years after the last pea crop. Kraft et al. (1974) reported outbreaks of *Fusarium oxysporum* f. sp. *pisi* race 1 in a field when a susceptible variety was grown again after a period of 40 years.

In the current paper we examine the contribution of crop frequency and number of years without either pea or other legume crop (hereafter called 'crop interval') to the severity of root rot disease in pea crops. The results of a field survey conducted between 1985 and 1987 will be discussed. Field observations are complemented with results of crop rotation experiments.

Materials and methods

Field survey (1985–1987).

Normally, crop interval (number of years between consecutive pea crops) and crop frequency are unequivocally related. In practice, where technical or economical constraints dominate agricultural activity, these criteria may diverge. In order to test the possible relation between root rot in peas and the crop interval or the frequency of legume cropping, 46 fields were sampled in 1985, 48 in 1986 and 51 in 1987. In 1987, 24 extra fields, with a known frequency of legumes, were assessed at flowering. The fields were in the traditional pea-growing areas of the Netherlands, i.e. in the North and the South. Various soil types were represented in the survey, with heavy clay predominant in the North, whereas clay and loam to sandy loam were typical in the South. Of each field only one hectare was investigated. Legumes were represented by peas (*Pisum sativum* L.), dwarf beans (*Phaseolus vulgaris* L.) and lucerne (*Medicago sativa* L.); field beans (*Vicia faba* L.) were not found in the rotation of the fields.

The degree of root rot in crops was assessed at different times during the growth period. Root rot assessments discussed in this paper were made on 1 ha per field, at flowering. Groups of 5 plants were sampled at 10 sites along a W-path through the field, 50 plants per field. Roots were dug up to a depth of 20–25 cm, carefully cleaned from soil, and washed before disease assessment. Root disease index, used as a measure of root rot severity, was scored on a scale of 0–5, with 0 for healthy roots and 5 corresponding to 100% rotten roots of dead plants. For details see Oyarzun (1989).

The data of this survey were analyzed using GENSTAT 5 facilities. (Multiple) regression analysis and linear or higher degree models were tested. Disease index (0–5) was sorted out in disease classes and fields were arranged in histograms according to their index value.

Greenhouse and laboratory experiments.

Between 1985 and 1986, complementary studies on the relation of crop frequency and root rot severity were performed with bioassays in pots. Results of bioassays proved to be well correlated with the intensity of root disease in pea under field conditions (among others, Sherwood and Hagedorn, 1958; Olofson, 1967; Biddle, 1984).

Soil samples were obtained from field experiments at the Research Station for Arable

Farming and Field Production of Vegetables (PAGV) at Lelystad. PAGV fields consist of a deep, calcareous clay soil (pH KCl = 7.7; $CaCO_3$ = 7.2%) with a moderate content of organic matter (2.8%).

In other cases samples were obtained from commercial fields.

Per field a soil sample was composed by mixing 50 cores, taken to 25 cm depth, and passed through an 8-mm mesh sieve to standardize aggregates. Relations between water content and matric water potential of soil samples were determined on a pF-device.

In 1979, a single field experiment was started at PAGV to monitor yield and diseases of peas, field beans, and dwarf beans in continuous culture (CC–). Samples from these treatments are coded as CCP, CCF and CCB respectively. In 1982, a field experiment was started with the vegetable type of the three legume species in a rotation of: potato – legume – sugar beet – legume – spring wheat – legume. The same legume was grown on the same plot in consecutive years. Soil from these plots is coded as AR.

Fields where only two vining pea crops (CA8) or one crop of field bean or dwarf bean (ECC) had been grown since land reclamation were also included. Except on CA8 (last peas in 1982/83), soil samples were taken after the harvest of the legume crop.

Experiment 1. Frequency of legumes. In 1985, after seven (CCF and CCB), two (AR) and one (ECC) crop of field bean and dwarf bean the plots were sampled and bioassayed. In the winter 1986/87, a bioassay was performed with samples from AR plots after two crops of pea, field or dwarf bean. Five additional bioassays were performed with CA8 samples and nine with CCP.

Ten seeds of the cultivar Finale treated with Thiram (1.5 g a.i./kg) were sown at a depth of 4 cm in 2.5-l pots; five pots per soil sample. The pots were incubated at 20/15 °C day/night temperature and soil moisture was kept at field capacity, approx. 26% (g/g) at a density of 1.2 (g cm^{-3}), by daily replenishment. At the green flowerbud stage plants were harvested, roots washed and assessed for root rot severity. For details, see Oyarzun (1989).

Experiment 2. 1987. The ability of the specialized microflora of 'continuous culture soil' to induce root disease on other legumes was studied. For a number of different legumes – dwarf bean, pea, field bean, lupin (*Lupinus luteus* L.), lucerne (*Medicago sativa*) and white clover (*Trifolium repens* L.) – development of root rot was determined after 6 weeks growth in soil from fields which had been cropped for 8 consecutive years with pea, dwarf bean or field bean (CCP, CCB and CCF respectively). Test crops, in four replications, were randomly allocated to each soil sample. The methods and growth conditions in this experiment were the same as described above.

Experiment 3. 1987. Since field beans were not grown in the commercial fields sampled, soil samples from 13 pea fields, with a known variation in inoculum potential of pea root rot (Oyarzun, 1989), were assayed with field bean, cv. Alfred, and pea, cv. Finale. Five pots were used per soil sample and sown with five seeds per pot. Method and experimental set-up were as in Experiment 1. Root disease indices were assessed at flowering and compared.

Experiment 4. A series of ten soil samples, from different parts of the Netherlands and with a varying crop interval of pea, was especially examined for the presence of *Aphanomyces euteiches*, a pathogen known for its long survival as oospores, especially in the organic soil fraction (Mitchell et al., 1969). The soil samples were separated in an organic and an inorganic fraction by wet-sieving. Unsieved soil and small quantities of the

organic and inorganic fractions were used to cover the root of pre-germinated pea of approx. 4 cm long, in Petri dishes. The soil was brought to saturation and the plants incubated for 8 days at 26 °C in darkness. Yellow brown softrot and the presence of distinct oospores in root tissue indicated the presence of *A. euteiches*.

Results

Field survey (1985-1987)

Notwithstanding the fact that the fields were in the traditional pea growing areas of the Netherlands, more than 60% had a pea-free interval longer than 5 years. Frequencies of just one or two legume crops in 18 years were most common (Fig. 1). The crop immediately preceding the pea was a cereal in over 50% of the cases; sugar beet preceded peas in nearly 30% of the fields, and potatoes or other crops in less than 20% (Fig. 2). The disease intensity showed no relation to the crop species immediately preceding the pea crop (χ^2, n.s.), as illustrated in Fig. 2.

Fig. 3 shows that severe root rot still occurred after a very long interval (longer than 9 years), whereas healthy crops on fields with a relatively short pea interval (0–4 years) were no exception. The correlation of the interval and the disease index was rather poor. Only for 1986 did the correlation reach any significance, but even then the length of interval only explains 4% of the variance in root rot (R^2; $P < 0.05$, Table 1). In the very wet years of 1985 and 1987 no significant correlation was established.

Regression analysis produced a poor but significant correlation between the frequency of legume (including peas) cropping over a period of 18 years and the development of root rot in dry peas (adjusted $R = 0.30$, $n = 169$, Table 1). The linear correlation of the root rot severity with the frequency of all legumes was slightly lower than for the pea frequency alone ($R = 0.30$ and 0.35 respectively; $n = 169$). Multiple regression analysis yielded a small increase in the quality of root rot severity prediction (variance accounted for increasing from 11.7% to 13.5%) when the pea frequency and interval were added to a linear model. Higher degree models did not improve the relationship.

When grouping the fields according to both legume cropping frequency and severity of

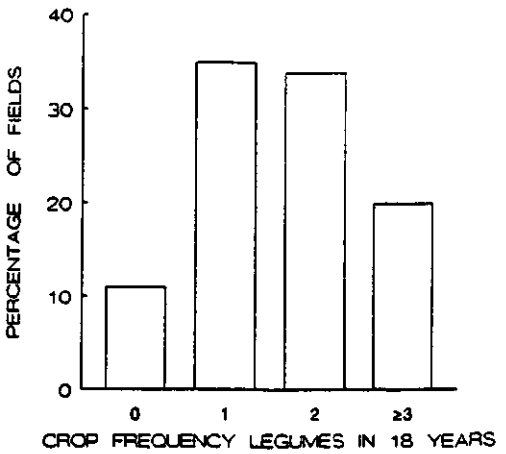

Fig. 1. Percentages of fields with different frequencies of legume crops over the last 18 years. Field surveys 1985–1987.

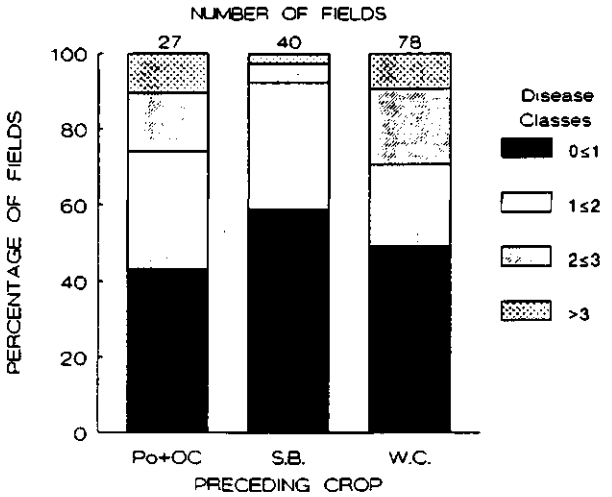

Fig. 2. Percentages of pea fields per disease class according to the root disease index (0–5) at flowering and to the crops immediately preceding pea. Field survey 1985–1987. Po + OC= potatoes and other crops; S.B.= sugar beet; W.C.= winter cereals.

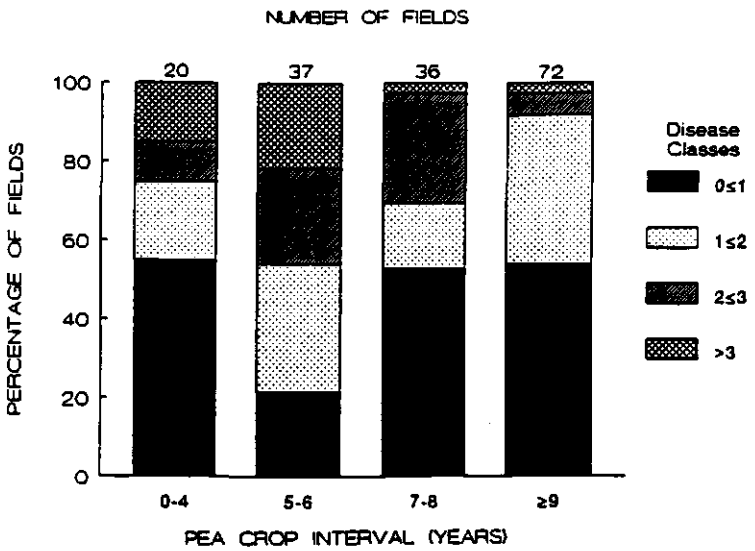

Fig. 3. Percentages of pea fields per disease class according to root disease index (0–5) at flowering and the lengths of the pea crop interval. Field surveys 1985–1987.

root rot in the crop at flowering, a shift to higher root disease indices with increasing cropping frequency was observed ($\chi^2 = 23.4$, df = 9, $P < 0.001$, Fig. 4). But even with a frequency of legume cropping of ≥ 3 in 18 years, field crops were evenly distributed over the root disease classes. The chances of obtaining either a healthy or a severely damaged crop seem to be equal.

Table 1. Field surveys 1985–1987. Correlation coefficients of root disease index of peas and pea interval (I), frequency of pea crops over 18 years (pea/18y) and legume frequency over 18 years (leg/18y). Figures per year, and for three years combined. (The percentage of variance of root rot indices explained by the length of pea interval combined with the cropping frequency is based on R2–adjusted.)

Factor	Year and number of fields							
	1985 (46)		1986 (48)		1987 (51)		1985–1987 (169)[a]	
	%Var	R	%Var	R	%Var	R	%Var	R
Interval	ns	ns	4	–0.25*	ns	ns	8	–0.29***
pea/18y	12	0.37**	10	0.35**	4	0.23*	12	0.35***
leg/18y	9	0.35**	9	0.32*	ns	ns	9	0.30***
I + pea/18	9	0.36**	9	0.35**	2	0.24*	14	0.38***
I + leg/18	7	0.33*	15	0.46***	ns	ns	13	0.38***

ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$.
[a] Including 24 supplementary fields.

Greenhouse and laboratory experiments

Experiment 1. Table 2 shows a low and similar degree of root rot in soil samples after continuous field and dwarf bean. Frequency of the crop did not affect the level of root rot significantly ($P > 0.05$, $n = 24$). In 1987, the root disease indices obtained in samples from AR fields were low and independent of crop effects ($P > 0.05$, $n = 18$).

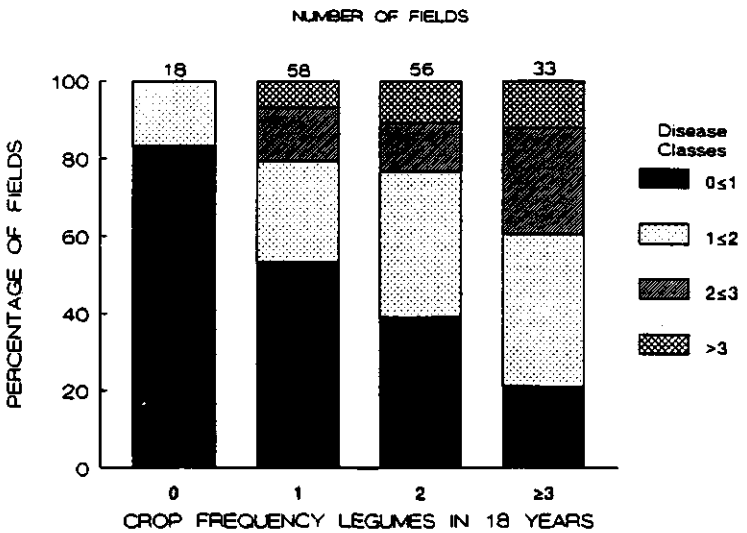

Fig. 4. Percentages of pea fields per disease class according to the root disease index (0–5) at flowering, and the frequencies of legumes in the rotation during a period of 18 years. Field surveys 1985–1987.

On soil sample CA8, with only two preceding vining pea crops, the mean root disease index was higher than on CCP ($P < 0.05$) with 7 years of continuous pea growing. This contrasts with the low DI after two pea crops on AR.

Experiment 2. Table 3 shows a similar infection pattern for peas on CC-samples as in Experiment 1. Root rot was most severe for plants on soil with a cropping history of the same species. Field beans on a soil sample with continuous cultivation of this crop showed a much lower root disease index than peas and dwarf beans on their respective continuous cropping soils. Lupins were never severely attacked. Lucerne and white clover, which were still in the vegetative phase at the moment of assessment, remained free of disease and were not included in the analysis.

Experiment 3. When the development of root rot was assessed for both peas and field beans, a significant positive correlation ($r = 0.67$; $n = 13$, $P < 0.05$) was observed (Fig. 5). If the aberrant point (1.2, 3.2) was excluded from the analysis because of its high deviation, correlation clearly increased ($r = 0.81$; $n = 12$, $P < 0.001$).

Experiment 4. Table 4 shows that *A. euteiches* survived for a considerable period. Even after 9 years without pea the pathogen was still viable, and its presence could be demonstrated in the organic and inorganic fraction of the soil. The only one sample with an interval of over 10 years had a lower root disease index than all but one of the other samples.

Table 2. Mean root disease index (DI: 0 = healthy; 5 = dead) of peas on soil samples from fields (CC–, CA8, AR, and ECC) with varying cropping frequencies of pea, faba bean and phaseolus bean, over a period of ten preceding years.

Frequency (10 years)	Pea	DI	Faba bean	DI	Phaseolus bean	DI
7	CCP[a]	3.0	CCF	1.0	CCB	0.6
2	CA8[a]	4.0	AR	0.7	AR	1.4
1	ECC	–	ECC	0.1	ECC	1.3
2b	AR	1.4	AR	1.0	AR	1.1

[a] Mean root disease calculated from several bioassays. *T*-test indicates means differing significantly ($P < 0.05$).
[b]1986/87 experiment; the other assessments were made in 1985/86.
– not performed.

Table 3. Mean root disease index (DI: 0 = healthy; 5 = dead) of different legumes grown for 6 weeks in pots with soil taken from fields with a cropping history of eight consecutive years of peas (CCP), dwarf beans (CCB) and field beans (CCF).

Soil code	Pea	Dwarf bean	Field bean	Lupin	Lucerne	White clover
CCP	3.8	0.8	1.3	0.5	0.0	0.0
CCB	1.0	3.4	1.0	0.4	0.0	0.0
CCF	0.8	1.5	1.9	0.4	0.0	0.0

For all comparaisons LSD: 0.47 ($P = 0.05$). Data on lucerne and white clover not included.

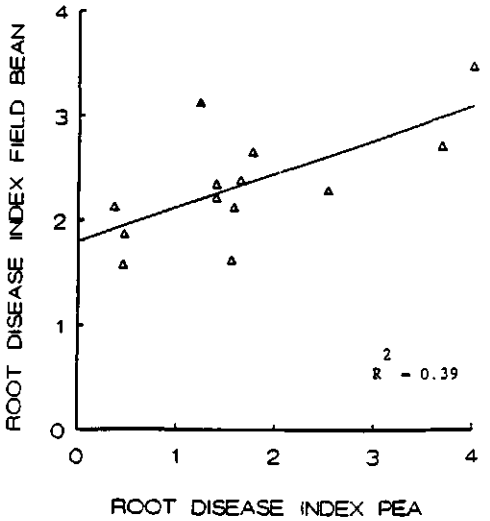

Fig. 5. Correlation between root disease indices of pea and of field bean. Experiment 3.

Table 4. Root disease index of peas (DI: 0 = healthy; 5 = dead) grown on soil samples from fields with pea intervals (years) of increasing length and the presence of *Aphanomyces euteiches* in different soil fractions.

Pea interval	Soft rot DI	Soil fraction		
		Unsieved	Organic	Inorganic
0	3.5	+	+	+
4	3.7	+	+	–
6	5.0	+	+	+
6	3.0	+	+	+
6	3.1	+	+	+
8	3.1	+	–	–
9	1.5	+	–	–
9	3.0	+	+	–
9	3.3	+	+	+
14	1.6	+	–	–

+ = Oospores of *A. euteiches* present in roots; – = *A. euteiches* not found.

Discussion

The data illustrate the low predictive value of the pea crop interval in relation to root rot inoculum potential in fields. In practice, the mere length of the interval gives little information about the suitability of a field for pea growing. In pots, root rot was significantly ($P < 0.05$) more severe on CA8 samples, 2 years after the last pea crop, than in soil samples from continuous pea for 7 years (Table 2).

The value of a 6-year pea rotation can be ascribed to a combination of delayed build-up

and exhaustion, in non-host years, of root rot pathogens which keeps damage within economically acceptable limits. The limitations of the interval criterion are illustrated by the slow breakdown of survival structures of many pathogenic fungi, of which *A. euteiches* is a striking example. Under field conditions oospores can survive a period of 20 years without a pea crop and still retain their virulence (Sherf and Macnab, 1986). The frequency of pea growing over a certain period was slightly better correlated (Table 1) with the level of root rot in pea in the field (% variance accounted for = 11.7, n = 169) than crop interval (% variance accounted for = 8.2). But clearly these low values make interval and frequency of limited use as predictor of root rot for practical purposes.

Results of the experiment with soil from the continuous cultivation fields of specific legumes are in accordance with the well established fact that peas and beans do not support continuous culture. The results also pointed to some specificity of each crop's pathogenic microflora, implying specialization of pathogenic species (Oyarzun et al., 1993). This suggests that growing another legume crop in rotations in which one legume dominates, has a phytosanitary value somewhat comparable to unrelated crops in the rotation. However, in practice, when different legumes are regularly grown in a rotation, populations of pathogens might accumulate potential against more than one crop. The fair degree of correlation of the root rot severity with the frequency of peas or legumes in general (Table 1), and the good correlation between root rot disease indices of field bean and pea root rot on soils which had a history of pea growing only (Fig. 5), are warnings that in practice one should not rely too much on the 'favourable' effect of specialization of pathogens. It is but of limited use to distinguish between the different legume crops from the point of view of planning crop rotation.

It became apparent during the survey that current infestation levels of the soil (measured as disease severity on field and test plants) were as much a characteristic of a specific field as of a crop rotation (see e.g. the high DI after only two pea crops on CA8, but not on AR, Table 2). This field effect corresponds with results published by other workers such as Curl (1963), Burke and Hagedorn (1968) and Zerlik (1979). Interactions between field characteristics and the rate of increase of soil infestation have been known for a long time (MacMillan, 1919; Walker and Snyder, 1934). Suppressive and conducive soils to plant disease have been well recognized (Baker and Cook, 1974), but many different names have been used to describe this phenomenon (Huber and Snyder, 1982). The concept of soil receptivity has been proposed (Alabouvette et al., 1982; Bouhot and Joannes, 1983) to account for modulating effects of the soil biotic and abiotic environment on inoculum potential. Build-up and breakdown of soil-borne pathogen populations do not only depend on growing a susceptible host or not, but also on the biological, chemical and physical properties of the soil in which the crops are growing.

More research is needed to deepen the knowledge about the role of the soil in limiting root diseases in peas (Oyarzun and Dijst, 1991).

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Chapter 5

Seed infections of dry peas, a potential source of soil contamination by foot and root rot fungi

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Abstract

Several fungal species causing root rot of pea occur as seed infection or seed contaminants. These infections constitute a source of primary inoculum and facilitate the introduction of pathogens in non-infested fields. All pathogens reported are able to survive in and cause infections from the soil. Seed dressing with fungicides kills, or often only temporarily inhibits the infection of roots by the pathogens. Significant correlations were found between the infection severity of the seed lots with field emergence and with the number of seeds produced per plant ($P \leq 0.05$). The quality standards valid for commercialization of certified pea seed in the Netherlands are discussed on the basis of their consequences for the infestation of new fields, epidemiology of pea root rot and the pea production.

Additional keywords. Fungicides, pea production.

Introduction

Within the group of pathogenic fungi causing root rot of pea, several species, e.g. *Mycosphaerella pinodes* (Berk. & Blox.) Vesterg., *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema, *Fusarium oxysporum* Schlecht. f. sp. *pisi* Snyd., *Stemphylium botryosum* Wallr. and *Botrytis cinerea* Pers. ex Fr., have the capacity to infect pods and be seed-borne (Richardson, 1973). The use of infected seed can lead to yield loss in pea crops (Wallen, 1974, Kempenaar et al., 1991).

Seed infections are a complicating factor in interpreting results of research on diseases caused by soil-borne pathogens. In May 1985 a project was started at the Research Station for Arable Farming and Field Production of Vegetables, PAGV, Lelystad to develop a bioassay in the greenhouse to assess the risk of root rot in field soils. In May 1986, the Centre for Plant Breeding and Reproduction Research, CPRO-DLO, Wageningen started a breeding programme to identify sources of resistance and to cross root rot resistance into current cultivars of, primarily, dry peas. This project aimed at the increase of partial resistance. Screening of breeding lines becomes more

difficult when the seeds are already contaminated with the pathogens to be used later for inoculation. Fungicides which kill or confine the seed infecting pathogens (e.g. systemic fungicides such as benomyl) unfortunately also counteract infection in young plants by the same pathogens used as inoculum in screening for resistance.

In view of this background it was desirable to examine the causal agents, type, frequency and severity of seed infections. A seedling symptom test was performed under greenhouse conditions with seed samples of which data were known from a field survey by PAGV (Oyarzun, 1987) to allow comparison of both data sets.

Materials and Methods

Fourty five seed samples (five cultivars) of dry peas were collected from 38 growers in April-May, 1986 (commercial samples, indicated by C). Of each sample the certification quality score given by the Netherlands Seed Certification Authority (NAK), was known. This score is based on the proportion of spotted and damaged seeds (x). About 60% of all samples fitted in NAK score class 3 ($10 < x < 20\%$), 25% in class 2 ($6 < x < 10\%$) and 15% in class 1 ($x < 6\%$). The germination capacity of the samples was determined by the NAK. Most of the C-samples were treated with a combination of two or three fungicides against seed and/or soil pathogens (thiram with benomyl, sometimes supplemented with fosetyl-Aluminium). Some samples were only treated with thiram, and only a few were not treated at all.

The development of the crops growing from these seed lots was observed during the season. Emergence, disease development and yield were assessed (Oyarzun, 1987). The seed producers supplied untreated seed samples of the same five cultivars for research purposes (research samples, indicated by R).

Health and emergence of both commercial (C) and research (R) samples were screened in a seedling symptom test in sterile silver sand (Neergaard, 1977). Immediately preceding this test, random sub-samples from the commercial samples were washed in tap water until no further release of fungicide in the water was perceptible. This provided a derived sample with only a fungicide residue on or in the seed (W-samples). Four random subsamples of six seeds were collected from C- and W-samples, and eight from R-samples. These were sown in silver sand in heat sterilized pots.

Germination and growth took place in a greenhouse at 18/14°C day/night temperature, 60-80% relative humidity and 16 hours light per day during four weeks. Under these circumstances the plants developed six to seven leaves. Subsequently the plants and the non-emerged seedlings were uprooted and rinsed. The plants, the germinated but non-emerged seeds and the ungerminated seeds were visually assessed for infection. Infection index 0 stands for completely healthy cotyledons and foot region (i.e. the zone of cotyledon attachment, 5 mm of hypocotyl and 5 mm of epicotyl), index 10 represents heavily attacked or fully rotten cotyledons or foot region. Emergence was scored. The averages of subsamples and samples were calculated per cultivar, and statistically compared using Student's t test. Rank correlations between parameters from the seedling symptom test in the glasshouse and estimates of emergence and the number of seeds produced per plant (indicated as 'field yield') of

the same seed lots, sown in the field, were calculated with Spearman's rank correlation coefficient.

Symptoms as documented by Hagedorn (1984) were used to select 17 of the 45 samples to isolate pathogens from diseased parts of the foot region of young plants or from the cotyledons of attacked seeds. Small pieces of tissue were disinfected in 1% sodium hypochlorite and plated in Petri dishes on water agar and Czapek-Dox agar. The Petri dishes were incubated 7 days at 22°C with 18 hours NUV light (λ = 365 nm) per day. The fungi were transferred to potato dextrose agar and identified. Some of the identifications were checked by the Mycology Department of the Plant Protection Service, Wageningen.

Results

Emergence and its relation with infection index. The percentage emergence of the R-samples was significantly lower than that of the C- and W-categories with cultivar 1 only (Table 1). With cultivars 4 and 5, C- samples had a significantly lower emergence percentages than R-samples. The decreased fungicide load of the W-samples never led to lower emergence. The W-samples of cultivars 2 and 4 had a significantly better emergence ($P \leq 0.05$) than the C-samples. In the R-samples of cultivars 1 and 3 lower emergence was accompanied by a higher infection index, while germination of the R-samples of the other cultivars, notwithstanding a high infection index, was better than in the corresponding C-samples.

Table 1. Results of the seedling infection test in the greenhouse and the NAK seed qualification per cultivar.

cv.	NAK-class[a]			emergence (%)				infection index[b]			
	1	2	3	C	W	R	C<W[c]	C	W	R	C<W[d]
1	16	21	63	93	90	0	44	2.0	2.1	6.2	44
2	-	-	100	91	98	94	17	1.6	1.3	5.8	17
3	14	43	43	85	87	75	43	2.3	3.1	7.0	71
4	75	25	-	72	86	94	0	4.2	2.7	7.6	0
5	-	34	66	89	93	100	0	2.3	1.8	5.4	0

[a]) NAK-class: 1 = spotted fraction smaller than 6%; 2 = smaller than 10%; 3 = smaller than 20%. Percentage of samples per cultivar and per class.
[b]) Infection-index: scale 0 (completely healthy), 10 (highly infected).
[c]) Percentage of W samples showing a better emergence than C samples. C = seed sample treated with current fungicide doses; W = seed sample with residuals of fungicides remaining after rinsing; R = test sample, non treated.
[d]) Percentage of C samples with an infection index smaller than W samples.

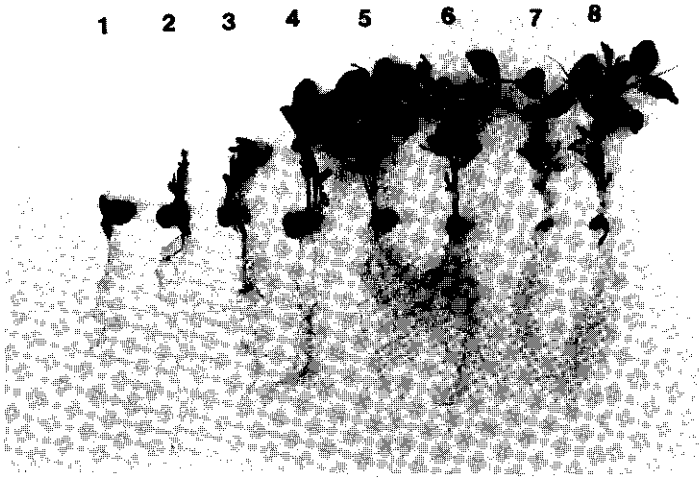

Figure 1. Seed infections on 23-day-old pea seedlings from the R-subsample of cultivar 3. The black to brown discoloration of the cotyledons spreads out from cotyledons to the stem axis. The infection index is illustrated in decreasing order, 9 to 3, from plant 1 to 8. The reduced stem and root growth caused by infection is clearly visible.

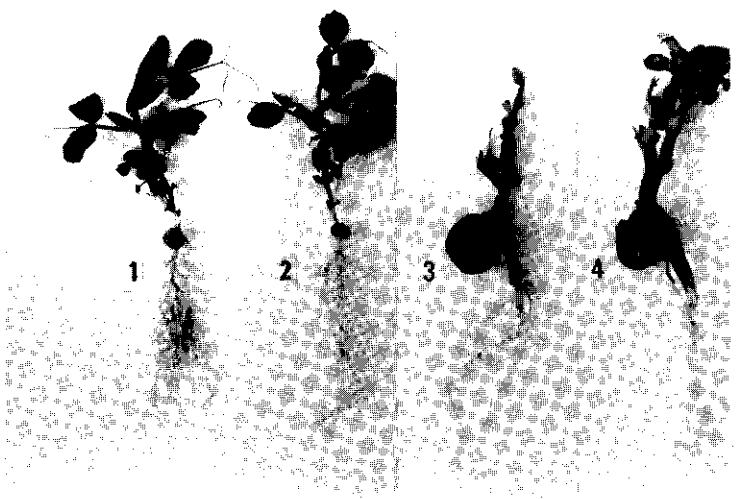

Figure 2. Seedlings (1 and 2) from the R-subsample after seed dressing with Benomyl and Thiram. The cotyledons have a normal yellow colour and the lesions are strongly restricted. Seedlings 3 and 4 are a close-up of plants 2 and 3 in figure 1. The rot originating from the cotyledons affects the epicotyl and extends to the root.

Effect of fungicides on infection index. Reduction of the fungicide load to the residue level did not affect the infection index of any of the cultivars. With cultivar 3 the score of the C-samples was lower than of W in a large number of cases. Without seed treatment (R) the infection soon became serious and led to high infection indices. The differences between the average infection indices of the C- and W-samples and those of the R-samples were significant for all cultivars ($P \leq 0.05$). The disease symptoms in the R-samples generally were those known as root rot, and resulted in reduction of the growth of the young plant (stunting; Fig. 1 and 2).

Correlation between seedling symptom test and field data. The number of samples of cultivar 1 permitted the computation of correlations between parameters in greenhouse and field (Table 2). The infection index of C-samples was negatively correlated with emergence in the greenhouse and with number of seeds per plant in the field. No correlation with field emergence was found. The W-samples showed a negative correlation between infection index and the three parameters considered. The emergence of W-samples in the glasshouse test was positively correlated with field emergence and yield. Similar trends were observed for cultivars 2 and 3, but the limited number of samples did not allow statistical analysis.

The quality standards. The NAK germination figures, which are based on tests in river sand, did not correlate with any of our parameters. Neither did the NAK seed quality scores correlate with the infection index.

Table 2. Spearman's rank correlation coefficients for the relation between greenhouse test results and field performance of the seed lots of cv. 1.

	1 infection index- C	2 infection index- W	3 emergence C	4 emergence W	5 emergence field	6 seeds/ plant
1	X					
2	0.45	X				
3	-0.57	ns	X			
4	ns	-0.76	ns	X		
5	ns	-0.46	ns	0.46	X	
6	-0.58	-0.60	ns	0.59	ns	X

ns: non-significant at $P \leq 0.05$. Two cases with extreme values of yield (seeds/plant) were discarded from calculations. C = seed sample treated with commercial fungicide doses; W = seed sample with residuals of fungicides remaining after rinsing.

Isolation and identification of pathogens. Depending on the cultivar, pathogen isolates were obtained from 0-33% of the total number of samples. Three species of root rot pathogens were isolated from the commercial C- and W-samples of the five cultivars: *Mycosphaerella pinodes, Phoma medicaginis* and *Stemphylium botryosum* (Table 3). The C and W-results were joined. From cultivar 4 only *Penicillium* spp. were isolated. C-samples from two cultivars, which were treated with a mixture of three fungicides, nevertheless yielded isolates of *Mycosphaerella, Phoma* and *Stemphylium*

(not shown). In the R-samples three more pathogens were found, *Fusarium solani* (Mart.) Sacc., *Fusarium avenaceum* (Fr.) Sacc. and *Rhizoctonia solani* Kühn. The pathogens were isolated from 33-100% of the samples selected for characteristic root rot symptoms (Fig. 2). In more than half of the cases, depending on cultivar, the R-samples yielded isolates of the above mentioned root rot pathogens and saprophytes such as *Alternaria* spp., *Trichoderma* spp. and *Penicillium* spp.

Table 3. Root rot pathogens isolated from infected cotyledons and from pea seedlings in five cultivars.

pathogens	cultivars									
	1		2		3		4		5	
	C+W	R	C+W	R	C+W	R	C+W	R	C+W	R
M. pinodes	+		+	+	+	+				
P. medicaginis		+	+	+	+	+		+	+	+
S. botryosum	+	+		+						
% isolations/total[a]	25		33		43		50		33	
% pathogens[b]	75		50		33		0		100	

[a]) percentage of samples used for isolation relative to the available total per cultivar.
[b]) percentage of samples yielding isolates.

Discussion

Seed transmission of root rot pathogens and effectiveness of fungicides. In the literature transmission of foot rot fungi by pea seed, and its extent are well documented (Hare and Walker, 1944; De Tempe, 1969; Richardson, 1973; Ali et al., 1982; Biddle, 1986). Our observations confirm published data. The untreated seed samples showed serious seed infections, which caused considerable stunting of seedlings (Fig. 1).

Fungicide treatment with a combination of two or three fungicides limits the attack of the young plant, but cannot prevent it in all circumstances. The residual dose (W-samples) led to a better emergence than the original dose (C-samples), possibly due to a phytotoxic effect of the fungicide dose applied. This effect is corroborated by the observation of an increased percentage of stunted seedlings in the C-samples, often with pink-red lesions in the cotyledons. Fungicide damage was also observed in the field in 1986. The degree of dose reduction in the W samples is unknown, but it is likely to be substantial. Nevertheless, the residual dose (most probably the part of the original dose of systemic fungicide which has passed through the seed coat towards embryo and cotyledons) was effective against the seed pathogens and it was less phytotoxic than the normal dose. This conclusion is only valid for the situation under

study. The protection which the residual dose can provide against soil-borne pathogens (which were absent in the test) is likely to be minimal. Phytotoxic effects can be magnified by incorrect application of the fungicides. Seed coating with a cocktail of various chemicals is widely used as a preventive measure. However, it is known that protection against soil pathogens which attack from the soil remains inadequate, that the protective effect is no longer visible in later growth stages, and that the effect depends largely on weather conditions (Ester and Gerlagh, 1985). All these arguments emphasize the lack of effective protective treatments.

Seed quality standards and test methods. NAK scores did not correlate with the infection index, nor with the emergence in the seedling test in the greenhouse. The same applied to the NAK germination figures of the samples. This shows that the "spotted" classes and germination indices are poor measures to assess seed quality from a seed health perspective. The lack of correlation between the germination percentage, as assessed with the blotting paper method, and the vitality of the seed is known (Perry, 1970). The necessity to eliminate the resistance of the living seed to increase the sensitivity of the blotting paper method for the detection of seed infection was reported (Limonard, 1966), but this method is not applied. As regards seed health in the Netherlands, the application of a more sensitive method than the blotter test has been advocated before. De Tempe (1968b, 1969) recommended the agar plate method to assess the infection frequency of pea seed.

Seed infections and their potential consequences for root rot epidemiology. The epidemiological consequences of seed infections are obvious. A light pea seed infection with *Ascochyta* spp suffices to trigger a strong epidemic in the crop (De Tempe, 1968a). No detailed research has been published about the distribution and spread of pea root rot with seed. It is probable that an effective distribution of inoculum takes place by the use of contaminated seed. The distribution is at random, and may introduce the initial inoculum or increase the inoculum already existing. The effect of seed infection of a single plant on its neighbours is unknown in detail. Some root rot fungi (e.g. *Fusarium solani* f.sp. *pisi*), starting from the seed, can infect the root system so extensively, that tens of neighbouring plants can be infected by spread through the soil (Bywater, 1959). *Ascochyta* spp pycnidiospores produced in leaf lesions, can be spread by rain splash, and *Mycosphaerella* ascospores can be dispersed by turbulent air. Seed infections are important too, because *Mycosphaerella, Phoma* and *Fusarium* spp. can survive in the soil with resting structures, notably chlamydospores (Reiling et al., 1960; Nash and Snyder, 1962). The persistence of chlamydospores in the soil permits the fungi to survive without loss of vitality and pathogenicity during a crop-free period of five years, recommended for peas. After some years of pea growing the gradual build-up of inoculum in the soil from lightly contaminated seed can lead to total crop failure. It is surprising to find a correlation between the infection index in the seedling test in the greenhouse and seed yield per plant in the field, notwithstanding many other contributing factors.

The frequency of seed infection with root rot pathogens in five popular cultivars was remarkably high. Peas are attractive in crop rotation. It is of the utmost importance to prevent that root rot problems become preponderant again, as in the past. Despite the

very limited pea acreage, heavily contaminated commercial fields were found. An essential requirement to prevent further spread is the use of clean seed. We consider the tolerance currently applied (10% with '*Ascochyta* and *Mycosphaerella*') as unacceptably high. The routine screening procedures of NAK need to be re-examined. The production year 1985 was characterized by exceptionally unfavourable weather for pea growing. The seed quality of the harvest 1985 therefore probably reflected an extreme and unique event, but similar circumstances occurred in 1987. The decision to lower the seed quality standards, as taken after the root rot year 1985, is certainly a risky one, which may lead to a serious increase of root rot problems. In 1989-1990, field and greenhouse experiments (Kempenaar et al., 1991) with infected seed lots reconfirmed the negative influence of this factor on production and underlined the importance of healthy seed.

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Chapter 6

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Bioassay to assess root rot in pea and effect of root rot on yield

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Abstract

Infection of pea roots by soil-borne pathogens causes foot and root rot. In 1985 research was started to develop a method to predict the root rot likely to occur in prospective pea fields. In a bioassay the pea cultivar Finale was sown in a composite soil sample from each field in pots under standardized conditions in the greenhouse. The plants were removed at the green bud stage and the severity of root rot recorded. Between 1985 and 1988 approximately 200 field pea crops were monitored for root rot development. Forty-eight fields were bioassayed in 1986, 51 in 1987 and 30 in 1988. Each year, root rot readings in the bioassay and disease severity readings at field sampled plants at flowering and green pod were linearly correlated ($P < 0.001$). As the degree of root rot in the field crop increased, there was a proportional lower yield. In heavily infested fields, up to a 50% yield reduction occurred.

The bioassay in pots proved to be a reliable method for predicting root rot severity in sampled pea fields.

Additional keywords: *Pisum sativum*, disease prediction, soil-borne pathogens.

Introduction

Many soil-borne fungal species cause 'foot and root rot', hereafter called root rot, of agricultural crops. Peas are extremely susceptible to some of these pathogens. Root rot causes early stagnation of root growth and symbiotic nitrogen fixation; it limits the uptake of water and nutrients. The infested crop is stunted and matures prematurely. If the growing season is unfavourable, root rot can cause crop failure (Reiling et al., 1960; Riepma, 1967; Tu, 1987). Genetical resistance nor chemical control are effective in root rot management. Only avoidance of fields with high disease potential can prevent the problem.

Many techniques are available to quantify fungal populations in the soil (Menzies, 1963). The use of selective growth media permits the isolation of specific fungal genera from soil, but then their pathogenicity still has to be established. In addition, use of selective media does not take into account the relative soil tilth, suppressiveness, or fertility which directly influence root rot disease severity.

Baits, mostly pieces of vegetable material, permit the isolation of specific soil pathogens, their quantification and the assessment of their inoculum potential. Examples include apples to isolate *Phytophthora* spp. (Duncan et al., 1987), carrot disks for *Thielaviopsis basicola* (Yarwood, 1946), pieces of potato for *Pythium aphanidermatum* (Stanghellini and Kronland, 1985), *Fusarium solani* var. *coeruleum*, *F. roseum* var. *sambucinum* and *Phoma exigua* var. *foveata* (Tivoli et al., 1987).

These methods are unsatisfactory in the case of root rot, where several fungal species are involved. To deal with this problem, growing plants may serve as a selective substrate

to sample the pathogen flora of a soil, in a bioassay to determine the inoculum potential of the soil (IPS). By standardizing the infection conditions, reproducible results can be obtained. The final disease intensity results from:
– density and virulence of the pathogens;
– competitiveness of the pathogens in relation to the other soil microflora;
– susceptibility of the test plant;
– physical and chemical characteristics of the soil;
– environmental conditions.

The first two factors make up the 'inoculum potential' of the pathogen, which was defined by Garrett (1956) as 'the energy available for infection of a host at the surface of the infection-court'. This potential is modulated by biotic and abiotic soil factors, and the result is called 'inoculum potential of the soil', IPS (Mitchell, 1979; Alabouvette, 1989). Data on the magnitude of the IPS permit the estimation of root rot risk of a particular field. IPS can be expressed by a value for disease incidence, e.g. with wilt diseases, or by severity of the infection as in the case of root rots.

Bioassays have been in use for vining peas for many years. In 1957 Johnson published a bioassay with pots in a greenhouse which gave a good estimate of the contamination of the soil examined. Sherwood and Hagedorn (1958) described a method to estimate the potential for common root rot caused by *Aphanomyces euteiches*. This method is still in use in the USA. Good results have been obtained with this method in Sweden (Olofson, 1967) and the UK (Biddle, 1979, 1984). The British Processors' and Growers' Research Organization (PGRO) offers a commercial test to pea growers. In Canada, a version of the bioassay is the 'window method', applied and financed by the industry itself (J.C. Tu, personal communication).

The lack of means of control, the increasing limitations imposed upon chemical pest control, lack of a short-term perspective for producing root rot resistant cultivars (Gerlagh, 1985), changes in harvesting methods, which lead to higher quantities of trash remaining on the field, and the uncertainty about the length of a rotation period for adequate reduction of soil inoculum potential, led to a fear of increasing root rot problems with increasing pea acreage in the Netherlands. Consequently the development of a greenhouse bioassay in pots to assess the inoculum potential of soil (IPS) of prospective dry pea fields was an objective in pea disease research in the Netherlands between 1985 and 1988. The research strategy has been elaborated elsewhere (Oyarzun, 1991).

Materials and methods

Development of the bioassay. In developing the bioassay, the criteria recommended by Bouhot (1979) and Bouhot and Bonnel (1979) have been taken into account. Peas are in general very susceptible to root rot, and have already been used successfully as test plants (Sherwood and Hagedorn, 1958). Since seed exudates activate soil pathogens (Cook and Snyder, 1965; Harman et al., 1978; Norton and Harman, 1985), seeds were sown directly instead of using young pregerminated plants. Pre-soaking of the seeds may lead to a decrease in infection (Short and Lacy, 1976).

The environment must allow disease to manifest itself maximally for any level of soil contamination. To achieve this, the conditions which contribute to maximum speed, selectivity and sensitivity of the test were determined. Subsequently the method was standardized. Finally the criteria for assessment of the disease were formulated.

Field selection in root rot research. In 1985, 46 pea fields (26 located in the North and 20 in the South of the Netherlands) with varying severity of root rot were examined at flo-

wering time. Particular attention was given to the types of symptoms and the disease patterns in the fields. A significant number of sampled fields had never been or had long ago been cropped with peas.

In 1986 and 1987 the bioassay results were compared with results from evaluating root rot in the field. In 1988 and later, field research was done to validate the bioassay in practice.

The fields, 48 in 1986 and 51 in 1987, were situated in the traditional legume growing regions of the North and the South of the Netherlands. Selections of test fields were made according to the following criteria: a pea crop in the respective year; at least one legume crop during the last 10 years; a large diversity of soil types and soil properties among fields; data available on cropping history and on physical and chemical soil properties.

Sampling procedure and soil preparation. In each field, only 1 ha was taken for sampling. The sampled area corresponded to the most homogeneous part of the field, excluding 10-m-wide field borders. Fields were sampled after ploughing in the autumn preceding the pea crop, or in early spring. In the sampled area, 50 subsamples of 20–25 cm depth were taken with an auger (5 cm diameter), passing through the field in a W-pattern. The 50 subsamples were combined, mixed and stored in a plastic bag at 5 °C until use. If samples were too wet, they were first dried by exposure to ambient air. Before testing, samples were crumbled and passed through a 0.8-cm mesh sieve which assured good homogenization of the sample. Of each sample the actual water content and that at field capacity (pF = 2) were determined. Soil water potential was determined by filling 100-ml cylinders with soil and placing them on a pF table (Anonymous, 1976). The soil density used in determining water potential was the same as for filling the pots in the bioassay.

The test plants. The cultivar Finale was used for all bioassays. Seed of the highest quality standard was further selected for size (7–7.5 mm) and absence of lesions and fissures. Subsequently, the seed health was verified in an agar test following ISTA procedures. Before sowing, the seed was either treated with thiram (TMTD, 1.5 g a.i./kg) or soaked for 10 min in a 1% solution of NaOCl, followed by two rinses with tap water.

Test procedure. Each soil sample was distributed over four 2.6-l pots. Each pot first received a 1-cm layer of moist riversand and then the soil was added. To prevent crust formation and internal leaching when water was added, and to limit evaporation, the surface of the test soil in each pot was covered with a 0.5-cm layer of perlite. Pots were filled, care being taken to pack the soil homogeneously according to a standard method (Slangen, 1979). Twelve seeds were sown per pot at 4 cm depth. The soils were gradually brought to field capacity and then placed in the greenhouse. The greenhouse climate during each test was maintained at the following limits: temperature 17–20 °C; air humidity 80–90%; light, shading when the radiation outside was more than 400 W/m^2; from October to March additional light (60 W/m^2) for 12 h per day when the light intensity outside was less than 100 W/m^2.

During germination loss of water by evaporation was prevented by covering the pots. After emergence, the number of plants per pot was reduced to ten. Soil moisture was adjusted daily to field capacity. The quantity of water needed was added at the top by a specially designed automatic water dispenser as shown in Fig. 1. A few times a week water was added from the bottom. The position of the pots was rerandomized at least three times a week.

Assessment of root rot severity in the bioassay. When test plants were in thirteenth leaf

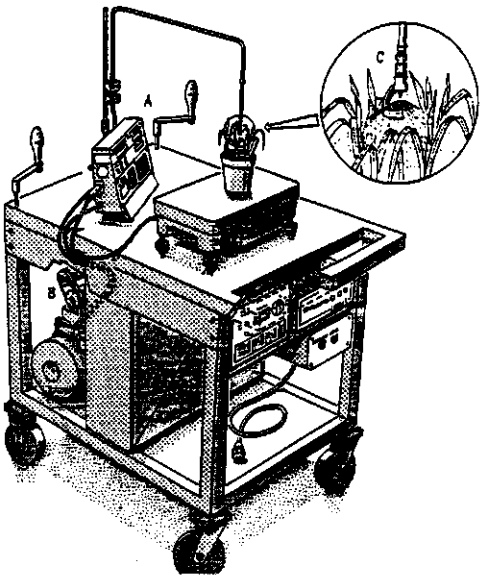

Fig. 1. Design of the automatic water dosage unit. Target weight is introduced manually or automatically (A). Difference between target weight (e.g. pot weight when soil water potential is -0.01 MPa) and current weight caused by evapotranspiration generates an electric signal which activates a valve linked with a water tank under pressure (B). When the difference disappears the valve closes instantaneously. A micro dosage unit (C) provides a fine water spray over the foots of the plants.

stage (green flower bud present), they were removed and their roots were carefully washed free of soil. Root rot severity per plant was scored on a 0–5 scale; 0 = healthy and 5 = roots 100% rotten. The Root Disease Index per plant (DIp) consisted of a weighted sum of the ratings of epicotyl, cotyledons, xylem and roots. Cotyledons represent the cotyledons themselves plus 1 cm of both epicotyl and roots. DIp was calculated using the formula:

$$DIp = 0.35 \times DI\ epicotyl + 0.20 \times DI\ cotyledons + 0.10 \times DI\ xylem + 0.35 \times DI\ roots$$

The Root Disease Index (DI) per sample is the weighted average

$$DI(sample) = [\overset{a}{\Sigma}[\overset{b}{\Sigma}DIp]/b]/a$$

where a = pots per sample and b = plants per pot.
DI characterizes IPS of the sample.

Field assessment of root rot. In 1986 and 1987, root rot in the field was assessed at three growth stages, when the plants had 7–8 leaves, at flowering (more than 50% of the plants with open flowers) and at early ripening (no more flowering; the lower pods filled but still green). Each sample consisted of sets of five plants taken at ten sites on a W path through the field, 50 plants in total. Plants were uprooted to a depth of 20–25 cm and for 10 cm at

each side of the row. The roots were washed free of soil and assessed for root rot. Visual estimation of the percentage of root affected by rot was scored on a scale (0–5) with 0 for white roots and 5 for 100% discoloration of underground parts or dead plants.

Fields were grouped according to root rot severity and the percentage of fields in each of four disease classes, negligible, slight, moderate and heavy disease, was determined. For comparison, results of the bioassay were grouped in the same way.

Crop stand and yield parameters. After emergence, crop stand was assessed by counting all plants at ten sites of 2 m length in two rows, 40 m in total. At the last sampling period yield-determining parameters (such as number of culms per plant, pods per culm, and the number of seeds produced per m^2) were scored. The thousand-kernel weight was supplied by the farmer. The yield was estimated by multiplying the number of seeds per m^2 by the thousand-kernel weight. This calculated yield was preferred over the yield as indicated by the grower. Since only part of the field was sampled, and since the farmer always looses some yield during harvest, the calculated yield is supposed to give a better estimate of the real dry grain production of the sampled area.

Time of execution of the bioassay. The possible deviation in IPS values of samples from autumn or spring was examined on 18 fields in 1986/1987. Test plants in spring were assessed for root rot when the first flowers had opened.

Bioassay for practice. In 1988, after the validation phase, bioassays were performed with soil samples taken without area restriction on 30 fields by workers of the Bedrijfslaboratorium voor Grond- en Gewasonderzoek (BLGGO) at Oosterbeek and compared to crop samples.

Data analysis. Analysis of data from bioassay and field assessments were performed by DAVE (data processing package at PAGV) or using facilities of GENSTAT.

Results

Field and crop data. Crop husbandry considerations did not lead to expecting limitations to pea growing on the selected fields (Oyarzun, 1991). In both years soil types varied from sandy loam to heavy clay; except a few peat and sandy fields, soils were alkaline with pH 7–7.5. Fields were well drained, but in 1987 water logging occurred in heavy clay due to abundant precipitation and a low infiltration rate.

In 1986, 75% of the seed lots were treated with fungicides, of which more than half with a mixture of thiram and carbendazim. In 1987 all seed was treated, of which 40% with metalaxyl or fosetyl-aluminium. In 1986, the most popular cultivar was Finale, in 1987 the semi-leafless cultivar Solara came up. In both years the average pea-free interval preceding the pea crop was longer than the 5 years (corresponding to a 6-year rotation) considered adequate for a healthy crop (Timmer et al., 1989). The legume share in the total crop rotation was modest (Table 1). Typically the soil was ploughed in October preceding the next pea crop and ploughing depth averaged 22–25 cm. The seedbed was prepared in March after harrowing, and seeds were sown at a depth of 4–5 cm (Table 2). The difference between intended (aim) and realised (real) seed depth is an indication of seedbed condition. The same applied to the difference between theoretical and real emergence, which was remarkably large in 1987, especially in the North (Table 2) where heavy clay soils prevailed.

Table 1. Share of different cultivars in the pea fields sampled in 1986 and 1987, the average number of years without peas (interval) preceding the crop, and the average frequency of peas and legumes in general over last 18 years. Data split up for the North and the South of the Netherlands.

Year	Region (n)	Share of cvs (%)			Pea interval	Pea in 18 years	Legume in 18 years
		Finale	Solara	Others			
1986	North (33)	73	7	20	7.6	1.5	1.9
	South (15)	33	21	46	7.9	1.4	2.3
1987	North (32)	68	21	11	6.6	1.2	1.4
	South (19)	25	47	28	7.8	1.2	1.9

n = number of fields in the region.

Root disease symptoms of test plants and of field crops. Three disease symptom categories could be distinguished: dark brown dry rot, black root rot and soft rot. Dark brown dry rot was the most frequent symptom. In 1986, test plants grown in some soil samples showed pronounced black root rot. This disease, caused by *Thielaviopsis basicola*, led to almost complete failure of the crop (Oyarzun, 1987). In both years, 1986 and 1987, soft rot occurred in the field and in the test plants. Later *A. euteiches* was isolated from such plants. The most representative root rot symptoms in test plants are illustrated in Fig. 2a–c.

Validation of bioassay results. In 1986, the linear correlation between DI of plants grown in the bioassay and in the field at the young plant stage was low (r = 0.50) but statistically significant. No correlation was found in 1987. With field plants in flower or immature pod stage, the relation between bioassay and field results is clear, and is best represented by a straight line (Figs 3a–d).

In 1986, the DIs from the bioassay were generally higher than of the fields. In 1987 the obverse was true. In 1986, the assessment in the field at the beginning of ripening was hampered in some fields by senescence of the crop. In 1987 the crop was still rather green at the last assessment date (mid July).

Classification of fields according to field rating of root rot severity and greenhouse bioassays. In 1985, root rot was generally slight (Table 3). Only 6% of the crops had moderate or heavy root rot. The relation between root rot in the field and in the bioassay is not

Table 2. Data on sowing and emergence. Figures represent the average values of the parameters for pea crops in the North and the South-West of The Netherlands. Theoretical field emergence is obtained by correcting the seed density by the germination capacity of the seed.

Year	Region	Seed rate (kg/ha)	Seeds per m^2	Depth		Field emergence (plants/m^2)		
				Aim	Real	Theor.	Real	% Real
1986	North	201	62	4.3	3.8	56	49	88
	South	191	57	4.2	4.7	54	50	93
1987	North	210	59	4.6	3.8	54	44	81
	South	190	57	4.6	4.7	54	50	93

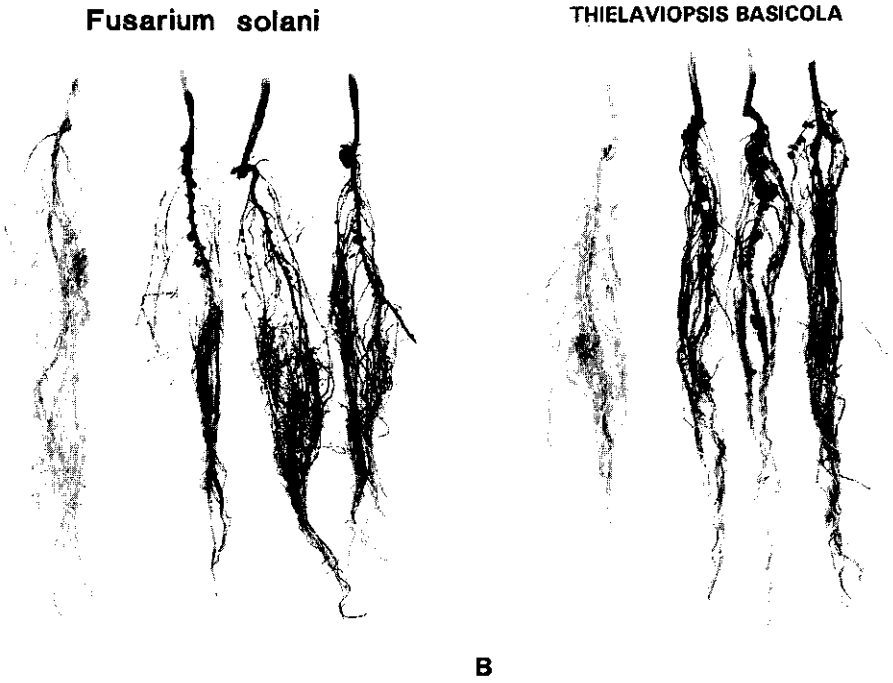

Fig. 2. Illustrations of the most representative pea root rot symptoms. Left, healthy pea roots; right = three diseased roots. (A) Root rot caused by *Fusarium solani* f.sp. *pisi*. Initial infection occurs near the area of seed attachment. Lesions enlarge and run together until epicotyl and tap root become completely shrunken and dark brown in color. Note blackened, degenerated nodules and the shrinking of epicotyls by the collapsing of dead cortical cells. (B) Black root rot caused by *Thielaviopsis basicola*. Infection primarily affecting taproot and lateral roots but no nodules. (C) Common root rot caused by *Aphanomyces euteiches*. Complete collapse of epicotyls and disappearance of the cortex. Strong reduction of the root system because the pathogen kills branch roots.

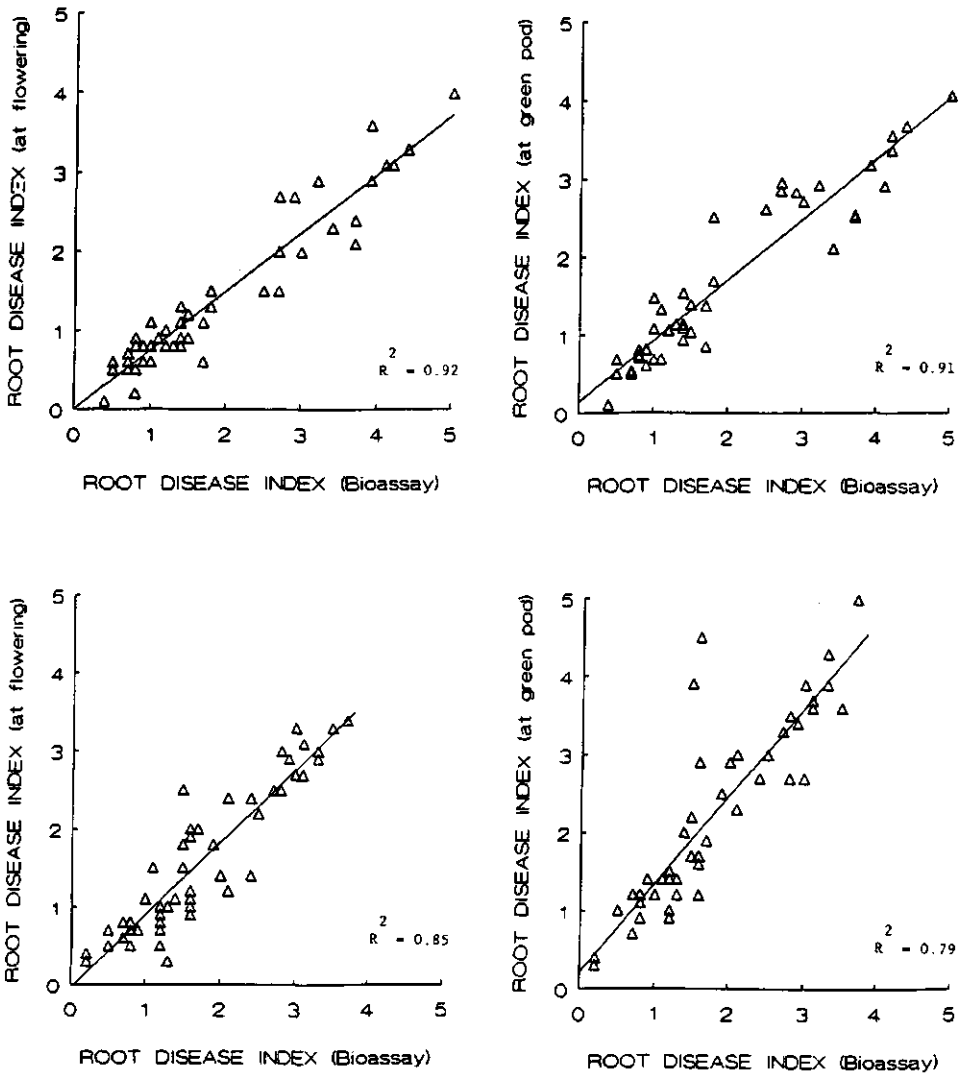

Fig. 3. Relationship between root disease index in a greenhouse bioassay and in field crops in 1986 and 1987 at flowering and green pod stages. (a) 1986, flowering; (b) 1986, green pod; (c) 1987, flowering; (d) 1987, green pod.

the same for 1986 and 1987. In 1986, DI in the field, at early ripening, ran parallel to the bioassay (χ^2-test, n.s.), but fields with very heavy infestation had lower ratings in the field than in the bioassay. In 1987, the fields with heavy attack were twice the number expected from the results of the test. Prolonged moist weather conditions caused heavy root rot even on slightly infested fields.

Relation between root rot severity and yield. Notwithstanding the high variability of fields, growth conditions and other factors, the correlation between yield and root rot (DI

Table 3. Percentage of fields in root disease classes according to the bioassay (BA) and to field assessment at flowering (FL) and ripening (RI) stage. In 1985 root rot was only assessed in the field.

Disease class	DI	1985	1986			1987		
		FL (46)[a]	BA (48)	FL (48)	RI (48)	BA (51)	FL (51)	RI (49)
Negligible	0–1	66	33	50	36	24	39	20
Slight	1–2	28	31	23	29	42	28	34
Moderate	2–3	2	13	15	24	22	25	22
Heavy	>3	4	23	12	11	12	8	24

[a] Number of fields.

at flowering) was significant ($P < 0.001$) with $r = 0.60$ and 0.64 for 1986 and 1987, respectively. Fig. 4a,b shows that an increase of 1 point in DI represents approximately a yield loss of 1 tonne per ha.

In 1986, the pea yield on fields with negligible disease was about 7 tonnes per ha. In the field with the most severe disease the yield did not reach 3 tonnes, even with an application of more than 200 kg N per ha. Disregarding the fields which were not harvested in 1987, yield losses due to root rot accounted for about 50% of the yield depression in fields in the class 'heavy disease'. In 1987, *Mycosphaerella pinodes* was the dominant foliar disease. It reduced thousand-kernel weight to 190–310 g, with an average of 260 g, more than 40 g lower than normal. This resulted in an average yield 1 tonne lower than in 1986 and a maximum calculated yield at the same level as the average in 1986.

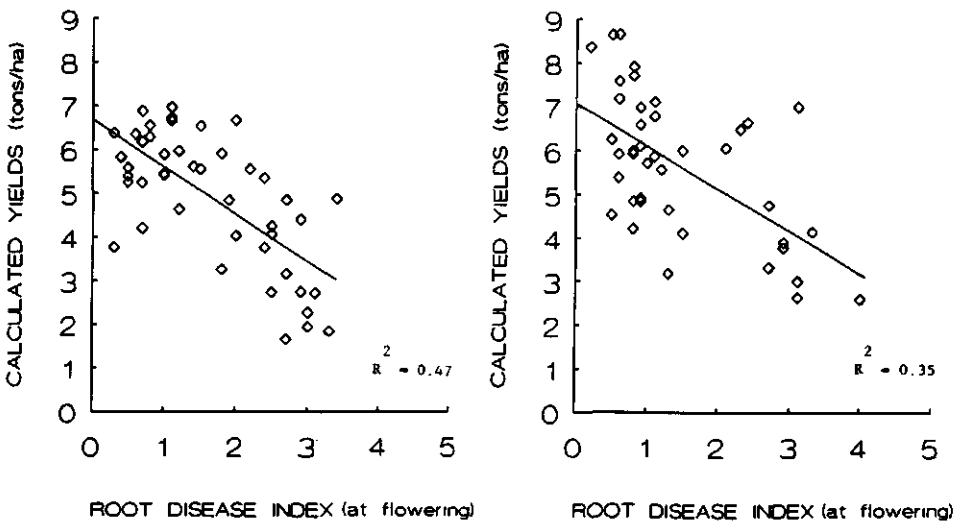

Fig. 4. Relation between the root disease index of pea at the flowering in the field and the calculated yield. (a) 1986; (b) 1987.

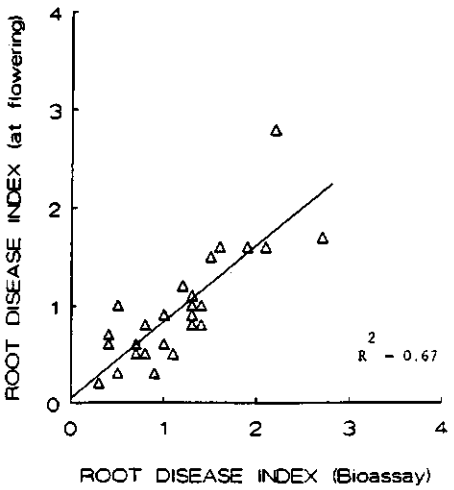

Fig. 5. Relation between root disease index of pea in bioassay and in the field in 1988. Field assessment was at the end of the flowering stage.

Time of bioassay execution. Paired differences between bioassay results of 18 soils in autumn 1986 and spring 1987 were not significant ($P < 0.05$, $n = 17$). The correlation between the two sets of data was good ($r = 0.92$, $n = 16$).

Bioassay for practical purposes. Though the percentage of the variation explained in 1988 was lower ($R^2 = 0.67$, $n = 30$) than for the 1986/1987 experiments, the correlation was significant ($P < 0.001$) (Fig. 5).

Discussion and conclusions

The years 1986 and 1987 were extreme for pea production. The year 1986 was very dry and sunny, with little disease. In 1987, continuing wet weather caused a catastrophe with regard to pea diseases and yields. Notwithstanding very different weather conditions in 1986 and 1987, the relation between test results and field assessment at flowering and ripening stages was good. Under the favourable 1986 circumstances, root rot depended on the quantity and vigour of the inoculum in the soil, since the environment did not predispose the crop to root infection. However, even in 1986 root rot occurred on some fields with good agricultural characteristics. Riepma (1967) described the same phenomenon. Bad years such as 1987 show that some management decisions, such as sowing on frozen soil or sowing on soils which easily get waterlogged, can greatly increase the occurrence of root rot and consequently infestation of the soil with root rot pathogens. As an example, the DI of test plants grown on soil samples of a field lightly contaminated before the 1987 pea crop increased from 1.5 before to 4.4 after the pea crop.

Characteristics of the bioassay. The bioassay gives an indication of the combined effect of all pathogenic fungi in the soil, and thus is aspecific. However, it is possible to modify the test conditions in such a way that a specific pathogen will dominate. At the start it was known, that *Fusarium solani*, *Phoma medicaginis* and *Pythium* spp. were the most com-

mon components of the root rot complex in the Netherlands (Schreuder, 1949; IPO, 1960–1970). The purpose of the bioassay therefore was to predict damage by this complex.

In bioassaying soil, high temperature was used by Kobriger and Hagedorn (1983) and saturation followed by drying to wilting point by Sherwood and Hagedorn (1958) to stimulate specific rot symptoms. We did not intervene to stimulate susceptibility of the test plants to specific pathogens. Conditions were created for optimal rooting in the available soil mass. Nevertheless, test plants showed conspicuous symptoms of infection caused by *T. basicola* and *A. euteiches*. It was also noted that the pea cyst nematode, *Heterodera göttingiana*, produced cysts in the pea roots within the test period. A great number of fungus species were identified in infected roots of test plants which corresponded with isolations from field plants (Schreuder, 1949; Riepma, 1952). A great advantage of bioassaying soil is that no seed-borne pathogenic species, such as *Phoma medicaginis* var. *pinodella*, *M. pinodes*, *Ascochyta pisi*, or seed contaminants such as *Fusarium* spp. will be scored if not present in the soil.

In root infections several fungus species are normally present. This explains why the IPS is assessed indirectly as a severity (DI), representing estimated percentages of attack, and not as a quantity of propagules per gram of soil corresponding to a certain percentage infection, as e.g. IPS(50): the number of propagules necessary to reach 50% infection. The latter is advised for individual pathogens (Bouhot, 1979; Rouxel, 1988).

IPS depends on biotic and abiotic properties of the soil (Alabouvette, 1989), which implies that IPS(50) must be substrate/field specific. Relating IPS to a number of propagules and using this number for various soils seems unwarranted.

Practical execution: Sampling. Sampling procedures depend on the distribution of the pathogen populations to be surveyed. In field observations (data not presented) disease patterns were often homogeneous. Observed within-field heterogeneities mostly reflected reparcellation, an old cultural practice among farmers in the Netherlands. Nevertheless we systematically sampled according to a W-pattern, as if the pathogens were clustered (Mihail and Alcorn, 1987). This sampling pattern is not always the most efficient (time, work), but it surely is the safest one. We took a sample every 200 m^2. Headlands were excluded. In comparable research situations one sample per 4000 m^2 has also given good results (Reiling et al., 1960; Olofson, 1967). The more homogeneous the distribution of the pathogen, the less intensive sampling may be. Then large samples instead of a big number of smaller ones give a more representative measure (Johnson and Curl, 1972).

The disease indices of test and field plants proved to be highly correlated. It should be kept in mind that soil sampling for the bioassay and disease rating of the field plants were always carried out in only 1 ha of the most homogeneous part of the field, and both according to the W-pattern. Omitting these precautions in 1988 led to a considerable decrease of R^2, the coefficient of determination; however, the linear correlation was still highly significant.

Test period. A bioassay must combine rapid production of reproducible results with simple procedures. A test period of 5–6 weeks is long and demands much space and labour. Experiments in 1988 have shown that the test period can be reduced by 10 days using early flowering cultivars (Oyarzun, 1991). The idea of using pots with a self-regulating moisture regime, as developed by Wisbey et al. (1977) or Snow and Tingey (1985), combined with tubes which use considerably less soil (Maduewesi and Lockwood, 1976), has been further elaborated into an automatized bioassay system (Oyarzun and Dijst, 1991).

Translation of bioassay results into a message to the grower. In formulating an advice regarding field-dependent root rot risks, cultural practices and other factors which could probably influence disease development should also be taken into account. The relative weight of each factor varies from year to year. This applies to the position of peas in the crop rotation with regard to root diseases and to the equivalence of the most frequently grown legumes as hosts of the root rot pathogens. In the dry year of 1986, the effects of legume frequency in the rotation on root rot were more pronounced than in the wet year of 1987 (Oyarzun and Hoogland, not published). In rainy years, physical constraints of the soil influence plant health to a larger degree. On heavy soils, root rot problems can easily occur due to waterlogging; 48 h of water saturation suffice to induce heavy root rot (Biddle, 1984). These factors are especially important when IPS is light or moderate. According to Rush and Kraft (1986) the effect of stress factors is to reduce the latent period. It is also possible that stress reduces plant resistance to such a degree that a lower level of inoculum potential suffices for the development of disease (predisposition).

Predictive value. The regression line of DIs of bioassay on field can be used to predict the probability of root rot in the crop. A perfect relation has an angle of 45° (1:1 line) and little dispersion. The variability of data and the deviation of the fitted line from 45° depend on the time of disease assessment in the field, the growing conditions of the crop, soil and climate, and on the conditions under which the bioassay is carried out. Pooling the bioassay and crop disease indices for 1986 and 1987, at flowering (Fig. 6), allows calculation

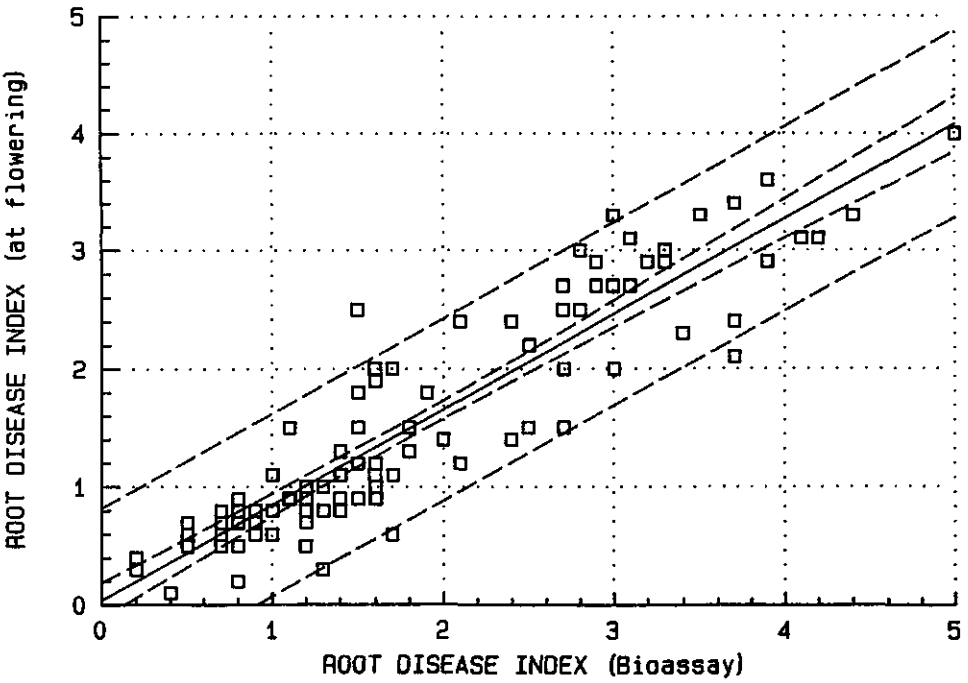

Fig. 6. Confidence interval of the population of regression lines (inner lines) and prediction interval (outer lines) for root disease index of individual field crops at flowering for a given bioassay result.

of a confidence interval for the fitted line and a prediction interval for individual points. In the climatologically extreme years of 1986 and 1987, individual differences with the fitted line on the average were not more than 20% at each point on the 0–5 DI scale (Fig. 6). Erroneous estimates thus seem improbable, but they may occur under extreme conditions. Thus a DI of 1.5 in the bioassay can correspond to heavy root rot in a rainy season. With a DI of >2.5 (root rot severity >50%) it is better to choose another field in all circumstances.

Economical considerations in the formulation of advice. In the Netherlands, pea should enlarge the flexibility of very narrow rotations, in which there are hardly any crops other than wheat, potato and sugar beet. Therefore the net financial result of pea has to be at least equal to wheat, the least attractive main crop. In this comparison, the price ratio pea/wheat and the yield stability of pea are decisive factors. Under favourable conditions, modern cultivars on commercial fields can yield 7 or even 8 tonnes dry seed (14% humidity) per ha (CEBECO, personal communication). On fields with a DI >3 yields of about 3 tonnes per ha were not uncommon.

Conclusions

Three years of field-oriented research have led to the following conclusions:
1. Values of the IPS as determined by a bioassay in pots in the greenhouse fit well with disease intensity in pea crops.
2. Soil sampling and the bioassay itself can be carried out in the autumn preceding the pea growing.
3. With test plants in pots the most common root rot pathogens of pea present in soil are successfully baited.
4. The bioassay produces a measure of the IPS and thus provides an indication of the suitability of a field for growing peas. This information may serve as an instrument in an integrated programme to control root rot.
5. In general the price/cost ratio of peas compared to cereals decides the short-term risk to be taken. In the field, light root rot infections also cause measurable yield depressions. Growing peas on slightly contaminated fields can endanger the long-term continuity of pea production, a risk which may be more important than a slight yield depression.

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Chapter 7

Computerized equipment to control soil water matric potential and soil temperature in inoculum potential assays of soil-borne pathogens

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Abstract

Computerized equipment to control soil temperature and soil water matric potential, ψ_m, at high soil hydric conditions was developed and evaluated. A series of experiments demonstrated the accuracy and reproducibility of the equipment's performance and its adequacy for the assessment of the inoculum potential of soil-borne pathogens in soils with different characteristics. Control of soil water potential is achieved by variation in the height of the water table in a medium with high water conductivity supporting the soil. The equipment consists of double-walled tanks, permitting the adjustment of soil temperature. It is provided with sensors, control software and valves for automatic operation. In a growth chamber at 24°C, with RH 70% and irradiation of 90 $W.m^{-2}$, ψ_m could be maintained in dynamic equilibrium for pF values ranging from 1 (-1 kPa) to 2 (-10 kPa) in various arable soil samples during the four to five weeks period of the bioassays. During the fourth week of pea or iris growth at pF = 2, the system controlled ψ_m within an amplitude of 0.4 pF-units. Between replications, variance was approximately 0.1 at pF = 2, decreasing with increasing ψ_m. Soil temperature in the system could be maintained at a constant level with a variance below 0.1, within an amplitude of 0.3° C.

Additional keywords: *Fusarium solani*, *Thielaviopsis basicola*, *Rhizoctonia solani*, root rot, pea, cauliflower, flowerbulbs, environmental factors.

Introduction

Usually, plants are used in bioassays of the inoculum potential of soil-borne fungi in soil (IPS). Constant conditions are necessary to reduce variability and to ensure reproducibility (Bouhot, 1979; Mitchell, 1979). Little has been published about the technical requirements to meet such conditions. Plant physiologists and soil scientists contributed to the development in this area (Haan and Barfield, 1971; Alverez and De Datta, 1977; Snow and Tingey, 1985).

In pot experiments, the control of water conditions in soil is tedious and time consuming, particularly if different types of soil are involved in one experiment (Oyarzun, 1993). It demands the construction of a water retention curve at a given soil density, before testing a soil. Only then, the desirable level of the soil water potential can be approximated by weighting (Emberger and Welty, 1983; Hering et al. 1987; Oyarzun, 1993). The term soil water potential, ψ_s, in this case is used as equivalent to soil water matric potential, ψ_m, because the soil solution, except in saline soils, is often quite diluted

(Bolt and Bruggenwert, 1976). Therefore the osmotic component of the soil water potential can be neglected. The matric potential, ψ_m, has been traditionally represented by the value of the $\log_{10}$ of the absolute value of the moisture tension (1 cm = 1 mbar = 0.1 kPa), a parameter known as pF (Bannister, 1976). We use the pF in this paper.

Soil water status is a major environmental factor affecting diseases caused by soil-borne pathogens. The stimulating effect of high soil water potential, ψ_s, on root rot has been attributed to a higher exudate production (Kerr, 1964), triggering propagule germination (Cook, 1981), shortening the path to the roots for swimming zoospores (Duniway, 1976), or limiting oxygen supply to the growing root (Schmitthenner, 1970) thus predisposing the roots to infection (Miller and Burke, 1985; Smucker and Erickson, 1987).

Soil temperature affects root infection (Benedict, 1969; Doornik, 1981). At the infection court, temperature is probably more buffered than soil water potential. Most root rot fungi are able to establish infection during the whole growing season (Mitchell, 1979) due to their wide temperature tolerance. For pea root growth, optimum soil temperature is about 18-20 °C. For most pea cultivars, optimum air temperature ranges from 16 to 24 °C (Kay, 1979). Within this range of temperatures, *Thielaviopsis basicola* and other pea root rot pathogens show maximum infection activity (Hagedorn, 1984; Rothrock, 1991).

The regulation of soil temperature is crucial for rot in flowerbulbs caused by *Rhizoctonia solani* AG-2-tulip (provisional indication, Schneider, pers. comm.) which has maximum pathogenicity at 9° C (Doornik, 1981; Schneider and Dijst, 1992).

Our aim to determine soil receptivity (SR, ranging from conduciveness to suppression of disease) to soil-borne pathogens necessitates the determination of IPS in a great number of field soil samples. Several systems were developed (Perroux, 1979; Miller and Burke, 1985; Oyarzun, 1993), but these were not considered appropriate for our purpose. In this paper we describe equipment designed to control ψ_m and temperature in a range of conditions suitable for the determination of IPS in different soil types. The performance of the equipment is illustrated by selected experiments.

Materials and Methods

General description of the equipment. The equipment consists of four units: a test unit, a water control unit, a temperature control unit and a computerized interface (Fig. 1). The current values of ψ_m are registered by mini-tensiometers and the soil temperature by thermocouples.

The test unit consists of tanks, internal size: 240*40*320 mm, which can be filled with soil in bulk or in subunits (mini-pots, available in various sizes). The water flows into the unit from the bottom and leaves the tanks by evapotranspiration only. As a transport medium for water upward to the soil, a florist's foam block (Smithers-Oasis, pore volume 98%, density $\approx$ 16 kg.m^{-3}, W.H.C $\approx$ 60%) of 240*40*200 mm is fitted at the bottom of the tanks (Snow and Tingey, 1985). Soil is placed on the foam block without separation for use in bulk, or with separation by nylon cloth for use in mini-pots. Top irrigation can be applied through mini-nozzles.

Mini-pots. For experiments with pea seedlings, six mini-pots made of black or transparent plastic material (40*40*120 mm), were placed in a row at the top of the foam block. The

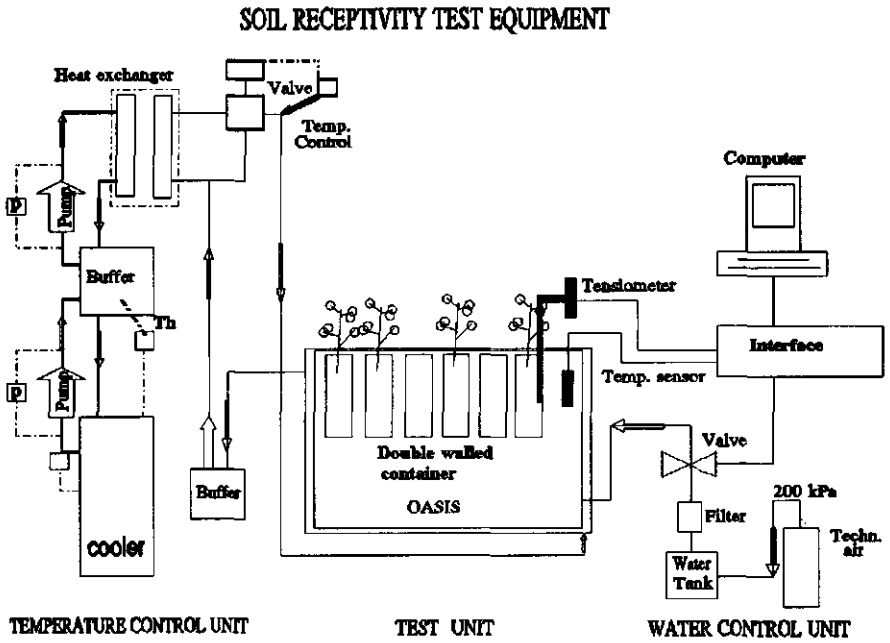

Fig. 1. A diagram of the computerized test equipment used to control soil water potential and soil temperature in bioassays testing plant roots with soil-borne pathogens to determine receptivity of soils.

pots were sealed at the bottom with a nylon cloth (Mauritz, monodur polymide) with a mesh of 10 μm.

Water control unit. To regulate water dosage, the opening period, T, of the valves is calculated. Every 10 min, the required value of pF, the set point (SP), is compared to the current value of a weighted average (LL) of pF calculated on the basis of one reading (M) per min and transformed to a moving average (L):

$$L = (L*0.6) + (M*0.4), \qquad LL = \sum_{t=1}^{10} (L)/\ 10 \qquad T = \frac{(LL - SP)}{3} \quad \text{(sec)}$$

$$T = 0 \quad \textit{if}: \quad SP > LL \quad \textit{or} \quad LL > SP \quad \textit{and} \quad LL(t\text{-}1) > LL(t)$$

This algorithm can be modified if necessary. Magnet valves (Stagair D-7121; range: 0 - 4 bars) were installed for automatic regulation of water flow. If a supply of water is required the valves open automatically. The water is filtered before reaching the tank. For

irrigation, a 1:1 mixture of boiled and demineralized water is used. The water reservoir is kept under pressure by technical air.

The temperature control unit. A double-walled tank of stainless steel permits control of soil temperature. In this unit the temperature of the cooling fluid is controlled by a refrigerator and a heat exchange device. The main pump (Calpeda Pump, C-CN; max. flux 4.6 m^3/h; max. working pressure of 6 Bars) drives the liquid through the heat exchanger to a common tube along the wall of the growth chamber. The tanks are connected to this tube by secondary tubes. At the operating pressure of 1.3 bar approx. 40 $l.h^{-1}$ circulate per tank, which is the equivalent of 17 times the content of the double tank wall. To avoid deformation of the tank walls by pressure of the fluid, a decompression device, a buffer, is placed at the end of the test units. From this buffer a second pump (Pumpec GA 200, 0.35 kW) returns the liquid to the unit. The device permits cooling to below zero.

Interface and programme. Operating programmes were written in MS Q-Basic and include registration, control, calibration, statistics and graphics. Set points are entered into the programme and used to automatically operate the water control unit. The system stores the signals from the sensors at a rate of 1 per minute and uses them to calculate LL, and comparing LL with SP every 10 minutes.

Measuring ψ_m. The current values of ψ_m are registered by mini-tensiometers. These were constructed using ceramic cups, 40 mm long and 4 mm internal diameter, connected to an electronic sensor (Honeywell 130PC-pressure sensor, with reduced sensitivity to temperature) which translates negative pressure (0 - 100 kPa) into a differential voltage.

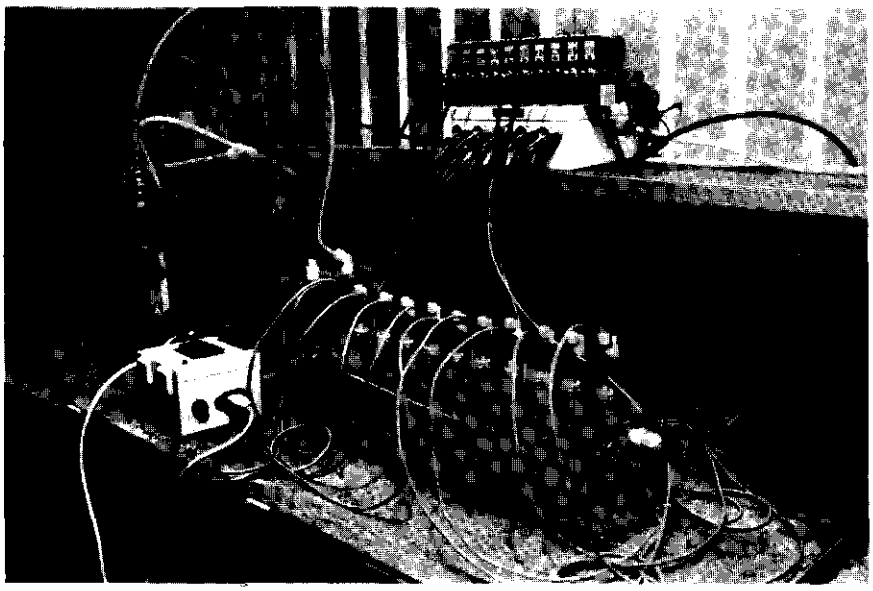

Fig. 2. Pressure chamber used for the calibration of the mini-tensiometers.

The mini-tensiometers are suitable for measurements in the range of zero to 80 kPa. The tensiometers are calibrated in a pressure chamber (Fig. 2). The ceramic cups are freed from air and submerged in air-free water, in the pressure chamber. A vacuum pump is attached to the chamber and a series of negative pressures are set. At equilibrium the value of the signal is stored. For each sensor a straight calibration line is calculated. The tensiometers are inserted in the upper 40 mm of the soil in a layer of fine sand, used to enhance the contact between soil and sensor.

Measuring soil temperature. Soil temperature is registered by an electronic NTC thermocouple (25°C, 10kΩ). For calibration, the sensors are inserted in water at zero and 25° C to set the scale. The fitted line is stored and used to calibrate the measurements.

Experiments. In all experiments, test soils were sieved (mesh 4 mm) previous to use. Four replicates were used in each test, placed randomly in the growth chamber. For pea root rot experiments with *Fusarium solani* and *Thielaviopsis basicola*, tanks were filled with 6 mini-pots each containing 176 ml of soil. Per pot, four surface-disinfected pea seeds, cv Finale, were sown at 20 mm depth and plants were grown for four weeks. For *Rhizoctonia* experiments, 1.3 l of soil was bulk-loaded directly on the foam. In the 120 mm deep soil, either a row of five iris bulbs cv. Blue Magic was planted at 9-15 mm depth, or a row of five radish seeds, cv Salido, or two rows of 11 cauliflower seeds cv. Oberon were sown at 15 mm depth and grown for five weeks.

Before the start of each experiment, all material coming in contact with soil and pathogens, including the ceramic cups, was pasteurized in water at 70° C, submerged in ethanol 70% and washed with sterile water. Tanks were disinfected in a warm soap suspension, 70° C, washed with tap water and surface sterilized with ethanol 70%. To eliminate solutes and acidifying effects of the foam, the foam blocks were rinsed for 24 hrs in running tap water, followed by rinsing in sterile tap water.

Stability of ψm control. Irrigation methods were compared as to their effect on the stability of ψ_m. Water was supplied to the tanks either at the bottom via a foam block of 'Oasis ', or at the soil surface through mini-nozzles (Fig. a/c).

The stability of ψ_m was investigated at different levels and in different soils. We present ψ_m data obtained from four different soil types at pF 1.1, 1.5 and 2.0 during the second to fourth week after pea sowing (Table 1). Similarly, the registered outputs of an experiment with pea on four soils are presented to show differences in time to reach equilibrium after changing SP (Fig. 4). Daily deviation in current pF values from the SP at pF = 2 during the fourth week of pea growing is illustrated (Fig. 5 a/d)).

Variability of disease development. An experiment with pea was carried out to compare variability in root rot development between mini-pots within one tank (within tank variance) with variation between mini-pots in different tanks (between tank variance) using two naturally infested soils. Mini-tensiometers were inserted in one of the pots at the middle of the tank. The tanks were then placed at random in the growth chamber. The water potential was set for weeks 1 to 4 at pF 2, 1, 1.5 and 2, respectively. One month after sowing, root rot disease severity (DI) was assessed on a scale running from

0 for healthy roots to 5 for dead plants or completely rotten roots.

Cross contamination within tanks. The possibility of reciprocal contamination between mini-pots in a tank was examined for two pathogens, *Fusarium solani* f.sp. *pisi* (Fsp) and *Thielaviopsis basicola* (Tb). A loam soil, steam-sterilized at 121° C, 0.12 MPa, was infested with a mixture of different isolates of Fsp and Tb at doses increasing by a factor 10 from 10 to 10^5 spores per g dry soil. One infestation level was assigned to a tank. Each tank contained 4 mini-pots filled with the infested soil and 2 with non-infested soil. Per pot, four surface-disinfected pea seeds cv. Allround and Colette for Fsp and Tb, respectively, were sown at 2 cm depth. Water content was adjusted to 26% (approx. pF = 2). After emergence the pots with sterilized soil were placed between the pots with infested soil. A tensiometer was placed in a pot with infested soil in the centre of a tank. Soil water potential was first adjusted to pF = 1 to equilibrate all the objects for a day, and than lowered to pF = 2. Root rot severity was assessed 5 weeks after sowing and isolations of pathogens from roots were made on Czapek-Dox or selective media.

Effect of ψ_m on pea root rot. These experiments aimed at elucidating some inconsistencies in reports on the relation between the severity of infection caused by Fsp and the soil water regime. In a first greenhouse experiment, a loam soil, highly infested with a complex of pathogenic fungi including Fsp, was used to determine the effect of ψ_m on root rot. Germination, rate of leaf formation and plant weight were determined in heat sterilized soil. The ψ_m was adjusted daily by adding water after weighting. A second experiment was carried out with the system, using the same heat-sterilized soil, to assess disease severity after artificial infestation with Fsp in a range from pF = 1.0 to field capacity. The soil was infested with a mixture of conidia from 21-day-old cultures of Fsp isolates. The isolates originated from monospore cultures and were grown on Czapek-Dox agar at 25° C under black light (λ = 365 nm) for 12 h per day. The soil was inoculated at a rate of $5*10^3$ conidia per gram dry weight. At each pF level, 2 tanks with 6 mini-pots were used and treated as described before. Mini-sensors were attached to the middle pot and water regimes set at pF 1.0, 1.3, 1.6, 1.9 and 2.2. The air temperature was maintained at 24/18° C. Light intensity was 90 W/m^2 during 12 hrs per day. Disease was assessed four weeks after sowing.

Soil temperature control. The performance of the equipment in controlling soil temperature is illustrated with an experiment at two soil temperature levels, 9 and 11.5° C, at 14° C air temperature.

Results

Stability of ψ_m. Top and bottom irrigation of water yielded a very different pF pattern in time (Fig. 3a,b). In bulk-loaded soils, top irrigation of radish and cauliflower yielded the same pF pattern (data not shown) as pea in mini-pots. With top irrigation, the maximum pF values correspond to the SP value. The amplitude of the cycles increased with an increase in water demand by the growing plants. If water was supplied from the bottom, a constant value was reached and maintained in almost the whole experiment,

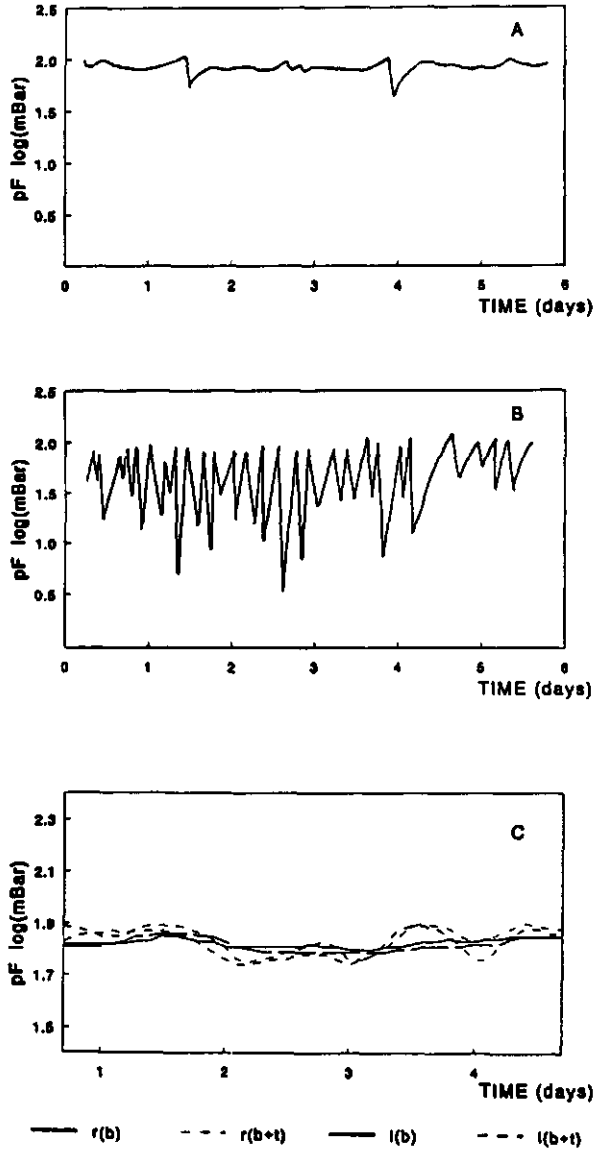

Fig. 3. Variation of soil water potential in time, expressed in pF values, during the fourth week after sowing, using (A) bottom irrigation and (B) top-irrigation of peas grown in mini-pots at pF = 2.0, and using (C) bottom irrigation (b) of iris (i) and radish (r) in bulk-loaded soil and a combination of top- and bottom irrigation (b+t) of radish and iris in bulk-loaded soil at pF = 1.9.

regardless of plants being grown in mini-pots or in bulk-loaded soil (Fig. 3a,c). By combining top- and bottom irrigation, the stability in ψ_m was comparable to bottom irrigation alone as shown for iris and radish in Fig. 3c.

The stability of ψ_m was further investigated in different soils and at different pF levels. In the mini-pots with pea seedlings, the pF was mostly very constant and at low pF differed hardly between soils (Fig 4). Soils differed in time needed to reach equilibrium.

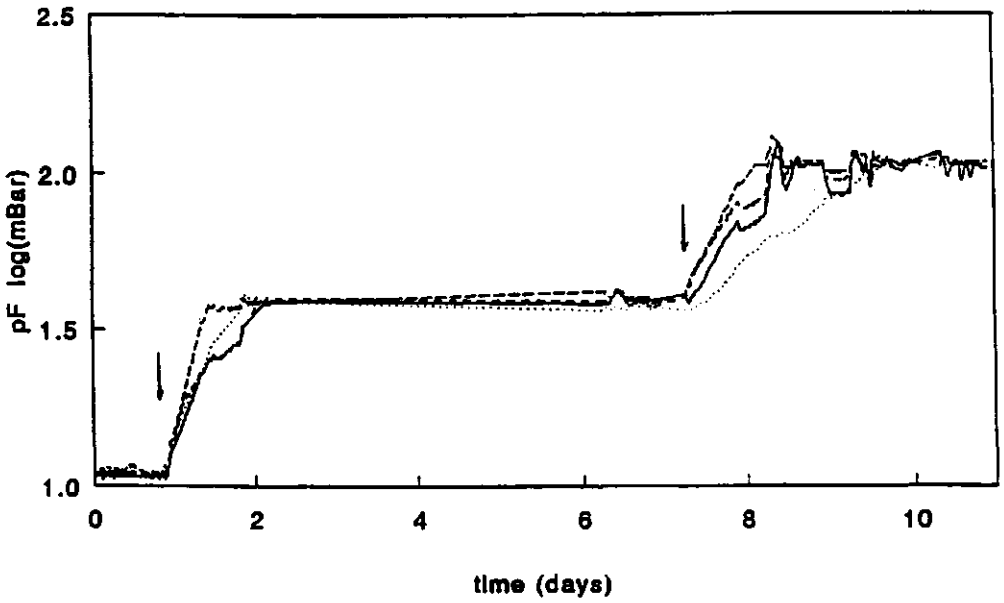

Fig. 4. Soil water potential patterns expressed in pF values, in time, for four different soils showing the time needed to reach equilibrium after changing set values (arrows). pF values set at 1, 1.5 and 2.0.

The standard deviation (s_x) for replicates was small and increased with higher pF set points in combination with older plants (Table 1). The SPs were approximated with an accuracy of $\approx$ 0.1 pF. Variation in pF increased with increasing evapotranspiration. At pF = 2, the amplitude of the cycle did not exceed 0.3 pF units for 4-week-old, strongly transpiring plants. Figure 5a illustrates the situation. Site differences within the growth chamber, such as proximity to the inlet of circulating air or irregularities in light intensity, can disturb synchronization and cause differences in amplitude of the cycles between replicates (Fig 5b/d).

Four-week-old pea plants use a relatively large amount of water. Table 2 presents the water used (g d^{-1} per tank) for 5 different natural soils. The figures were calculated using a flow rate per valve of 4.5 -5.0 ml per second. The coefficient of variation indicates that large differences in transpiration can occur between replicates per soil in the growth chamber. Assuming a pore volume of 50%, the amount of water used per pot was calculated to reach values of 2 times the pore volume when growth is vigourous.

Table 1. Control of soil water matric potential, ψ_m . Averages and standard deviations of ψ_m, expressed as pF units, of 4 replicates (6 means per day) per soil calculated for four soils (S1 to S4) during the first (set point, SP: pF = 1.1), second (SP: pF = 1.5) and third (SP: pF = 2.0) week after emergence of pea. Accuracy is the SP-weighted difference of the average pF and SP.

	2st week		3nd week		4rd week	
Set point: pF:	1.1		1.5		2.0	
	Av	s_x	Av	s_x	Av	s_x
Soils						
S1	1.15	±.009	1.56	±.020	1.98	±.064
S2	1.16	±.024	1.53	±.069	1.98	±.074
S3	1.18	±.039	1.57	±.033	2.00	±.070
S4	1.13	±.011	1.53	±.060	1.93	±.092
Accuracy	0.05		0.03		0.01	

Table 2. Averaged period of watering, T, (sec day^{-1}), the coefficient of variation (cv %) and the calculated water use (w.u. in g H_20 per tank per day) by pea plants in the fourth week after sowing in 5 natural soils (S1/S5). The last column indicates the relation between the volumes of daily water use, wv, and the pore volume of the sample, pv, at the indicated soil density (dens.).

	T	cv	w.u.	dens.	wv:pv
	$s.d^{-1}$	%	g/tank/day	$g.cm^{-3}$	
Soils					
S1	235	27	1057 - 1175	1.0	2:1
S2	213	42	959 - 1065	1.0	2:1
S3	119	22	536 - 595	1.1	1:1
S4	69	49	310 - 345	1.1	1:2
S5	56	27	252 - 280	1.0	1:2

Table 3. Root rot disease index (DI) in pea (0 = healthy, 5 = dead) in two naturally infested soils. Per soil, the variance of the score of disease severity is compared between tanks of the system (blocks) and within tanks (mini-pots used as subplots). ns = non significant; *** = P ≤ 0.01

	Soil infestation:			
	Slight	Heavy	f	P
Means (DI)	1.64	4.27	216	***
Var between blocks	0.39	0.38		
Var within blocks	0.28	0.23		
f	1.39	1.60		ns

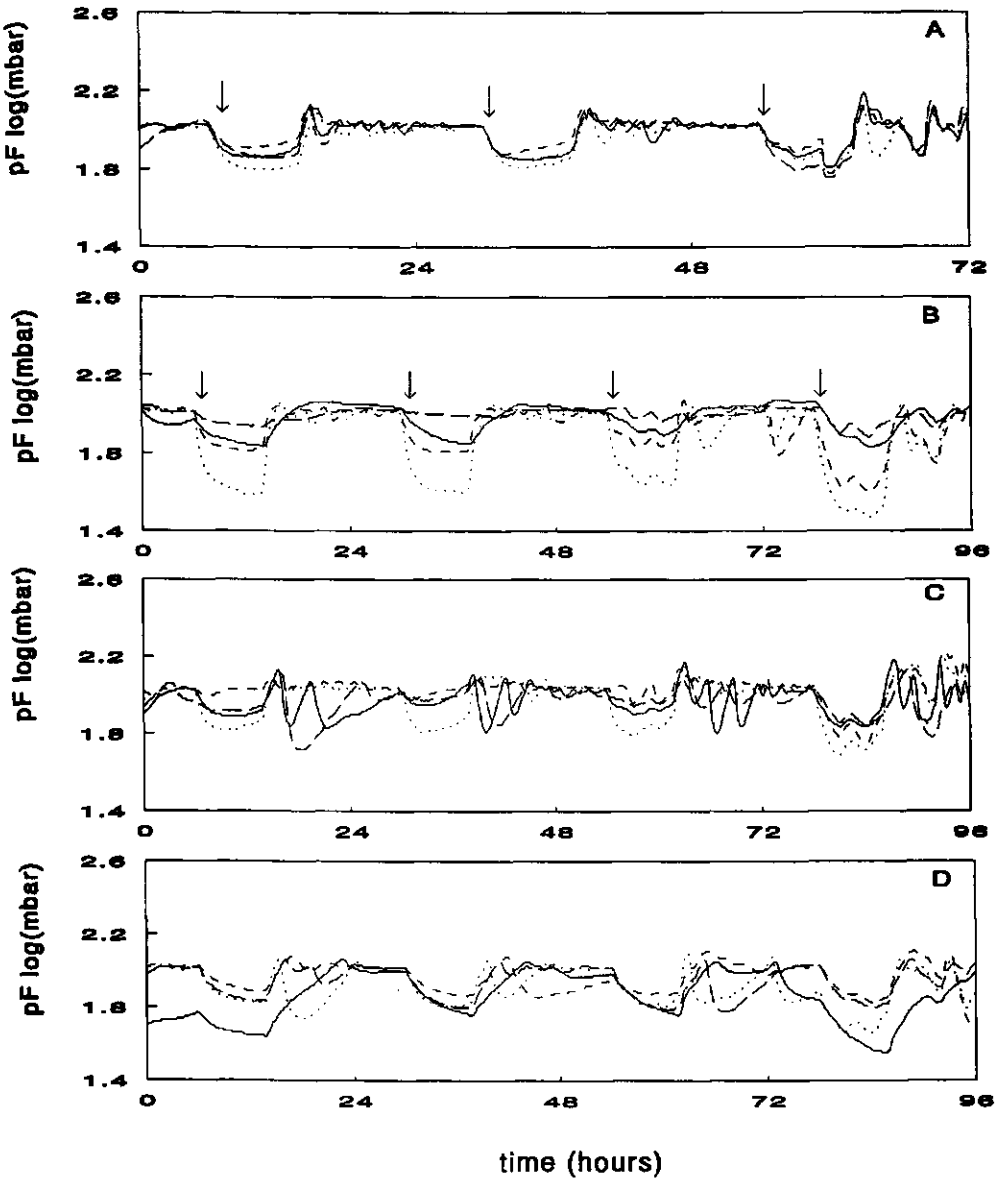

Fig. 5. Variations in amplitude of soil water potential patterns during three or five days in the fourth week of pea growing in four different soils. The arrows indicate the start of pF decrease after rising the water table to compensate sudden increases of evapotranspiration. (A) sandy loam, (B) loam (C) sand and (D) clay. pF values set at 2.0. In each graph the patterns of four replicates are given.

Variability of disease development. The variation in pea root rot severity between tanks was not larger than between mini-pots within tanks ($P \leq 0.05$) as calculated for two soils differing in natural infestation level ($P \leq 0.01$; Table 3).

Cross contamination within tanks. No symptoms of root rot developed in pea plants in the mini-pots with sterilized soil placed within a tank next to mini-pots containing soil infested with Fsp or Tb, regardless of the level of soil infestation (Table 4). No pathogens were isolated from the roots or epicotyls of plants growing in sterilized soil.

Table 4. Average disease index (DI, 0 = healthy, 5 = dead) after infestation of sterilized soil with increasing amounts of spores of *Fusarium solani* f.sp. *pisi* and *Thielaviopsis basicola*, and the standard deviation (s_x) of four replicates. No root rot symptoms developed in plants in the non-infested objects (plants growing in pots with sterilized soil placed between infested pots) and no pathogens were found after screening for contamination.

	Infestation level: Log_{10} (conidia.g^{-1} dry soil)									
soil treatment	1		2		3		4		5	
	DI	s_x	DI	s_x	DI	s_x	DI	s_x	DI	s_x
Fsp-infested	1.7	±.5	2.8	±.2	3.0	±.2	3.6	±.4	4.2	±.3
Non-infested	0		0		0		0		0	
Tb-infested	2.4	±.4	2.9	±.4	3.0	±.3	4.4	±.4	5.0	
Non-infested	0		0		0		0		0	

Effect of ψ_m on pea root rot. In a previous experiment with manual ψ_m control, pea root rot caused by a complex of fungi, including Fsp, in natural soil strongly increased with ψ_m (Fig. 6). With automatic control of the soil water regime, however, no differences in *Fusarium* root rot occurred in sterilized soils between pF 1.3 and 2.2. Only at pF = 1 the infection was more severe ($P \leq 0.05$; Table 5).

Table 5. Root rot severity of 4-week-old pea seedlings, expressed as disease index (DI, 0 = healthy, 5 = dead), growing at several pF levels in a soil inoculated with *Fusarium solani* f.sp. *pisi*. s_x = standard deviation. Values followed by the same letter are not significantly different according to the Duncan test ($P \leq 0.05$).

pF	1.0	1.3	1.6	1.9	2.2
mean DI	4.11[a]	3.45[b]	3.53[b]	3.46[b]	3.53[b]
s_x	0.18	0.04	0.03	0.07	0.21

Table 6. Soil temperature stability. Average daily maximum and minimum soil temperatures and their standard deviations during one week, calculated from 30 registrations per day per replicate for the set temperature of 9° and 11.5° C. Air temperature in the growth chamber was kept at 15° C. In the tanks flowerbulbs were growing on a sandy soil.

Soil T°C		Day 1	2	3	4	5	6	7
9	max	9.2 ±.07	9.2 ±.06	9.2 ±.06	9.2 ±.05	9.1 ±.06	9.1 ±.04	9.3 ±.05
	min	9.0 ±.05	9.0 ±.00	9.0 ±.05	8.9 ±.05	8.9 ±.02	8.9 ±.03	9.0 ±.00
11.5	max	11.5 ±.06	11.4 ±.06	11.4 ±.06	11.3 ±.05	11.4 ±.06	11.4 ±.06	11.4 ±.06
	min	11.5 ±.06	11.4 ±.03	11.4 ±.05	11.4 ±.05	11.4 ±.06	11.4 ±.05	11.4 ±.06

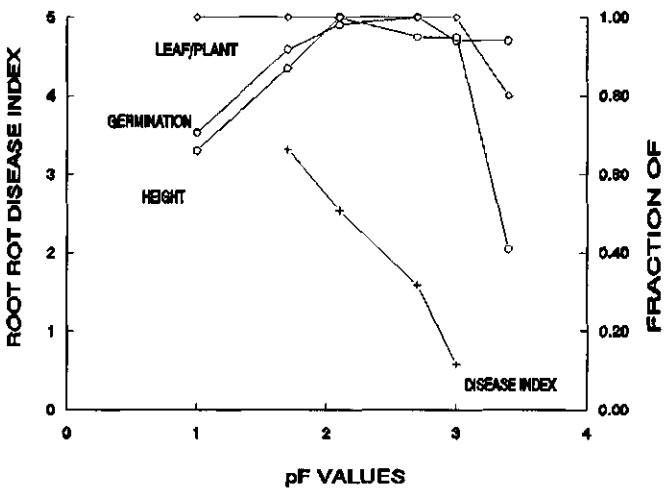

Fig. 6. Effect of soil water matric potential, expressed in pF values, on root rot disease index in pea (DI, 0 = healthy, 5 = dead) grown in a naturally infested soil and on germination rate, plant height and number of leaves per pea plant in the same but heat-sterilized soil, expressed as a fraction of the maximum value registered, evaluated one month after sowing.

Soil temperature control. In previous experiments the equipment was provided with a cooling unit, Frigomix, which had a pump capacity of 16 l/min at 0.3 bar and a temperature stability of 0.5° C, to control temperature within ± 1° C in up to 20 tanks. A fall of approx. 2° C below the setpoint was observed daily during the light period. With

the current temperature unit, capable to control 100 tanks, this temperature effect caused by transpiration is suppressed. The equipment controls the soil temperature at the aimed level within a narrow range (Table 6). Between replicates, temperature variation remained within $\pm$ 0.07° C. With 14°C air temperature, the amplitude in soil temperature around the setpoint of 9° C was approx. 0.3° C and around the setpoint of 11.5° C it was negligible. Thus, the amplitude was greater when the difference between air and soil temperature was greater. The same precision was observed in a sand and in a clay soil. On the wall of the tanks and on the soil surface some condensation developed at 9° C, because their temperature was below the dewpoint.

Discussion

Stability of ψ_m control. Maintaining ψ_m in dynamic equilibrium is possible only to a limited extent, because several factors interfere. The method of water supply has a large influence. We obtained the most constant ψ_m in the upper 40 mm of soil with an upward water supply through the soil. If top irrigation is necessary, large fluctuations in ψ_m can be avoided by a combination of top- and bottom irrigation, which also avoids undesirable effects of partial saturation of the soil surface by top irrigation. Using top irrigation, the amplitude and frequency of the ψ_m cycles increased drastically, despite the rather frequent ψ_m adjustment. This result illustrates the variation of soil water potential which usually occurs in pot experiments when this is daily adjusted by weighting. In our system with top irrigation we set a maximum acceptable drought level, whereas in manually watered pot experiments the soil may become drier than intended. Using bottom irrigation, the water flows upward continuously by capillarity, compensating water loss due to evapotranspiration, and maintaining ψ_m in a dynamic equilibrium.

The success in achieving a stable ψ_m is affected by the hydraulic properties of the soil, the amount of water demanded for evapotranspiration and the set value of ψ_m. The lower pF is set, the more stable the response. Until the fourth week ψ_m was very stable at pF $<$ 2. The small soil volume used in the experiments implies that, with the advance of time, roots will exploit soil water faster and that the differences between SP and LL will increase, even when ψ_m is adjusted six times per hour. Furthermore, relatively small differences in water transport into the soil and water use will provoke a strong fall in the unsaturated soil hydraulic conductivity. The extent to which this occurs depends on soil type, water demand and set value of ψ_m. The water conductivity of a drying sandy soil is lower than of clay. A logarithmic relation exists between unsaturated conductivity and volumetric water content (Stockton and Warrick, 1971). At a pF of 1 to 1.5, the differences observed between replicates (n) or between soils were negligible and the precision (n/s_x) high. In the fourth week, at pF = 2, the mentioned effect becomes evident (Fig. 5). During the dark period ψ_m is in equilibrium. At the start of the light period the deviation from the set point increases rapidly and the system reacts by rising the water table until current LL(t) $\leq$ LL(t-1). During the fourth week, when the peas were growing vigorously, more than 1 l of water was used per day per tank, a high amount relative to the pore volume of the soil. In the growth chamber, site dependent differences in evapotranspiration occurred, causing some loss of synchrony in the ψ_m patterns of the replicates (Fig. 5b/d). Nevertheless, the accuracy of the system is high,

i.e. the quotient: (X -SP)/SP, with X for the average of registered pF values, is about zero. In most of the examined soils, the ψ_m remained fairly constant with a maximum amplitude of 0.4 pF units.

Variability of disease development. In terms of pea root rot development in the mini-pots, the results (Table 3, 4 and 5) indicate that replicates were fairly equal, despite variation in site. Further reduction in the number of replicates can be considered.

Cross contamination within tanks. Miniaturization, with mini-pots as sub-units within a tank, implies a larger risk of cross contamination. The results point to the absence of such contamination. We attributed this to the unidirectional water flow, the relative immobility of root rot pathogens (Miller and Burke, 1974; Rush and Kraft, 1986), and also to the short duration of the test. The use of small pots reduces the test period because root rot symptoms develop faster than in large pots (Muadewesi and Lockwood, 1976).

Effect of ψ_m on pea root rot. In natural soils, an increase in *Fusarium* root rot under dry conditions was reported (Cook and Papendick, 1972). However, in our experiments, pea root rot in natural soil, caused by Fsp in combination with other root rot pathogens, increased with increasing ψ_m (Fig. 6). It seems important to know whether pea root rot is caused by Fsp alone or by Fsp in combination with other root rot pathogens. To investigate the effect of ψ_m on infection by Fsp alone, tests were carried out in sterilized soil. The results show that infection by Fsp alone is always severe and that the ψ_m regime has little effect in the absence of other biotic soil factors. Only at pF = 1, Fsp root rot severity was significantly higher, possibly because of limited aeration.

Our results in sterilized soil may not be in contradiction to the results reviewed by Cook and Papendick (1972). In natural soil, optimum germination of Fs propagules occurs at high humidity. In some soils, the higher the soil humidity the more the germination tube is affected by the antagonistic microflora (Cook, 1981). Our results show that, if biotic factors are eliminated, Fsp infects at a relatively high soil water content.

Soil temperature control. Even if the equipment was used at its maximum capacity of 100 tanks, the variation between replicates and the amplitude in soil temperature were quite small. This implies a great improvement in temperature control as compared to the cooling units used earlier (Oyarzun and Dijst, 1991). The type of soil did not affect the accuracy of control. Soil temperature could be controlled with high precision, probably due to the individualized flow of cooling fluid. Initially, because of the pressure needed to circulate the cooling liquid, tank walls tended to be deformed. By a free flow of the liquid into a common buffer the problem was solved. Undesirable condensation of water on the walls of the tanks was observed when they were cooled below the dewpoint.

Comparing systems. Several systems have been developed to grow plants under constant conditions, such as mist chambers (Smucker and Erickson, 1976), ceramic irrigation cups (Read et al., 1961), ceramic plates (Miller and Burke, 1974; Perroux, 1979) and osmotic systems (Williams and Shaykewich, 1971). A system based on PEG solutions separated by a semi-permeable membrane was proposed for study of root rot diseases (Wisbey et al., 1977). However, such a membrane can be a substrate for micro-organisms, cracks easily, and the contact with the soil deteriorates by changes in evapotranspiration and by shrinking or swelling of the soil. The system developed by Perroux (1979), in which

water under pressure, flows to the soil through a double wall consisting of ceramic plates, seemed attractive. However, we considered it inadequate for our phytopathological experiments since (i) in such a system roots tend to grow along the surfaces of the walls (ii) the system is rather difficult to clean, and (iii) it is expensive to discard contaminated parts. While experimenting with ceramic plates to irrigate soil, we observed that the contact between soil and plate was disrupted because the evapotranspiration demand of the plants rapidly exceeded the water conductivity of the plates.

A constant soil temperature has often been achieved using the classical Wisconsin tanks, a water bath with controlled temperature in which subtanks are submerged. Such a device was also used to assess disease (Benedict, 1968) but control of ψ_m was always done manually. Our equipment, with the test unit based on the system of Snow and Tingey (1985), allows a free manipulation of the tanks, automatic control of ψ_m and individual regulation of soil temperature per tank.

The advantage of our equipment is that it (i) accurately differentiates IPS, between soils (Table 3), (ii) is relatively simple to operate, and (iii) does not require work after the start of the experiment. All parts in contact with soil and pathogen can be cleaned completely or they are cheap enough to be discarded. The latter applies to the foam, the mini-pots, and the ceramic cups of the tensiometer.

From our results we conclude that the equipment (i) successfully controls ψ_m in different soil types, (ii) works with relatively small amounts of soil and (iii) allows elements of the test unit to be handled individually. The demands of constancy and reproducibility were successfully met.

For its use in a drier water potential range (pF > 2), the equipment needs some modification. Mini-tensiometers can be replaced by plastic ceramic blocks and the algorithm for water dosage modified. Lower transpiration demand by choice of other plant species or more humid climate conditions would contribute to maintain ψ_m in dynamic equilibrium for a longer time.

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Chapter 8

Determination and analysis of soil receptivity to *Fusarium solani* f.sp. *pisi* causing dry root rot of peas

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Abstract

A procedure was developed to differentiate field soils according to their receptivity, ranging from suppressive to conducive, to *Fusarium solani* f. sp. *pisi* (Fsp), one of the most important soil-borne fungi causing dry root rot of pea. Experiments were carried out using samples of natural soil collected from commercial fields which had a low inoculum potential of root rot in peas in previous years. In bioassays, with computer-controlled soil water potential, light intensity, air temperature and relative humidity, dry root rot severity responses to a range of inoculum levels were determined. Disease severity on different soil samples, at the same infestation level, showed that soil as a substrate strongly affects the inoculum potential of Fsp. In selected samples which only produced dry root rot, the amount of native *Fusarium* in pea rhizosphere soil was uncorrelated with root rot severity. Univariate and multivariate models were examined for their adequacy to describe and compare disease response data. Principal component analysis carried out on data of sample by Weibull fitted disease responses or on data of sample by average disease responses, produced a similar receptivity order ($P=0.01$) of the samples, but fitted values increased the quality of ordinations. Cluster analysis followed by a canonical variate analysis permitted to classify the tested soil samples in groups which significantly differed ($P=0.05$, based on χ^2) in soil receptivity for Fsp. The value of this technique for further ecological research is discussed.

Additional keywords. Bioassays, suppressive soil, conducive soil, disease reducing soil, inoculum potential, multivariate analysis, soil-borne pathogens.

Introduction

Previous field research on root rot of dry peas in the Netherlands showed that the cropping frequency of peas or legumes was poorly but significantly correlated with the intensity of root rot (Oyarzun et al., 1993b). The question was posed whether such a weak association with cropping frequency could possibly be explained by differences among fields in the degree of suppressiveness to the soil-borne pathogens of peas.

The role of the soil environment in modifying disease was illustrated by the existence of suppressive soils (Baker and Cook, 1974). The recognition that soil factors affect the intensity of disease caused by soil-borne pathogens stimulated research on this subject. Alabouvette et al. (1982) proposed the term *soil receptivity* (SR) to describe soils in their effect on inoculum potential, ranging from suppressive to conducive to a plant disease. Alabouvette (1986, 1990) stated that every natural soil has some potential to reduce disease. Therefore, SR is part of *inoculum potential* as defined by Garret (1970) when 'the energy available for infection of a host at the surface of the

infection-court' is affected by the biotic and abiotic environment.

Much research on the theme was directed to *Fusarium oxysporum*, agent of wilt disease in several crops (Louvet et al., 1976; Rouxel, 1978; Tramier et al., 1979; Cook, 1982; Alabouvette et al., 1982; Sneh et al., 1987). Other pathosystems were also studied (Wijetunga and Baker, 1979; Rouxel and Briard, 1988; Tivoli et al., 1990). No information was available on SR to root rot of pea, but some reports on SR deal with the same pathogenic species on other crops (Stutz et al., 1985; Lechappe, 1986) Little attention was given to quantification of SR, construction of a measure of receptivity for comparison of SR between soils and investigation of factors responsible for differences in SR (Corman et al. 1986; Doublet, 1986; Doublet et al., 1988).

The objective of this study was to develop procedures, including bioassays, to differentiate field soils according to their SR to root rot of pea. The most important root rot pathogens in dry pea, *Fusarium solani* (Mart.) Sacc.f.sp. *pisi* (Jones) Snyder & Hansen (Fsp), one of the dry root rot pathogens, *Thielaviopsis basicola* (Berk. & Br.) Ferraris (Tb) causing black rot and *A. euteiches* Dreshs (Ae) causing soft rot, were examined separately. This paper is limited to Fsp. We describe SR and compare methods to analyze receptivity data, we rank the tested soils according to their degree of SR and we classify them into groups differing significantly in SR.

Materials and Methods

Selection of fields, soil sampling. Fifty fields, mostly with commercial crops, were selected in which root rot had not developed or in which its occurrence had been slight (maximum root disease index (0-5) at flowering = 1.8) in previous investigations during 1986 and 1987 (Oyarzun, 1993). Five 'fields ' were part of the same experimental field at the Research Station for Arable Farming and Field Production of Vegetables (PAGV) but differed in the crop species grown in monoculture for the last 10 years.

During late autumn 1990 and winter 1991, all soil samples were collected after ploughing. Each field was two to three hectares in size. Excluding 10 m wide borders, soil samples were collected by taking 100 sub-samples of 250 mm depth with an auger, following a W-path through the field. Per field, the sub-samples were bulked to yield one soil sample, briefly 'soil' of about 100 kg. Soils were stored in plastic bags at 5 °C until further processing. Wet samples were gently dried by exposure to ambient air. When adequate moisture content was reached, samples were crumbled and passed through a sieve of 5 mm. If frozen, samples were crumbled and sieved prior to drying.

Natural Fusarium infestation level of the soil samples. The presence of *F. solani* and other fusaria in the samples was determined in the rhizosphere soil of pea cv. Allround, grown for 21 days on 27 of the samples, using plate dilution methods. Selective 'fusarium-media', SFA (Burgess and Liddell, 1983) or PPA (Nash and Snyder, 1962), were used to count the number of viable *Fusarium* propagules in soil or root macerate suspensions. Plates were incubated at 24°C in dark (SFA) or under NUV, λ = 365 nm (PPA). Counts were made after one week of growth. Doubtful cases were transfered to CDA and PDA for further identification. No attempts were

made to differentiate pathogenicity between the *Fusarium* isolates found.

Reference soil. To allow for comparisons between separate experiments, the same heat-sterilized soil was tested in all experiments, as a reference for experimental conditions along with the arable soils. The reference soil was a light clay soil, obtained from a field with good agronomic properties and originally highly contaminated with pea root pathogens. The soil was partially sterilized by heat treatment of 104 °C for at least 5 hours, eliminating pea root rot pathogens and other microflora (Van der Spek, 1967).

The soil receptivity test

To assay the effect of SR of each soil sample, the severity of root rot was determined in a susceptible pea cultivar over a range of inoculum densities of the pathogen, under standardized conditions (Rouxel, 1978, 1991).

Test pathogens. The inoculum of Fsp consisted of a mixture of conidia of three highly virulent isolates, Fs48, Fs04 and Fs14 (Oyarzun et al., 1993a). Monospore cultures of these three isolates had been conserved on Carnation Leaf Agar, CLA (Fisher et al., 1982). To obtain conidia, cultures on CDA were placed under 12 hrs NUV at 24°C. After 21 days, the conidia were washed from the agar surface and collected in 10 ml sterile demineralized water per plate. The mixture of conidia from the different isolates was filtered through a double layer of cheesecloth and conidial density was determined.

Treatments. From each sample, sub-samples were infested with either 10, 100, 1.000, 10.000 or 50.000 conidia per g dry soil, resulting in treatments further referred to as D1, D2, D3, D4, and D5, respectively. As a control, a non-inoculated sub-sample (D0) was treated with sterile demineralized water only. The inoculum suspensions were atomized into the soil using an air pressure of about 0.3 bar under continuous rotation of the soil in a plastic bag. After infestation, the samples were placed in dark at 5°C during 48 hrs until further use to obtain equal distribution of water through the soil sample.

Test plants. High quality seed of *Pisum sativum* cultivar Allround (70-75 mm diameter) was surface disinfected in 5% NaClO for 10 min and subsequently rinsed thoroughly in sterile demineralized water.

Experimental conditions. Black plastic mini-pots of 4 x 4 x 12 cm (w x l x h) were carefully filled with test soil. Four pea seeds were sown in each pot at 2 cm depth. Water was gradually added to the pots up to approximately field capacity. During germination temperature was kept at 20 °C and pots were covered to avoid water - losses. One day after emergence, the mini-pots were placed on top of a block of florist foam (Smithers-Oasis) in 4 x 24 x 32 cm test tanks. Each test tank contained 6 mini-pots representing the six treatments of one soil sample. The test tanks were part of a computerized system in a phytotron (Fig. 1) which automatically regulated soil water potential (Oyarzun et al., 1994). Light intensity, relative humidity and temperature were adjusted automatically to 90 $W.m^{-2}$ during 12 $hours.day^{-1}$, 80% RH and 22/18 °C day/night, respectively. During the first, second and third week after germination, soil water potential was adjusted to pF 1.0, 1.5, and 2.0, respectively. Soil water potential was monitored in the non-inoculated objects by electronic mini-tensiometers.

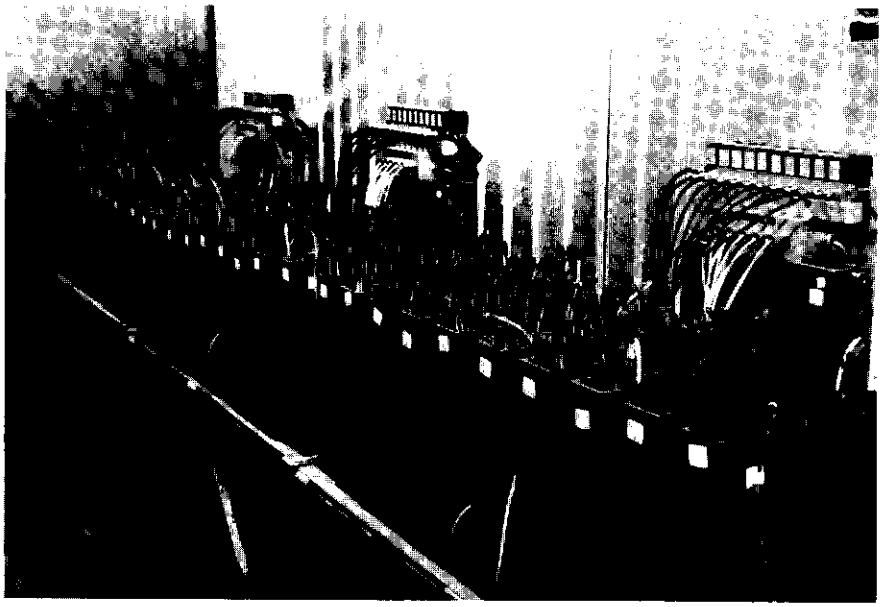

Figure 1. The equipment to test soil receptivity to *Fusarium solani* f.sp. *pisi*, causing dry root rot of pea, under standardized conditions.

Experimental design. Objects were placed in a block with soil samples as plot (tanks) and treatments (mini-pot) as subplots and replicated 4 times. The experiments had a split-plot design. A maximum of ten soil samples could be examined at each bioassay.

Disease assessment. After 3 weeks of growth in the tanks, plants were uprooted. Roots were cleaned with tap water and disease severity was assessed. The root rot disease index (DI) (0 = health, 5 = 100% necrotic root or dead plant) was calculated as the weighted average of scores of the affected cotyledon, epicotyl, roots and xylem (Oyarzun, 1993).

Inoculum potential of natural soil

The value of the root rot severity of non-inoculated objects (D0), due to infection by indigenous pathogenic fungi, was considered to be an estimate of the standard inoculum potential (IPS) of the soil (Mitchel, 1979).

Statistical differentiation of SR

To assess the degree of SR, the data on disease expression from the SR-tests of the field soil samples and the sterilized reference soil were statistically analyzed.

Comparing disease responses. Differences between soil samples in root rot severity over the range of infestation doses were examined by ANOVA in a conventional split-plot analysis and effects were separated by the LSD at $P=0.05$. Analysis of this type of data, where each sample originates from one single field without replicates, is valid

on sample level (Parkinson et al., 1971).

Parallel curve analysis (Digby et al., 1989) was used to investigate differences in disease responses of five soil samples originating from different monocultures.

Generating parameters to express SR. In search of parameters representing differences in disease response curves and SR between soils, a number of biologically meaningful models were tentatively fitted by numerical methods. Among others, Gompertz, logistic, exponential, and Weibull models (Hau and Kranz, 1990) were tested. In fitting the Weibull model (equation 1), the disease index was first converted into a 'health index' (HI= 5 - DI) and initial parameter values were obtained by logistic regression.

Equation 1.

$$HI = A * e^{-(Log(dose)*B^{-1})^{C}}$$

Where:

A = HI without artificial infestation.

B = Scale parameter. Log(dose) needed to reduce A to (e^{-1}) of its value.

C = value determining the shape of the response curve.

In this form of the Weibull model (Rawlings et al., 1985) the higher the values of the parameters, the lower IPS and the disease response to soil infestation. If C = 1, the Weibull model becomes the exponential model.

Models enable the calculation of fitted values of disease responses and parameter values which can be tested for their adequacy to characterize SR.

Characterization of SR by ordination. In search of the best procedure to assess SR, disease data were analyzed by Indirect Gradient Analysis techniques (IGA) such as Polar Ordination (PO), Principal Component Analysis (PCA) and Correspondence Analysis (CA).

For ordination, disease responses were represented by either (1) the abundances of plants in the five disease classes (the plant abundance per disease class was obtained by sorting individual root rot scores in five classes, (0-1), ..(4-5)), (2) the average disease response per inoculum dose or (3) the fitted values of disease responses obtained with the Weibull model. Plant abundance was compared by PO, using percentage dissimilarity (Ludwig and Reynolds, 1988) and by CA.

The average disease responses per soil*inoculum dose combination and the Weibull fitted disease responses were examined by PCA. In PCA, severity per dose was considered as an individual variable. So, six variables were considered: D0 = IPS = disease response on non-inoculated samples and D1, D2, D3, D4 and D5 = disease responses of the five inoculum densities. First, as required for PCA, the linear relationship between variables was confirmed. Then, PCA was carried out on a matrix of the sums-of-squares-and-products (SSPS), or variance-covariance.

Results of ordinations were compared with parameter values obtained after fitting data with the Weibull and the exponential model.

Clustering the soil samples in SR groups. Clustering among samples was examined

on a matrix of similarities using fitted values of disease responses by means of the average linkage method. The coefficient of similarity, S_{ij} of the *i*th and *j*th sample was calculated according to Digby et al. (1989), equation 2.

$$S_{ij} = \frac{1}{p} \sum_{k=1}^{p} S_{ij'k} \qquad \text{with:} \qquad S_{ij'k} = 1 - \left(\frac{x_{ik} - x_{jk}}{r_k}\right)^2$$

x_{ik} = disease response with inoculum dose k= D0...D5; r_k = range of k

The consistency of the clusters was examined by Canonical Variate Analysis (CVA) on a matrix of groups by fitted disease responses.

The multivariate analyses were carried out using procedures in CANOCO (Ter Braak, 1987b), Statistical Ecology (Ludwig and Reynolds, 1988), GENSTAT 5 (Digby et al., 1989) and Statistix (Anonymous, 1985). Biplots were constructed by using facilities of CanoDraw 3.00 (Smilauer, 1992).

Results

Selection of fields. Forty six field soils were tested for their SR to Fsp. Fifteen of these soils were discarded from further analysis because of severe natural infestation with root rot. The remaining 36 data sets used in further analysis consisted of 31 field soils and the reference soil, tested in each of the five experiments (Table 1).

Comparing disease responses. Data of the response curves of the 36 samples tested (including 5 times the reference soil) are summarized in Table 2. On the reference soil, the root rot severity of plants always quickly reached high values at D1 and D2. On the soils from arable fields, the SR-test generated a great diversity of root rot responses to the increasing infestation levels, D1-D5, (Fig. 2A).

For each of the five experiments, the results from ANOVA indicated that the disease severity per infestation level significantly ($P \leq 0.01$) depended on the inoculated soil sample (Table 2). Since dosage is a quantitative factor, its total sum of squares(SS) could be partitioned into linear, quadratic and higher components. The SS for interaction between soil sample and dose was partitioned accordingly. Significant interaction components were found between soil sample and linear, quadratic and higher order terms of dosage.

Some field soils reacted as conducive as the sterilized reference soil, whereas in others the disease was strongly reduced, even at the highest inoculum level. In some samples, even at D5 (50.000 conidia g^{-1} soil added) root rot severity remained significantly lower than the disease severity in the reference soil at D1 (10 conidia g^{-1} soil added). Absolutely suppressive soils sensu Baker and Cook (1974) were not found.

Table 1. Characteristics of the soil samples tested for SR to *Fusarium* f.sp. *pisi*: soil type, humus class, gravimetric water content, soil density, root rot severity, and population density of *F. solani*, *F. oxysporum* and *Fusarium* spp. in pea rhizosphere soil.

code	soil type[1]	humus class[2]	%H_2O [3]	S.d. [4]	DI [5]	F.s[6] 10E^4	F.o[6] 10E^4	F.spp[6] 10E^4
as	lcl	lh	29.0	1.1	0.5	0.88	0.01	0.01
ag	lcl	lh	28.1	1.1	0.8	0.01	0.01	0.01
bl	lcl	lh	23.9	1.1	1.0	0.01	0.84	4.30
bk	lcl	lh	32.0	0.9	0.8	1.71	0.01	0.60
gv	lcl	lh	24.6	1.2	0.6	nd	nd	nd
ha	lcl	mh	32.2	1.0	0.8	2.16	0.73	0.01
jg	hcl	mh	45.0	0.9	0.9	nd	nd	nd
jn	hcl	lh	38.0	1.0	0.9	2.88	0.01	43.00
ja	hcl	lh	31.1	1.1	0.7	0.01	0.01	3.80
kh	vllo	lh	24.3	1.2	1.3	1.76	2.33	0.01
lf	sa/pt	sh	39.0	1.0	0.4	0.01	0.66	0.70
ls	hlo	lh	25.2	1.1	0.5	0.17	0.01	0.70
lu	hlo	lh	23.6	1.0	1.1	4.00	0.01	8.50
mu	lcl	lh	33.6	1.0	0.6	0.01	0.61	0.70
rc	hlo	lh	29.7	1.1	0.9	1.00	0.01	0.01
rg	hcl	lh	36.0	1.0	0.9	0.01	0.63	0.01
jr	hcl	lh	33.3	1.0	1.0	nd	nd	nd
rh	lcl	lh	30.0	1.1	0.5	nd	nd	nd
ro	hlo	lh	28.9	1.0	1.3	6.50	4.98	8.30
dm	vllo	lh	23.4	1.1	1.2	nd	nd	nd
tr	llo	lh	22.0	1.3	0.6	14.83	3.55	2.20
to	llo	lh	22.0	1.3	nd	2.18	0.01	0.90
ve	hlo	lh	23.7	1.1	1.2	1.88	3.13	0.70
wa	llo	lh	25.8	1.1	0.9	0.01	2.10	6.40
wi	llo	lh	22.5	1.1	0.6	0.01	0.84	3.00
zu	hcl	lh	43.2	0.9	0.8	0.01	0.70	1.80
cv	hlo	lh	26.0	1.2	*	7.17	0.01	1.10
cb	hlo	lh	26.0	1.2	*	0.70	0.01	0.10
cf	hlo	lh	26.0	1.2	*	7.38	2.50	0.01
cm	hlo	lh	26.0	1.2	*	0.01	0.01	0.01
co	hlo	lh	26.0	1.2	*	0.10	0.01	0.10
re	hlo	lh	26.0	1.2	4.0	8.40	1.21	3.30

[1] Soil type: sa = sand; vllo = very light loam; llo = light loam; hlo = heavy loam; lcl = light clay; hcl = heavy clay; pt = peat.
[2] Humus class: lh = lightly humic; mh = moderately humic; sh = strongly humic.
[3] %H_2O: gravimetric water content of the soil sample at water holding capacity.
[4] S.d.: soil density used in the bioassay (g * cm^{-3}).
[5] DI: Root rot severity at flowering in the last pea crop: 0= no necrosis, 5 = 100% necrotic roots; * = No peas grown on these fields.
[6] Populations of *F. solani* (F.s), *F. oxysporum* (F.o) and *Fusarium* spp. (F.spp) (10^4 cfu*g^{-1} dry soil) in pea rhizosphere soil; nd = not determined.

Table 2. Root rot severity in one month old pea plants grown on natural field soil samples after infestation with increasing amounts (D1..D5) of inoculum of highly virulent isolates of *F. solani* f.sp. *pisi*, DO = not artificially infested (IPS).

Experiment nr.	Soil code:	IPS	Inoculum doses							
		DO	D1	D2	D3	D4	D5	S*D[1)]	LSD1[1)]	LSD2[1)]
I	bl	1.85	2.22	2.20	2.42	2.80	3.13			
	jg	2.72	3.17	3.40	3.55	3.67	4.43			
	ja	1.88	1.73	2.50	3.63	3.63	4.18			
	rh	1.92	2.17	3.00	3.10	3.85	4.13			
	zu	2.63	3.22	4.07	4.70	4.82	4.95			
	cm	0.22	0.73	2.30	2.92	3.90	4.05			
	rl	0.43	4.75	4.88	4.93	5.00	5.00	***	0.64	0.67
II	lf	0.75	1.75	3.10	4.30	4.78	4.80			
	tr	1.35	1.45	1.98	2.83	3.63	3.88			
	to	1.48	2.15	2.70	3.13	4.35	4.65			
	cv	1.08	2.38	3.35	4.72	5.00	5.00			
	cb	1.30	1.73	2.15	3.30	3.63	4.13			
	cf	0.25	0.85	1.30	2.63	2.85	3.28			
	co	0.98	1.55	1.98	3.13	4.60	4.82			
	r2	0.47	2.22	3.67	4.35	4.40	4.72	***	0.57	0.63
III	jn	1.35	1.92	1.88	2.61	2.78	3.28			
	kh	1.77	2.08	2.42	2.53	3.25	3.35			
	rg	1.52	1.55	1.88	2.50	2.83	3.13			
	jr	1.88	1.98	2.22	2.53	2.80	3.15			
	ro	0.70	0.98	1.20	2.05	2.67	2.67			
	wi	0.90	1.70	1.90	2.08	2.63	3.35			
	r3	0.03	2.47	4.32	4.55	4.65	4.78	***	0.48	0.53
IV	ha	1.27	1.50	1.27	1.48	2.17	3.05			
	mu	1.42	1.75	1.92	2.10	2.05	3.53			
	rc	1.08	1.02	1.20	1.17	1.48	2.85			
	dm	1.65	2.25	2.40	2.88	3.10	3.95			
	ve	1.05	1.10	1.02	1.15	1.77	2.58			
	wa	0.98	1.15	1.27	1.25	1.58	2.95			
	r4	0.00	4.40	4.80	4.95	5.00	5.00	***	0.37	0.42
V	as	0.98	1.02	1.38	2.05	2.60	3.38			
	ag	2.10	2.33	2.60	2.85	3.63	4.35			
	bk	2.45	2.78	3.22	3.70	4.13	4.65			
	gv	1.52	1.95	2.55	4.00	4.68	4.88			
	ls	1.00	1.25	1.15	1.50	2.17	2.45			
	lu	1.23	1.67	1.73	2.13	2.83	3.15			
	r5	0.15	3.65	4.13	4.40	4.55	4.88	***	0.50	0.58

[1)]. Per experiment nr., the interactions soil * dose (S*D) are significant at P=0.01 (***) ; Least Significant Differences at P=0.05: LSD1 for comparing disease severities at different inoculum doses per soil and LSD2 for all other comparisons.

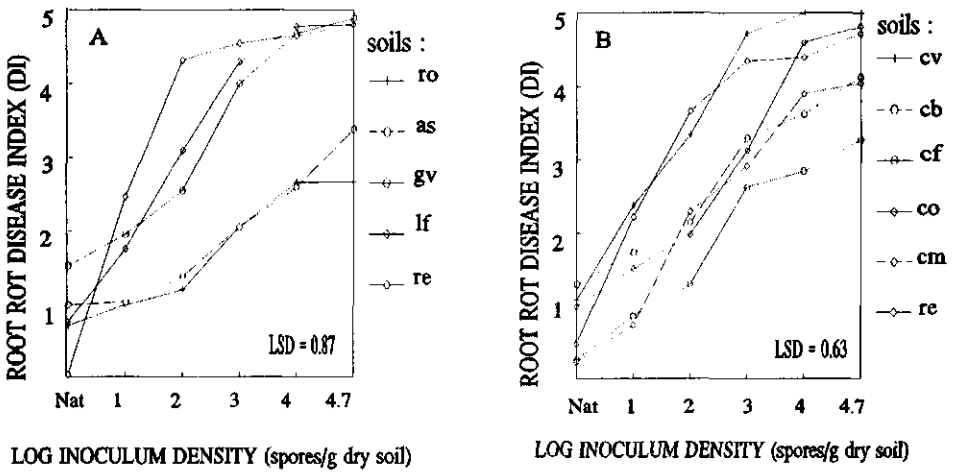

Figure 2. Root rot disease responses of pea to a range of increasing inoculum densities of *Fusarium solani* f.sp. *pisi*. A. Effect on four different natural field soils. B. Effect on five soil samples which originated from one field but differed in cropping history, monocultures of field beans (cv), *Phaseolus* beans (cb), flax (cf), onions (co) and maize (cm). In both experiments the same sterilized reference soil (re) was included.

Significant differences (P = 0.05) in the disease response curves, slope of disease progress and disease maximum, were found between the five soil samples originating from the same experimental parcel but differing in the crop species grown in monoculture during the last 10 years (Fig. 2B). Soil cv, from continuous cultivation of *Vicia faba* L., was the most conducive whereas in soil cf, continuous cultivation of *Linum usitatissimum* L., disease was most reduced.

Generating parameters to characterize SR. Curves of disease response to soil infestation varied in initial and maximum level, shape and rate. To quantify differences in responses curve parameters had to be determined. To this end, several models were examined. To compare and interpret responses, methods producing dimensionality reductions were also used.

Models with fixed shapes such as logit and Gompertz generally failed to fit the data. The Weibull and exponential models were more successful. With both models, the percentage of variance accounted for mostly exceeded 70%. The exponential model has a slope parameter (RX), whereas the Weibull model has two parameters to describe scale (B) and shape (C). Parameter values from Weibull and exponential modelling are presented in Table 3. With both models five soils had a rather low fit, variance accounted for being <65% (Table 3), mainly due to variability between

Table 3. Parameter values and percentages of variance accounted for by the Weibull and exponential models after fitting disease response to infestation of natural soil samples and sterilized reference samples with increasing doses of *F. solani* f.sp. *pisi* [1].

Soil	Weibull $HI = A*e^{-(L*B-1)^C}$				Exponential $DI = A + B*RX^L$			
Code	A	B	C	%VAR	RX	B	A	%VAR
as	4.03	4.99	2.46	92.0	1.50	0.43	0.48	91.9
ag	2.74	4.34	3.57	76.9	1.69	0.20	1.94	78.3
bl	3.01	6.79	2.13	39.9	1.40	0.30	1.65	40.9
bk	2.51	3.63	1.88	74.8	1.15	2.36	0.09	75.8
gv	3.39	2.87	2.71	94.0	1.04	20.6	-19.2	91.2
ha	3.66	5.08	5.83	82.5	3.15	0.01	1.31	81.9
jg	2.19	4.90	1.47	43.8	1.29	0.60	2.27	46.3
jn	3.59	6.19	1.41	79.0	1.12	2.70	-1.27	80.2
ja	3.33	3.86	1.75	74.1	1.09	5.50	-3.80	71.4
kh	3.23	5.86	1.53	69.1	1.16	1.69	0.08	69.9
lf	4.40	2.15	1.65	92.7	0.77	-6.26	6.88	91.0
ls	3.92	6.22	2.81	55.1	1.65	0.16	0.86	54.7
lu	3.63	5.65	2.10	58.3	1.33	0.67	0.62	59.0
mu	3.21	4.79	13.39	83.9	9.68	0.00	1.77	84.4
rc	3.89	4.93	11.08	77.4	8.75	0.00	1.10	77.5
rg	3.55	6.02	1.71	77.1	1.22	1.15	0.27	75.8
jr	3.13	6.94	1.75	80.0	1.30	0.53	1.33	80.1
rh	3.15	4.09	1.45	75.3	1.05	8.5	-6.60	75.4
ro	4.36	5.92	1.52	79.1	1.10	3.98	-3.37	78.0
dm	3.20	5.00	1.71	66.4	1.26	1.03	0.76	68.9
tr	3.71	4.15	2.04	88.6	1.20	2.10	-0.90	86.7
to	3.34	3.39	2.13	84.4	1.13	4.07	-2.55	86.2
ve	3.95	5.31	5.77	82.6	3.27	0.01	1.02	82.0
wa	3.85	4.92	10.34	73.2	7.02	0.00	1.14	73.4
wi	3.97	5.92	1.36	58.8	1.20	1.60	-0.50	61.3
zu	2.50	1.98	1.59	78.8	0.73	-3.21	5.76	77.3
cv	4.03	1.89	1.66	92.1	0.72	-5.38	6.38	91.7
cb	3.73	3.81	1.70	78.6	1.06	9.0	-7.80	77.9
cf	4.89	4.47	1.29	86.6	0.97	-21.0	21.1	86.0
cm	5.00	3.10	1.38	90.7	0.90	-10.7	10.7	89.4
co	3.79	3.25	3.22	92.8	1.20	3.1	-2.23	91.6
r1				nf	0.05	-4.53	4.95	96.8
r2	6.08	1.29	0.87	94.0	0.57	-4.62	5.05	93.9
r3	6.00	0.87	0.87	94.1	0.44	-4.97	4.94	93.7
r4				nf	0.12	-4.96	4.96	99.3
r5				nf	0.24	-4.44	4.60	94.6

[1] HI= Health Index; DI = Disease Index; L=(Log(dose)); nf = not fitted by the model.

replicates. The Weibull model failed to fit the data of three sterilized references because root rot increased more than 63% (B=0) at D1, i.e. when the percentage of healthy roots fell below 37% at D1. The exponential model fitted all data. However, as a result of its undeterminate upper limit, the change in shape from concave (RX<1) to convex (RX>1) changes the value of the asymptote (A) enormously, prohibiting its further interpretation.

Characterization of SR by ordination. PCA ordination carried out on a SSPS or a variance-covariance matrix of average disease responses, accounted for 80.6% of the variance by the first principal axis and 9.7% by the second one (n=36), 90.3% in total. Using Weibull fitted disease responses, the percentage of variance associated with the first two axes was 81.5% for the first and 13.6% for the second axis, a total of 95.1%, an increase of nearly 5% (n=33; Table 4). To visualize the relationships between variables and between variables and each axis, variable loadings were transformed into correlations (Table 4). Disease responses (D1-D5) are all highly correlated with the first principal axis, whereas D0 is the only variable highly correlated with the second axis. Besides, the second axis contrasted the responses associated to non- and low inoculum doses (D0, D1) with the high ones (D4, D5) indicating differences in disease rate (Madden and Pennypacker, 1979). Axis III, accounting for 3.4% of the variance, opposed the extreme responses (D0, D4, D5) to the central ones (D1, D2, D3) stressing curve shape differences between soils.
Axis IV and higher did not provide any further information ($P \leq 0.05$, χ^2).

Table 4. Percentage of the variance (P) accounted for by the first four principal axes and the correlation coëfficients of the disease variables (D0 - D5) with each axis after PCA of Weibull fitted disease responses.

	AXIS I	AXIS II	AXIS III	AXIS IV
P:	81.5	13.6	3.4	1.2
D0	- 0.22	- 0.94	- 0.25	0.04
D1	- 0.78	- 0.57	0.25	- 0.07
D2	- 0.96	- 0.10	0.25	- 0.03
D3	- 0.99	- 0.09	0.04	0.05
D4	- 0.97	0.14	- 0.13	0.12
D5	- 0.94	0.16	- 0.21	0.22

Figure 3 presents an ordination diagram of field samples, resulting from PCA on average disease responses without correction for IPS (D0), in a biplot using Euclidian distances (Ter Braak, 1987a). Fields are represented by asterisks and disease (variables D0-D5) by arrows. Arrows indicate the direction in which root rot severity increases. The first axis represents the overall increase of disease by infestation and the second axis the location of the response curve on the Y-axis, see Fig. 2. The variation in disease severity along the I-axis is the result of differences in the effect of the substrate (soil) on disease. Therefore the first PCA-axis can be regarded as a gradient of SR to Fsp. The value of the root rot severity on each soil can be assessed

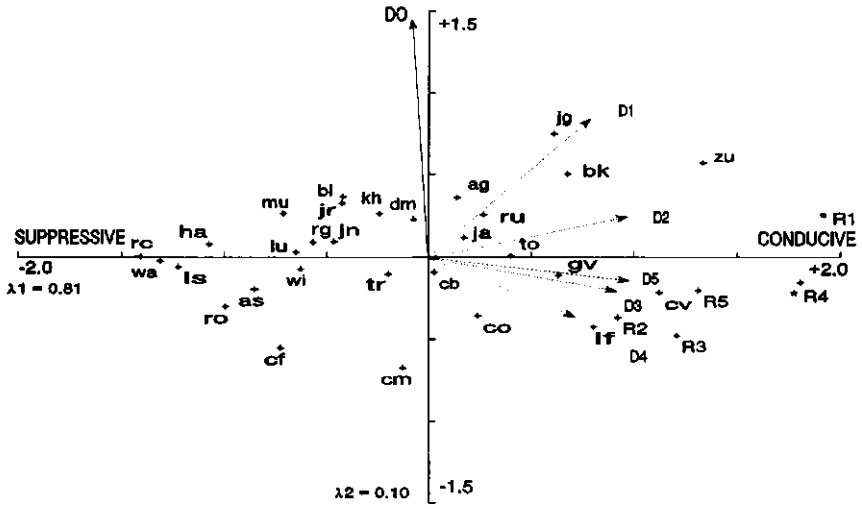

Figure 3. PCA biplot of SR data. Ordination diagram of the SR data with disease response variables represented by arrows and soil samples by asterisks. The biplot is constructed using Euclidian distances for optimal separation of soil samples. The direction of the arrows represents the direction in which the value of the response of the corresponding variable increases most and the length of the arrows equals the magnitude of the change in that direction.

Table 5. Order of soil samples according to their score on the first principal axis obtained by PCA on (Weibull) fitted root rot responses of pea to soil infestation with *Fusarium solani* f.sp. *pisi* before (A) and after correction (B) for IPS.

Suppressive ----------> conducive [I-axis; 81.5% var.]										
A:										
rc	ve	wa	ls	ha	ro	as	mu	cf	lu	wi
-3.0	-3.0	-2.9	-2.6	-2.2	-2.0	-1.6	-1.5	-1.4	-1.2	-1.2
rg	jn	bl	jr	kh	tr	cm	dm	cb	ag	ja
-1.0	-0.8	-0.7	-0.3	-0.2	-0.1	0.1	0.2	0.5	0.7	0.9
co	rh	to	jg	gv	bk	lf	r2	cv	r3	zu
1.0	1.0	1.3	1.8	2.0	2.0	2.3	2.4	2.9	3.1	3.5
Suppressive ----------> conducive [I-axis; 94.7% var.]										
B:										
mu	rc	wa	ve	ha	bl	ls	jg	jr	ag	lu
-2.4	-2.4	-2.3	-2.2	-2.1	-2.1	-1.9	-1.5	-1.3	-1.3	-1.2
rg	kh	jn	dm	as	bk	wi	ro	ru	ja	tr
-1.1	-1.1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.2	0.1	0.2
cb	to	zu	cf	co	gv	cm	cv	lf	r2	r3
0.6	0.7	0.7	1.2	1.4	1.5	2.9	3.4	3.7	4.5	6.2

by perpendicular projection of the sample point onto the arrows representing the variable (Ter Braak, 1987a). The natural IPS, i.e. disease severity at D0, runs almost perpendicular with respect to the first axis. Therefore the I-axis provides very little information on this parameter.

A SR order of the soils on the first principal axis in PCA of Weibull fitted values is presented in Table 5A. Soils with a high IPS (e.g. *zu, bk, jg* and *ag* in Table 2) were ordered in the same range as the more receptive ones (e.g. the reference soils *r2* and *r3*). When the value of the root rot disease severity of the non-inoculated object (D0) was subtracted from the disease severities at the increased infestation levels (D1-D5), the order of the soil samples was changed, especially the order of soils with a high IPS, Table 5B. Due to this correction the percentage of variance accounted for by the first principal axis increased to 94.7%.

Alternative ordinations. Analysis of soil samples according to plant abundance in disease classes by Polar Ordination (PO) using percentages of dissimilarity (Ludwig and Reynolds, 1988), produced an ordination strongly folded on the second axis. Correspondence Analysis (CA) performed on the same data yielded a poor separation of the variables on the first axis ($\lambda = 0.40$) and a cumulative percentage of variance accounted for by the first axis of 62.4. Furthermore samples showed some Arch effect. Detrended Correspondence Analysis (DCA) by segments confirmed the small gradient represented by the first CA axis ($S_x = 1.0$). Therefore, ordination of soils by CA was considered less appropriate to order this set of data (Ter Braak and Prentice, 1988; Ludwig and Reynolds, 1988). In fact a linear relation was found by pair-wise plotting of disease responses, giving a good reason to use PCA.

Comparing SR assessments. PCA ordination of non-fitted and Weibull fitted disease responses. Similar ($r = 0.94$) scores of the soil samples along the PCA first principal axis were obtained before and after Weibull fitting of the disease responses, GI and WI (Table 6). Using fitted data instead of non-fitted data, the percentage of variance associated with the first two axes increased by 5%, whereas the meaning of the configuration did not change. Furthermore, the second axes, GII and WII, which represent differences in disease response due to natural infestation, were almost perfectly correlated ($r=0.99$). The value of the natural IPS did not correlate with the first axes but it correlated highly with the second principal axis ($r=0.91$, $r=0.90$). In conclusion, Weibull fitting of disease responses improves the ordination by PCA.

Comparing PCA ordination with Weibull parameter values. The SR order produced by the first PCA-axes correlated well with the values of the Weibull scale parameter B ($r = 0.80$ and -0.75, $n = 33$). Multiple regression showed that by adding the location parameter A and shape parameter C the relation improved significantly (R^2-adjusted = 0.78 and 0.85; $P \leq 0.05$). The correlation between the first axes and the Weibull parameters was mainly caused by variation in parameter B which represents differences between samples in the scale of the disease response by inoculum doses (Table 7). The PCA first axis alone represents the same aspects of variation as the combination of the B and C Weibull parameters (scale and shape). Therefore, PCA ordering is simple and satisfactory to differentiate SR.

Table 6. Simple correlation coëfficient matrix (A) and Spearman rank correlation matrix (B) of the first two principal component axes created by PCA on average disease responses (GI, GII) and Weibull fitted disease severities (WI, WII), the parameters of the Weibull model: A, B, C, the exponential model slope parameter RX, and the values of the disease severities on not artificially infested samples (IPS) (n = 33; r= 0.325, P ≤ 0.05).

A: Simple correlation coëfficient:

	GI	GII	WI	WII	A	B	C	RX	IPS
GI	-								
GII	-0.05	-							
WI	-0.94	-0.07	-						
WII	0.10	-0.99	-0.00	-					
A	-0.09	0.86	0.01	-0.87	-				
B	0.80	-0.37	-0.75	0.41	-0.41	-			
C	0.42	-0.10	-0.54	0.18	-0.11	0.17	-		
RX	0.45	-0.13	-0.55	0.19	-0.10	0.22	0.98	-	
IPS	-0.10	-0.91	0.21	0.90	-0.91	0.24	0.03	0.02	-

B: Spearman rank correlation:

	GI	GII	WI	WII	A	B	C	RX	IPS
GI	-								
GII	-0.04	-							
WI	-0.94	-0.02	-						
WII	0.05	-0.99	-0.01	-					
A	0.15	0.90	-0.19	-0.89	-				
B	0.75	-0.38	-0.73	0.37	-0.21	-			
C	0.35	-0.23	-0.48	0.27	-0.26	0.22	-		
RX	0.71	-0.41	-0.73	0.45	-0.30	0.66	0.78	-	
IPS	-0.18	-0.89	0.23	0.88	-0.94	0.15	0.24	0.24	-

Table 7. Significances (t-student value = 2.09, df=29; n=33) and importance of the Weibull parameters A, B and C after partitioning variance (v.r), in relation to the order given by the first principal axis obtained by PCA on fitted disease responses.

	t-student	v.r	P
A	- 5.4	0.2	0.05
B	-10.7	134.8	< 0.01
C	- 6.2	37.9	< 0.01

The parameter RX of the exponential model was little correlated with the first PCA-axis and uncorrelated with parameter B of Weibull. However, it gave an almost perfect correlation with the shape parameter C of the Weibull model (Table 6A). Spearman rank correlation produced a considerable improvement of the association of RX with the PCA axes and Weibull's scale parameter B (Table 6B). Since this ranked RX only represents a partial aspect of disease response variation, the ordering of soils for SR by PCA is preferred.

Clustering soils in SR-groups. Cluster analysis was carried out by average linkage on a matrix of similarities between soils based on Weibull fitted disease responses. At 95 % similarity five groups were formed (Fig. 4). One of the groups contained only three soil samples with high IPS. This group was added to the group which neighboured it in the PCA receptivity gradient. Following the disease responses of the samples in each group, the soil groups were named 'Strongly reducing' (Str), 'Moderately reducing' (Mrd), 'Slightly reducing' (Srd) and 'Conducive' (C).

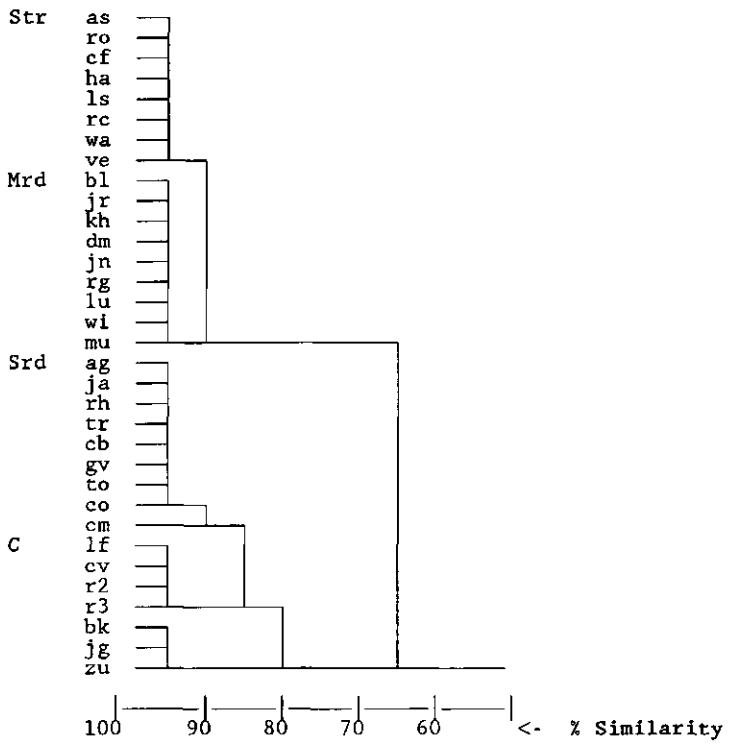

Figure 4. Cluster analysis of SR data. Dendrogram from Linkage Cluster Analysis on similarity coefficients of different soil samples (n = 33) obtained from (Weibull) fitted values of disease responses. Clusters: Str = strongly reducing; Mrd = moderately reducing; Srd = slightly reducing ; C = conducive.

Canonical variate analysis (CVA), a variant of discriminant analysis, carried out to investigate differences between groups showed maximum separation on two dimensions ($P \leq 0.05$, based on χ^2; $\lambda_1 = 11.6$ and $\lambda_2 = 1.35$, respectively representing the ratio of between-groups to within groups variation). In constructing Figure 5, Mahalanobis distances between the means of each group were adjusted for variation within groups and scaled in such a way that the canonical variate space within each group is the unity in all directions (Digby et al, 1989). A 'multivariate normal distribution' of data, i.e. soil samples normally distributed in a six-dimensional space,

was asssumed. A 95% confidence area could be drawn for sample scores with a radius equal to the squared root (SQR) of the χ^2 value at 95% with two degrees of freedom (axes), in this case 2.45. The circles drawn in Figure 6 include 95% of the soil samples in each group. This area is the confidence interval for the canonical mean, a point in the cluster representing minimal internal variance. The confidence interval for the means of the group equals 2.45/SQR(n), (n = number of soil samples in the group). The groups C, Srd and Str were well separated, but Mrd overlapped with Str (Fig. 5). The canonical means differed significantly.

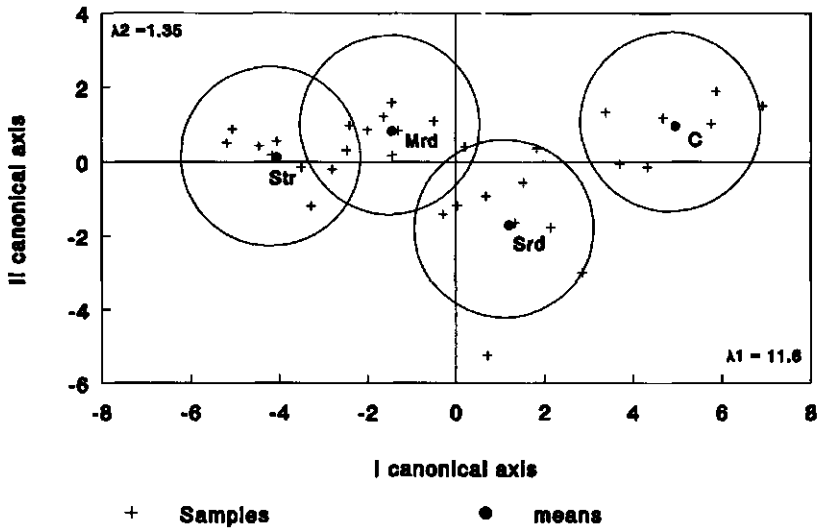

Figure 5. Canonical variate analysis. Plots of the first two discriminant function axes and the canonical mean of four groups of soil receptivity to *F. solani* f. sp. *pisi*. Axis units are expressed in units of standard deviation. Canonical means of groups: Str = strongly reducing; Mrd = moderately reducing; Srd = slightly reducing ; C = conducive. (n =33).

Relationship between IPS, SR and indigenous Fusarium spp in soils

The ranked values of these variables were compared by Spearman rank correlation. Analysis showed no significant association between the natural IPS of the soil samples, the degree of soil receptivity, expressed as rank on the first principal axis, and the amount of propagules of *F.oxysporum* and *Fusarium* spp. present in roots or rhizosphere soil. The lack of correlation between IPS and SR had been revealed earlier by values in PCA. The amount of *F. solani* in pea roots, however, was positively correlated with suppressiveness and inversely correlated with IPS (P = 0.05, n = 27).

Discussion

Qualitative differences in SR. The results show that the root rot disease responses, induced by infestation with Fsp, strongly differed between the soil samples. According to the definition of SR by Alabouvette et al. (1982), this variation in disease response means that differences in SR to Fsp in pea exist.

Production of disease responses. To assess differences in soil receptivity, the methods used for collecting disease data are crucial. Standardization of the environmental conditions, artificial infestation and careful experimental design are needed. SR for diseases caused by soil-borne plant pathogens has been investigated in several ways, usually with limited standardization of climatic conditions. To compare levels of SR, bioassays were carried out in a phytotron with standardized light intensity, temperature, and humidity of both soil and air (Oyarzun et al., 1994). Spore suspensions were used to avoid mixing substrates, as this may affect SR.

Corman et al. (1986) explored disease evolution in time, starting with different infestation levels and Perrin (1987) did so with a single fixed initial inoculum level. Rouxel and Regnault (1985), Rouxel and Briard (1988), Lucas et al., (1989) and Sarniguet et al., (1992) investigated disease responses to a range of artificially increased infestation levels in soils, determined at one time after planting. Our choice to test SR to Fsp according to the latter procedure was based on the consideration that the assessment of root rot severity is a destructive procedure. Mini-pots were used to obtain a complete exploration of the soil by roots, maximizing encounter of infection courts and pathogen, and thus permitting maximum expression of an inoculum potential. Testing time was kept as short as possible to avoid unfavourable root environment caused by over-rooting in the pots.

Differentiating SR. In characterizing SR, the problem is to distinguish between disease response curves in a variable soil environment. To this purpose disease responses were linearized (Wijetunga and Baker, 1979), or parameters obtained by fitting data, among which areas under the disease response curve, were analyzed by principal component analysis (PCA) (Campbell et al., 1980a) or cluster analysis (Campbell et al., 1980b). Rarely multivariate analysis had been used to characterize SR (Doublet et al., 1988; Lechappe, 1986).

The assessment of the disease responses, either as incidence or as severity, with or without time dependency, determines the procedures to differentiate SR. Corman et al. (1986) investigated the incidence of wilt in time, and calculated the survival probability as an indicator of SR. When disease incidence or disease severity were assessed as a function of inoculum, SR variation was illustrated as differences in response intensities between soils per infestation level (Albouvette et al., 1982; Alabouvette, 1986; Tivoli et al., 1987; Rouxel and Briard 1988). However, a quantitative discrimination of SR requires more than merely an illustration of differences per inoculum level. To assess SR, overall differences in disease response

curves should be investigated. Since we consider the elements level, shape and slope of the disease response curves to be essential characteristics of soil receptivity, we searched a procedure to deal with these aspects. Therefore, the first step consisted in fitting disease response curves. Fitted values and parameters, characteristic of the disease response on each soil, were retained for further analysis.

Models. To fit disease responses mathematical models may be employed which do not contain parameters with a biological meaning (Madden and Campbell, 1990). We preferred to employ models yielding parameters with a biological meaning.

Since variation in shape of response curves was found, models with predetermined shapes may not fit the data as found indeed for the Gompertz model. Flexible models such as the Weibull model fitted most of the data. However, the Weibull parameters for scale (B) and shape (C) did not always vary in the same direction (Table 3). Thus, each parameter separately remained inconclusive for ranking SR. Combination of parameters B and C did not yield unequivocal ranking of SR either.

The exponential model produced results similar to Weibull, but also fitted extreme responses. In particular, the model fitted curves from sterilized soils. The exponential slope parameter RX behaved similarly to the Weibull shape parameter C and, since C was regarded inadequate, so was RX.

Although the two models did not give a very high fit, the obtained values are satisfactory if diversity of responses and experimental variability is considered. The low percentage of variance explained by both models for some soil samples was ascribed to variation between replicates, but these were retained in the analysis because of their heuristic value.

Ordinations. If SR represents a gradient from suppressive to conducive effects of soils on soil inoculum potential, multivariate techniques offer an alternative to explicitize such a gradient (Ter Braak, 1987a). In PCA ordination a new variable (the first axis) was created as a linear combination of the disease responses. Figure 3 and Table 4 show that this first axis contains almost exclusively SR information. The dispersion of the soil samples along this first axis (Fig. 3) is caused by properties of the samples counteracting the effect of increased densities of the pathogen on root rot. The second axis mainly represents the disease variability due to natural infestation (D0 = IPS).

Doublet et al. (1988), presented SR ordinations to *Plasmodiophora brassicae* by correspondence analysis (CA). They obtained an ordination which was strongly folded on the second axis, the Guttman effect. Nevertheless, they projected samples on the first axis to represent ranks of SR. If more dimensions have to be used which are not orthogonal, then the data have to be detrended, a procedure which was omitted by Doublet et al. (1988). As CA was less adequate for our data, PCA was employed for further differentiation.

In *'Comparing SR assessments'*, it was concluded that Weibull fitting of disease responses improved the ordination by PCA. It was also concluded that the first PCA axis was more adequate for ordering soils than Weibull's parameters. When information of natural IPS (D0) was removed from PCA, the first ordination axis

represented shape and slope of the disease responses and the percentage of variance accounted for by this axis increased to 95%. However, neglecting IPS does not give better biological distinction and leads to the anomaly that samples which differ largely in soil inoculum potential, while yielding the same disease response to infestation, would be placed at the same SR level.

PCA generated variables were used to assess effects of environmental factors on disease incidence (Madden and Pennypacker, 1979; Campbell et al., 1980b; Hau et al., 1981; Schruh et al, 1987), plant losses (Stynes and Veitch, 1981, 1983) or to characterize microbial populations (Sarniguet et al., 1992) as reviewed by Hau and Kranz (1990). According to our results, it is an elegant way to express soil receptivity to soil-borne plant pathogens, being simple, reproducible and available in several computer programs.

PCA might be applied to develop an improved scale of SR which is standardized in its "zero-point" and measuring unit, and warrants further investigations.

Searching for factors causing differences in SR. To explore which factors may be responsible for differences in SR between soils, PCA can be employed. Grouping of soil samples according to receptivity offers an extra facility for such exploration. Clustering of data (Fig. 4) had not the intention to discriminate between variables responsible for group association. CVA was used to look for group coherence. Our analysis indicated three completely separated groups: conducive (C), slightly reducing (Srd) and strongly reducing (Str) soils. CVA results showed that most of the samples were intermediately receptive to Fsp (Fig. 5) as in Figure 3. Theoretically, it could be expected that IPS somehow represents soil receptivity. IPS and SR were uncorrelated for Fsp. This may imply that soil receptivity with regard to Fsp in pea works through a more specific antagonism. Increasing suppressiveness was positively correlated with the number of *F. solani* colonies in pea roots, whereas the latter was negatively correlated with the natural IPS. Besides, the amount of *F. solani* in rhizosphere soil was uncorrelated with IPS. This indicates that a saprophytic *F. solani* could be active as an antagonist.

Soil samples from closely related fields, differing in cropping history, were classified in significantly different SR groups. This result stresses the importance of the biological factor to SR.

Under the experimental conditions used, common root rot, a disease caused by *Aphanomyces euteiches* and *Pythium* spp., developed on a considerable number of non-artificially infested soil samples. High natural infestation by these fungi impeded the assessment of the effect of additional Fsp inoculum. For this reason such soil samples were excluded from statistical analysis, but all these samples could be considered to be highly conducive to root rot.

In natural systems, diversity is considered to be an important obstacle limiting disease in reaching an epidemic level. In agriculture, soil homogeneity and uniform crop properties are instrumental in obtaining high yield and quality. No stabilisation in an ecological sense can be expected in agricultural soil because of the short growing

periods and the continuous disruptions of the arable layer. However, even in such disturbed soils an interrelated complex of factors does exist which greatly affects the activities of pathogens on host plants (Palti, 1981; Schippers, 1991). The recognition of such properties of a soil is indispensable for understanding and use of biological control and for breeding plants for resistance to soil-borne pathogens.

The combination of procedures presented permits research dealing with the identification of ecological characteristics correlated with differences in SR. Further research should elucidate possible causal mechanisms.

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Chapter 9

Analysis and comparison of soil receptivity to *Thielaviopsis basicola* and *Aphanomyces euteiches* causing root rot in pea

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Abstract

Soil receptivity (SR) to *Thielaviopsis basicola* and *Aphanomyces euteiches*, two soil-borne pea pathogens, was examined in field soil samples. SR patterns, resulting from a Principal Component Analysis (PCA) on a variance-covariance matrix, differed between pathogens. Most root rot disease responses to inoculation were conducive to *A. euteiches* and suppressive to *T basicola*. A more comprehensive comparison of SR, including that for *Fusarium solani* f.sp. *pisi*, showed that in the same soils disease caused by *T. basicola* was strongly reduced, *Fusarium solani* f.sp. *pisi* intermediately reduced, whereas disease caused by *A. euteiches* was usually promoted. Inoculum potential of soil (IPS), determined by bioassay before the last pea crop in 1986 or 1987, and IPS determined during SR tests to individual pathogens in non-inoculated field soil samples in 1991, showed a significant increase due to soft root rot pathogens. No change in IPS occurred when disregarding this disease.

Additional keywords: *Pisum sativum*, black root rot, soft root rot, *Fusarium solani* f.sp. *pisi*, inoculum potential, suppressive soil, statistical analysis, ordinations.

Introduction

Thielaviopsis basicola Berk. & BR. (Tb) and *Aphanomyces euteiches* Drechs. (Ae), causal organisms of black root rot and soft root rot of peas, respectively, are well established in Dutch arable soils. Both pathogens attack pea soon after sowing and can provoke serious problems at each developmental stage of the crop.

In a previous paper we hypothesized that variability of root rot between pea fields (Oyarzun et al., 1993) could be in part caused by differences in soil receptivity (SR) to soil-borne pea pathogens. SR covers the effects of biotic and abiotic soil environment on the expression of pathogenicity (Alabouvette et al., 1982; Alabouvette, 1986). We reported significant differences between field soils in response to root rot disease caused by highly virulent *F. solani* (Mart.) Sacc. f.sp. *pisi* Jones Snyder & Hansen (Fsp; Oyarzun et al., 1994). No SR research was carried out yet on

Ae, one of the most serious pathogens in peas (Pfender, 1984). Studies on SR to Tb were made for diseases in tobacco (Stutz et al., 1985) and *Phaseolus* beans (Lechappe, 1986), but not in peas.

In this paper we present (i) results on SR for the two soil-borne pea pathogens Tb and Ae, examined individually, using devices and procedures described for Fsp (Oyarzun et al., 1994), (ii) a comparison of the SRs to these pathogens, including results obtained in a previous study for Fsp, and (iii) data on the change in the level of inoculum potential of soil (IPS) in fields assessed before the last pea crop in 1986-1987 and in 1991.

Materials and Methods

A set of 40 soil samples were tested for SR to Tb, including 32 soils previously tested with Fsp (Oyarzun et al., 1994). Twenty seven soils were tested for receptivity to Ae, including 23 samples previously tested with both Fsp and Tb. In both sets of samples, 5 soils were included which originated from fields in monoculture of different crop species.

Characteristics of the soils and SR test procedures were described previously (Oyarzun et al., 1994). Characteristics of additional field soils included in this study are given in table 1.

Table 1. Characteristics of the soil samples tested for SR to *T. basicola* (Tb) and *A. euteiches* (Ae): silt fraction; organic matter, gravimetric water content, soil density, root rot severity, and occurrence in field soil of Tb, Ae and *F. solani* (Fs).

code	Silt (%)[1]	organic matter(%)	%H_2O[2]	S.d.[3]	DI[4]	Tb[5]	Ae[5]	Fs[5]
bu	22	1.2	23	1.2	1.5	-	+	+
el	32	2.2	27	1.1	0.5	-	+	+
st	72	3.6	45	0.9	1.5	-	+	+
th	69	4.6	44	0.9	0.6	-	-	+
sm	44	2.0	35	1.0	0.5	-	+	+
lf	*	10.2	60	0.9	0.2	-	-	+
aa	53	3.7	40	1.0	0.7	-	-	+
ad	52	3.5	40	1.0	0.9	-	-	+
mt	30	1.8	25	1.1	0.7	-	-	+
hg	32	2.4	26	1.1	1.1	-	+	+
er	24	1.9	24	1.2	1.2	-	-	+
re	26	3.4	24	1.2	4.0	+	-	+

[1] Silt (%): texture fraction < 16 mu. * = peat.
[2] %H_2O: gravimetric water content of the soil sample at water holding capacity.
[3] S.d.: soil density used in the bioassay ($g.cm^{-3}$)
[4] DI: Root rot severity at flowering in the last pea crop: 0 = no necrosis, 5 = 100% necrotic roots.
[5] presence (+) or absence (-) of *T. basicola* (Tb), *A. euteiches* (Ae) and *F. solani* (Fs) in soil.

Indigenous population of tested fungi. The ocurrence of Tb in non-inoculated rhizosphere soils and in roots of 21-day-old pea plants was examined by plate dilution methods using selective Tb medium (Papavizas, 1964) or carrot disks (Lloyd and Lockwood, 1962). Presence of chlamydospores of Tb, conidia of *Fusarium* and oospores of Ae in roots was microscopically examined.

The presence of Ae in soils was investigated in Petri dish tests (Oyarzun and Van Loon, 1989). Ae was confirmed by isolation on diluted corn meal agar (CMA; Pfender, 1984). The 27 field soils tested for SR to Ae were not or slightly infested by this pathogen.

Soil receptivity tests. Pathogens: *Thielaviopsis basicola.* Two monosporic cultures of highly virulent isolates of Tb, Tb21 and Tb36, were grown separately on malt agar (MA) in the dark at 24° C for 21 days. Spore suspensions (endoconidia and chlamydospores) were obtained from 21-day-old cultures by washing the spores from the agar surface with sterile tap water. The suspension, with spores of the two isolates, was filtered through a double layer of cheesecloth and the density determined. The suspension was kept at 0° C until use. Soil samples were infested with the suspension to achieve a density of 100 - 1.000 - 5.000 - 10.000 and 50.000 conidia per g dry soil (D1-D5).

Aphanomyces euteiches. Two isolates, Ae1 and Rst1, were first grown on diluted CMA at 24° C. Zoospore suspensions were obtained by incubating 7-day-old CMA disks with the fungus for five days at 26° C in liquid maltose-peptone medium (MPM), aerated for 24 hrs, washed, and incubated in water for 48 hrs. Zoospore density was determined microscopically. The suspension was atomized into soil to reach a density of 50 - 100 - 500 - 1.000 and 5.000 zoospores per g dry soil (D1-D5). For the control treatments (D0) only sterile water was added to the soil.

Reference soils. As a reference for inoculum quality, heat sterilized soil samples were treated as test soils and used in each experiment. The reference for Tb consisted of an alkaline (pH-KCl 7.8), lime-rich (8% carbonate) and lightly humic (2.8% organic matter) light sandy loam with a high natural infestation by this pathogen. As a reference for Ae, a light sea clay soil from a pea monocropping plot was used. This reference was the same as described for Fsp (Oyarzun et al., 1994).

Test plants. Surface sterilized, high quality seed of the cultivar Finale, 7 - 7.5 mm diameter, was used in all tests.

Test conditions. Air temperature was kept at 24/18° C day/night with Ae and 22/18° C day/night with Tb. The tests were performed using computerized equipment (Oyarzun et al., 1994).

Disease assessment. Four weeks after sowing, plants were uprooted, roots cleaned with tap water, and root rot disease severity scored. Disease severity of epicotyl, cotyledon and root were scored individually, on a disease scale of 0 for healthy to 5 for 100% infected. A disease index (DI) was calculated as a weighted mean of these 3 components (Oyarzun, 1993).

Experimental design. After emergence, objects (dose*sample) were arranged in a block with test soil as plots (tank) and inoculum doses as subplots (minipots) and replicated four times.

Analysis of data. Split-plot ANOVA was carried out on root rot disease scores per experiment. Subsequently a Weibull model (Rawlings et al., 1988) was fitted to data, to obtain parameters describing disease behaviour in different soil samples. Previous to fitting the data, DI was converted into a Health Index (HI = 5-DI). With the average disease response per soil*inoculum dose combination and the Weibull fitted values, a Principal Component Analysis (PCA; Ter Braak, 1987) was carried out on a variance-covariance matrix. Weibull parameters were further used to interpret ordinations.

Comparing SR for Tb, Ae and Fsp. SRs for the pathogens Tb and Ae were compared to those for Fsp (Oyarzun at al., 1994) by a PCA on all available averaged disease responses (n=103). A restricted subset of 23 disease responses, obtained from samples tested with all three pathogens, was clustered by Average Linkage Cluster Analysis (Digby et al., 1989) using a matrix of similarity of samples (n: 3x23 + 3 references = 72). Canonical Variate Analysis (CVA) was carried out to examine the strength of the clusters.

The statistical analyses were made using procedures in Statistix (Anonymous, 1985a), Statistical Ecology (Ludwig and Reynold, 1988), GENSTAT 5 (Digby et al., 1989) and CANOCO (Ter Braak, 1987).

Determining temporal changes in IPS. IPS was calculated as the average root rot disease index (DI) obtained from non-inoculated soil samples used in SR-tests. In estimating IPS, DI values obtained when testing SR for Fsp were used too. Therefore data covered approximately 50 samples, excluding 5 samples from fields never cropped with peas. The IPS values obtained in the 1991 samples were compared with those determined by bioassays in 1986/1987 before the last pea crop was grown (Oyarzun, 1993). Analyses were carried out with the sign test and other non-parametric methods (Steel and Torrie, 1985).

Results

Indigenous population of Tb and Ae in soil. T. basicola. Determination of Tb in soil was hard to perform, because selective media and baiting the fungus with carrot disks did not work satisfactorily. In some cases, baiting the fungus with pea followed by microscopic observation permitted to demonstrate its presence in the soil. The number of samples from commercial fields containing Tb was low, only 4 out of all samples investigated. The fungus was found in 4 of the 5 soil samples from a monocropping experiment. *A. euteiches.* In the Petri dish test, the presence of oospores was detected in 20 samples. In SR tests, soft root rot occurred in the D0 treatment of 16 samples. In 14 out of these Ae was demonstrated, in two *Pythium* spp. only.

Table 2a. Root rot severity of one month old pea plants grown in field soil samples after inoculation with various amounts (D1-D5) of inoculum of *Thielaviopsis basicola*, D0 = non-infested (IPS).

Experiment nr.	Soil code	Doses D0	D1	D2	D3	D4	D5	SxD[1)]	LSD1[1)]	LSD2[1)]
I	r1[2]	0.73	1.75	2.45	2.90	3.55	3.98	***	0.52	0.53
	cb	0.80	0.90	1.28	1.75	2.65	3.93			
	cf	0.20	0.58	0.60	0.80	1.28	2.80			
	ja	1.00	0.95	1.18	1.33	2.25	4.03			
	rh	1.78	1.78	2.33	2.58	3.80	4.65			
	jg	1.55	1.98	2.18	2.40	3.03	3.88			
	le	0.45	1.05	1.05	1.28	1.50	1.88			
	lf	0.40	0.65	0.98	1.30	2.13	2.03			
	cm	0.48	1.20	1.30	1.40	2.10	3.03			
	cv	2.00	2.63	2.95	3.18	3.68	4.03			
	co	0.70	1.10	1.38	1.95	2.58	3.53			
II	r2[2]	0.00	2.25	3.80	4.43	4.58	4.95	***	0.53	0.59
	kh	0.68	0.80	1.23	1.58	2.23	3.78			
	jr	0.98	1.13	1.10	1.45	1.73	2.85			
	jn	0.55	0.65	1.13	1.30	2.10	3.23			
	hm	1.08	1.18	1.25	1.45	1.55	2.68			
	rg	0.63	0.60	0.73	1.18	1.35	2.73			
	ro	0.83	1.03	1.13	1.48	2.00	3.48			
	bl	0.60	0.83	0.85	0.83	1.15	3.18			
	zu	1.00	0.88	1.00	1.23	1.80	2.73			
	wi	0.50	0.85	1.28	1.78	2.40	3.48			
III	r3[2]	0.08	3.30	4.15	4.43	4.65	4.83	***	0.48	0.50
	bk	1.78	1.75	2.18	2.73	3.28	3.95			
	ls	0.60	0.83	1.03	1.93	2.13	3.93			
	as	1.03	0.95	1.23	2.15	2.65	3.40			
	aa	0.53	0.30	0.70	1.25	1.48	2.48			
	ad	0.23	0.45	0.60	1.10	2.40	3.28			
	rc	0.78	0.75	0.73	2.00	2.50	3.88			
	wa	0.75	1.07	1.15	1.40	2.05	3.45			
IV	r4[2]	0.00	2.85	3.70	4.58	4.75	4.88	***	0.48	0.54
	tr	1.45	1.00	1.28	1.55	1.85	2.45			
	to	1.00	1.00	1.00	1.78	2.05	3.13			
	ve	0.63	0.68	0.90	1.80	2.55	3.90			
	gv	1.73	1.78	2.98	3.38	4.18	3.75			
	lu	0.48	0.78	1.78	2.65	3.45	4.48			
	dm	1.10	1.85	2.38	2.55	3.58	4.20			
	er	1.00	1.35	1.98	2.65	3.53	4.75			

[1]. In each experiment, the interaction of dose and soil (SxD) was highly significant, $P \leq 0.001$ (***); Least Significant Differences at $P = 0.05$: LSD1 for comparing disease severity at different inoculum doses per soil and LSD2 for all other comparisons.
[2]. Reference soil.

Soil receptivity test. Analysis of data.

T. basicola. Of the 40 samples tested for SR to Tb, 7 were discarded because of high natural infectivity (Table 2a). *A. euteiches*. In the second test for Ae, the water flow to several tanks was perturbed by technical problems. We retained only 4 samples where perturbation did not occur and the reference (r2), though it was affected. Affected samples were tested again (table 2b).

Table 2b. Root rot severity of one month old pea plants grown in field soil samples after inoculation with various amounts (D1-D5) of inoculum of *Aphanomyces euteiches*, D0 = non-infested (IPS).

Experiment nr.	Soil code	Doses D0	D1	D2	D3	D4	D5	SxD[1]	LSD1[1]	LSD2[1]
I	r1[2]	0.03	3.05	4.08	4.88	5.00	5.00	***	0.52	0.53
	cv	1.85	2.65	3.78	4.45	4.63	5.00			
	cb	1.45	4.80	5.00	5.00	5.00	5.00			
	cf	0.80	1.23	2.03	3.40	4.95	5.00			
	cm	0.80	1.13	2.68	3.78	4.50	5.00			
	co	1.23	3.93	4.88	5.00	5.00	5.00			
	aa	0.48	4.13	4.20	5.00	5.00	5.00			
	ad	0.83	2.33	4.03	4.63	4.98	5.00			
	tr	0.68	1.43	1.60	3.78	4.53	5.00			
	to	1.03	2.33	4.05	4.95	5.00	5.00			
	ro	1.28	3.83	4.65	4.98	5.00	5.00			
II	r2[2]	0.08	1.93	2.78	3.68	4.55	5.00	***	0.50	0.56
	le	0.68	1.43	2.60	4.05	4.63	5.00			
	lf	0.93	3.00	4.50	4.98	5.00	5.00			
	ja	1.15	2.18	2.85	4.25	4.78	5.00			
	bk	2.80	3.65	3.50	4.40	4.40	5.00			
III	r3[2]	0.00	1.73	2.78	4.45	5.00	5.00	***	0.36	0.38
	as	1.05	1.28	1.83	4.03	4.93	5.00			
	bl	0.73	1.00	1.30	3.73	4.73	5.00			
	gv	1.95	4.63	5.00	5.00	5.00	5.00			
	jn	1.18	2.88	3.98	4.95	5.00	5.00			
	kh	1.13	4.30	4.95	5.00	5.00	5.00			
	ls	1.13	2.25	4.68	5.00	5.00	5.00			
	lu	0.98	2.80	4.90	5.00	5.00	5.00			
	mu	0.95	1.03	1.10	1.15	1.78	2.08			
	rc	0.93	1.08	1.10	4.83	5.00	5.00			
	rg	1.10	1.35	1.50	1.45	1.50	4.50			
	ve	1.00	0.85	1.48	1.50	2.48	4.93			
	wa	0.80	1.73	2.58	3.70	4.75	5.00			
	wi	0.83	2.18	4.03	5.00	5.00	5.00			

[1,2]. See Table 2a.

In all experiments, the interaction of dose and soil (SxD) was highly significant ($P \leq 0.001$; Table 2 a,b). Linear, quadratic and higher polynomial terms significantly contributed to describe the effect of the (log) dose on soils ($P \leq 0.05$, based on LSD). In the density ranges used, black root rot (Tb) increased less with increasing dose than soft root rot (Ae). To illustrate this effect the data of table 2a,b were rearranged in a contingency table of (i) the minimum dose needed to produce the first significant increase ($P \leq 0.05$) in rot severity and (ii) the severity provoked by the highest inoculum dose (Table 3). The two distributions clearly differed ($P \leq 0.05$, based on χ^2). A significant increase ($P \leq 0.05$) in disease severity was achieved with the lowest dose of Ae in most samples. For Tb the distribution was about equal over the three lower doses. For Ae, the maximum severity of 100% was produced by the highest dose in all but one sample. With Tb, it only occurred in 30% of the cases.

Table 3. Frequency distribution of soil samples according to (left) the lowest dose of *Thielaviopsis basicola*, Tb, or *Aphanomyces euteiches*, Ae, needed to achieve a significant increase ($P \leq 0.05$, based on LSD) of root rot severity in peas and (right) the maximum severity provoked by the highest dose (D5).

Pathogen	Dose $_{min}$ D1	D2	D3	D4	D5	DI at D5 1-2	2-3	3-4	4-5	n
Tb	10	9	13	4	1	1	6	19	11	37
Ae	22	4	2	2	0	0	1	0	29	30

In the reference soil, D1 of Tb produced severe black root rot (Fig. 1A), but in the natural soil rot severity was moderate, even at D5 (Fig. 1B). In general the receptivity pattern of soils tested to Tb was skewed towards strongly suppressive. Figs. 2 A,B show similar responses of soft root rot to artificial infestations with Ae in sterilized and natural soil.

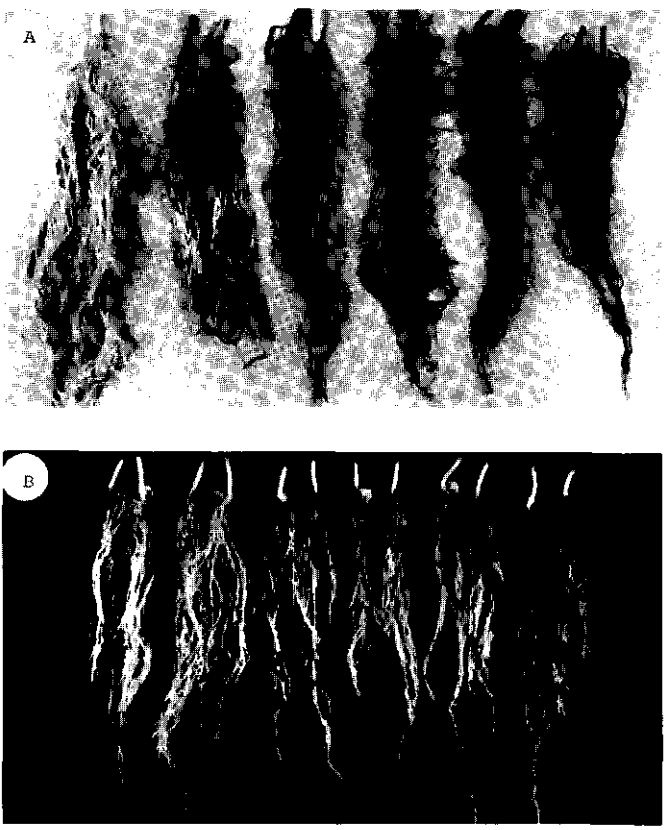

Figure 1. Symptoms of black root rot in pea at increasing infestation dose (from 100 to 50.000 spores g^{-1} dry soil, D1-D5) of *Thielaviopsis basicola* in a sterilized soil (A) used as a reference and in a field soil (B). At the left roots in non-inoculated soil (D0).

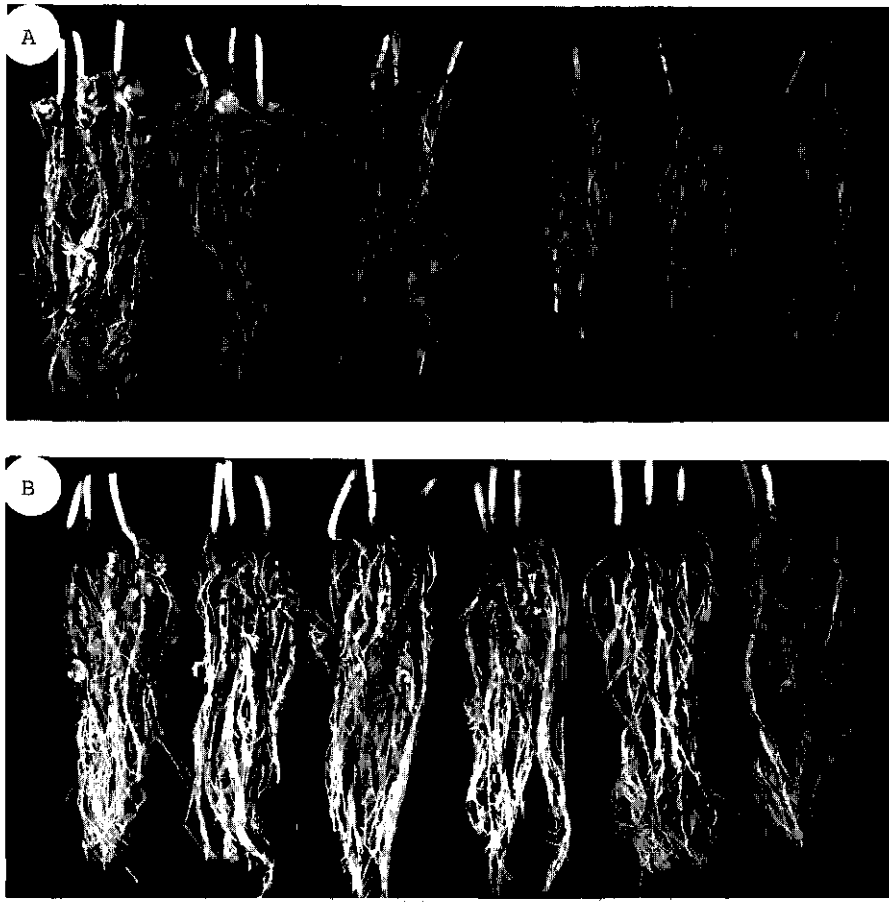

Figure 2. Symptoms of soft root rot in pea caused by increasing infestation dose (from 50 to 5000 zoospores g^{-1} dry soil, D1-D5) of *Aphanomyces euteiches* in a sterilized soil (A) and in a field soil (B) in which disease severity was strongly reduced. At the left roots in non-inoculated soil (D0).

Describing differences in receptivity. For both pathogens, the Weibull model described responses adequately but the fit, represented by the percentage variance accounted for, was better for Ae (Table 4). For Ae, all but one scale (B) parameter values were lower than the value of the maximum dose D5 (3.7). For Ae the large value of the shape parameter C ($C > 5$) found in most of the samples indicates strongly curved responses. For Tb, low responses to infestation were expressed by a scale parameter B slightly larger than 4.7 (the log(dose) value of D5) in almost 50% of the samples, and by large values of shape parameter C.

Ordination of disease responses. For Tb a PCA of average disease responses (4 replicates of S*D) yielded a first principal component axis representing 84% of the

variance, with an increase to 86% when using Weibull predicted responses. Second and third principal axes represented almost equally low amounts of information (Table 5). The second axis opposes samples associated with low inoculum doses to the two highest ones, indicating that differences in slopes operate at this level. The third axis opposes the central to the extreme variables, typical of the curvature contrast, but due to the high correlation of IPS with this axis, it mainly represents variation of disease

Table 4. Weibull parameter values and the percentage of variance accounted for by fitting health indexes (HI = 5-DI; DI = no necrosis, 5 = 100% necrosis) obtained as responses to soil infestation with *Thielaviopsis basicola* (Tb) or *Aphanomyces euteiches* (Ae).

$$HI = A.E^{-(\log[dose]/B)^C}$$

Soil	Tb				Ae			
Code	A	B	C	var%	A	B	C	var%
as	4.1	4.7	4.5	90	3.9	2.6	6.7	99
bl	4.3	4.8	13.7	89	4.2	2.6	7.3	98
bk	3.3	4.5	4.6	86	2.2	2.6	2.7	73
cb	4.2	4.5	6.2	92	3.6	1.5	7.1	99
cf	4.6	4.9	8.1	93	4.1	2.6	5.3	93
cm	4.2	5.0	4.8	73	4.3	2.5	3.9	88
co	4.2	4.7	4.5	64	3.8	1.6	6.1	94
cv	3.0	4.6	2.2	63	3.2	2.2	3.4	89
gv	3.4	4.1	2.5	76	3.1	1.6	8.9	99
ha	3.9	5.1	8.6	63	*	*	*	
jg	3.3	4.7	5.2	77	*	*	*	
jn	4.4	4.8	5.6	66	3.8	1.9	4.9	95
ja	4.0	4.5	9.4	89	3.9	2.3	3.7	94
kh	4.2	4.6	6.7	95	3.9	1.6	5.7	99
le	4.6	6.3	2.4	73	4.4	2.4	3.9	94
lf	4.5	9.2	1.6	62	4.1	1.8	6.6	95
ls	4.3	4.5	6.5	94	3.9	1.7	12.1	98
lu	4.5	4.0	4.3	94	4.0	1.8	11.1	98
aa	4.6	5.2	4.9	82	4.8	1.3	1.8	97
ad	4.7	4.6	5.5	90	4.2	1.9	7.4	93
mu	*	*	*		4.1	5.0	3.5	50
rc	4.3	4.4	6.2	84	4.0	2.5	15.5	99
rg	4.4	5.0	7.2	91	3.7	3.6	17.5	93
jr	4.0	5.0	7.3	69	*	*	*	
rh	3.2	4.2	7.2	74	*	*	*	
ro	4.1	4.7	7.5	83	3.7	1.7	4.5	98
dm	3.7	4.3	3.3	77	*	*	*	
tr	3.9	5.8	4.2	72	4.3	2.6	5.1	96
to	4.1	4.9	5.7	85	3.9	1.9	8.2	91
ve	4.4	4.4	6.1	93	3.9	3.2	11.1	93
er	3.8	4.1	5.9	92	*	*	*	
wa	4.1	4.7	7.4	85	4.2	2.4	3.6	96
wi	4.4	4.7	4.5	89	4.2	1.9	8.1	99
zu	4.1	5.1	6.8	69	*	*	*	
r1[1]	4.3	4.1	2.1	86	5.0	1.7	3.4	100
r2[1]	5.0	2.5	2.1	96	5.0	2.7	1.7	91
r3[1]	5.0	1.7	1.2	98	5.0	2.2	3.7	99
r4[1]	5.0	2.2	1.7	96	*	*	*	

[1]. reference soils; * = not determined

in naturally infested soil. For Ae, the same configuration was found (Table 5).
Figures 3 A,B show soil scores in ordination diagrams. For both pathogens the first and third axis were used to represent the gradients of SR and H0 (the health index of non-inoculated objects). The information represented by the first axis was lower for Ae, than for Tb. For Ae, a considerable amount of information is retained in the second axis (var = 17%; Table 5).

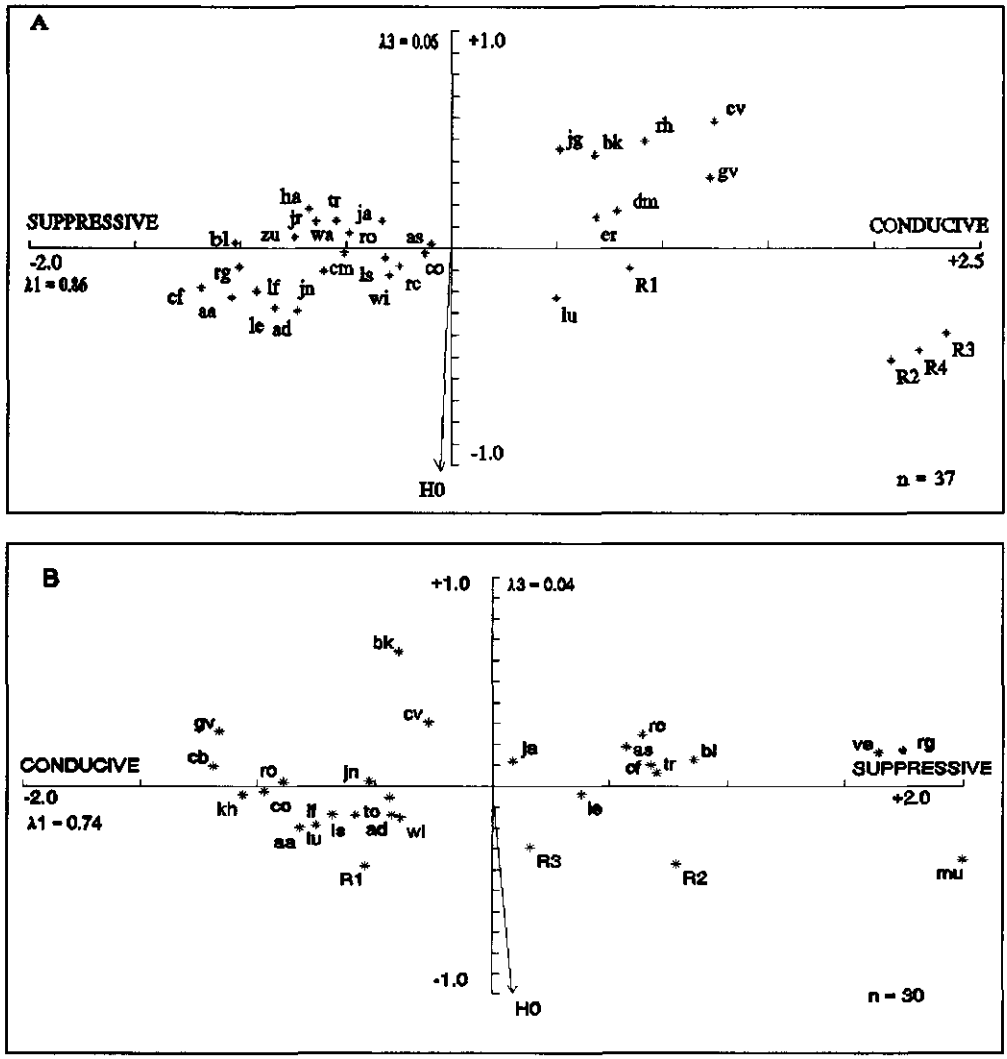

Figure 3. PCA ordination diagrams of SR of *Thielaviopsis basicola* (A) and *Aphanomyces euteiches* (B). Soil scores (*) are represented in the first and the third Principal Component axis. The biplot is constructed using Euclidian distances. The arrow indicates direction in which natural disease pressure of the soil decreases (H0).

Interpreting disease gradients by Weibull parameters. When the values of the sample scores on the first PC-axis (obtained on disease average) were regressed on the Weibull parameters A (location), B (scale) and C (shape or curvature), a high correlation was found (R^2-adj = 0.84 for Tb; R^2-adj = 0.89 for Ae). All the Weibull parameters contributed to the construction of the first axis (Student's t significant at P $\leq$ 0.05). Stepwise analysis of variance of the first axis stressed the importance of roughly similar contributions to R^2 of the B and C parameters for Tb and of parameter B for Ae in the information contained in the first axis (Table 6).

Table 5. Eigen values (λ) and correlation coefficients between disease response variables (HI's) and the first three principal component axes obtained from a PCA on a variance-covariance matrix. The latter was calculated from Weibull predicted disease responses to infestations with *T. basicola* and *A. euteiches* on different field soils.

	T. basicola			*A. euteiches*		
	I	II	III	I	II	III
(λ)	.86	.07	.06	.74	.17	.04
HI's						
H0	.09	-.02	-.99	.24	-.42	-.82
H1	.92	-.32	-.15	.88	-.43	-.07
H2	.97	-.23	.04	.95	-.23	.13
H3	.99	-.03	.05	.90	.41	.01
H4	.99	.11	.03	.80	.58	-.07
H5	.83	.54	-.06	.53	.52	-.34

Table 6. Regression coefficients (ß), Student's t-values (t) and the change in the coefficient of determination by stepwise adding Weibull parameters to the regression on the First Principal Component axis.

Predictor Variables	*T. basicola*			*A. euteiches*		
	ß	t	R^2-adj	ß	t	R^2-adj
A	-0.49	-3.8***	-0.02	0.49	4.1***	0.01
B	-0.58	-10.4***	0.54	1.25	14.8***	0.88
C	-0.20	-8.1***	0.84	0.04	2.1*	0.89

(*** P $\leq$ 0.001; * P $\leq$ 0.05)

Comparing SR between pathogens. A PCA was carried out on the total of 103 soil*pathogen combinations, including 36 tested to Fsp. In figure 4, the scatter of the soil scores along the first axis (var% = 83) contrasts SR for Tb and Ae, whereas soil scores for Fsp were more or less intermediate. To obtain more reliable information about SR differences for these pathogens, analysis was restricted to responses belonging to 23 samples tested for all the three pathogens and an averaged value of references (72 combinations). Cluster analysis of sample similarities, at 90% based on Weibull predicted responses, followed by discriminant analysis yielded four well separated cluster centroids (P $\leq$ 0.05, based on χ^2; Fig. 5). Response types for Tb, Fsp and Ae (Tb:Fsp:Ae) were differentially represented in the four groups: SM,

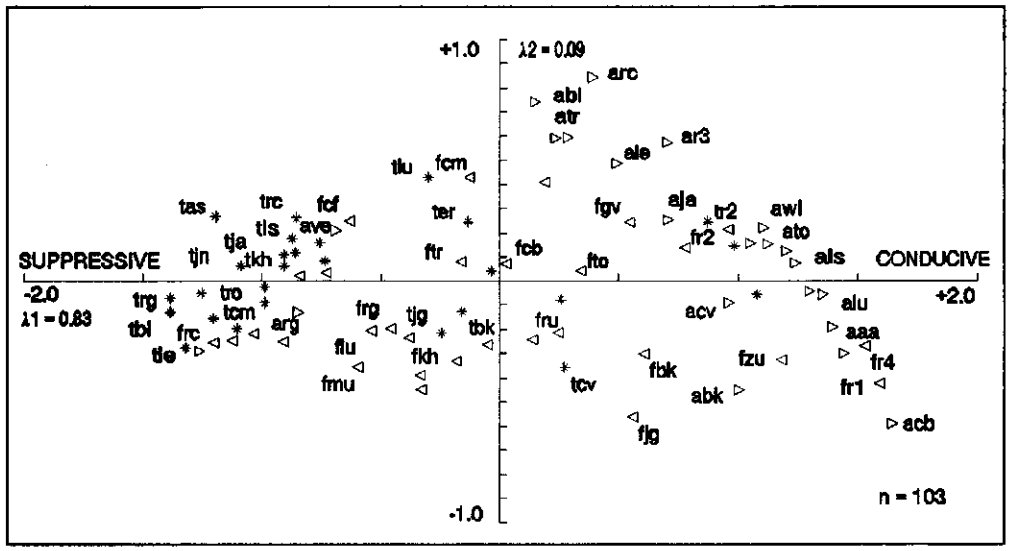

Figure 4. PCA ordination diagram of all pathogen*soil combinations tested for receptivity to *T. basicola* (*; n = 33 + 4 references), *F. solani* f.sp. *pisi* (◁; n = 31 + 5 references) or to *A. euteiches* (▷; 27 + 3 references) on the first and second PC axis using Euclidian distances.

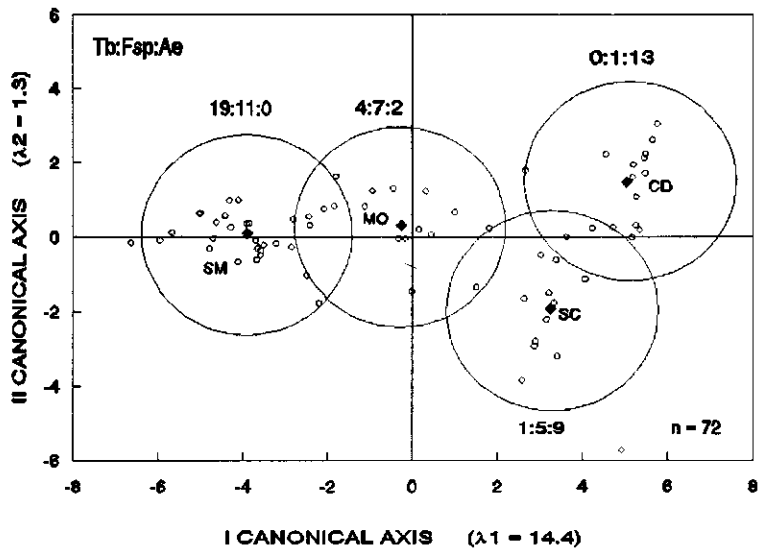

Figure 5. CVA plot of sample scores, the canonical variate means, and their 95% confidence area of four groups obtained by cluster analysis of soils tested for *T. basicola*, *F. solani* f. sp. *pisi* and *A. euteiches*, based on 90% similarity. Response types: SM = strongly to moderately disease reducing; MO = moderately to slightly disease reducing; SC = slightly reducing to conducive and CD = conducive.

strongly to moderately disease reducing 19:11:0; MO, moderately to slightly 4:7:2; SC, slightly reducing to conducive 1:5:9, including some of the references; and CD, conducive 0:1:13, including references and highly infested field soil.

Temporal changes in IPS. A significant increase of IPS was found ($P \leq 0.001$, $n = 50$) when the IPS of the samples determined, in 1986 and 1987 previous to growing the pea crop (Oyarzun, 1993), and in 1992 were compared by the Sign Test. In 1992 a higher IPS was found in 35 samples, with a strong increase in 19. In the latter category, 16 cases of soft root rot and 3 cases of dry rot occurred. The increase of IPS in the rainy year 1987 was almost entirely due to soft root rot pathogens (9 out of 10 samples). If soft root rot inducing soils were discarded from the analysis no change in IPS was found.

Discussion

Pathogen occurrence in soils. The occurrence of Ae in a relatively large number of soil samples, supposed to be free from or slightly infested with this pathogen, indicates the enormous potential of this pea root rot pathogen under Dutch conditions. Though most soils were alkaline, Tb, known to be favoured by alkalinity, was infrequent.

SR tests. Analysis of data. Our results confirmed the effect of the soil on the expression of pathogenicity of the pathogens studied ($P \leq 0.05$; LSD). The results also emphasize the contrast in receptivity of the same soil to different pathogens.

Special care has to be taken in interpreting SR gradients obtained by PCA. Because of the ordinal character of the constructed gradient, the position of a particular sample score on an ordination axis depends on the responses of the other samples. The more completely the gradient is represented, the more correct is the allocation of the sample. Therefore, when data of all sample*pathogen combinations were ordinated ($n = 103$), SRs for individual pathogens were better differentiated (Fig. 4). The differences in SR, could be more fully appreciated by comparing the composition of the clusters and the distances of cluster centroids as produced by discriminant analysis (Fig. 5) of those soils ($n = 72$) which were tested for the three pathogens. The contrast in SR for Tb (low receptivity) and Ae (high receptivity) was remarkable. SR to Fsp was higher than to Tb (Figs. 4 and 5).

Such differences in SR to individual pathogens suggest the existence of multiple mechanisms selectively affecting the pathogens or of common mechanisms affecting the pathogens in a different way. For Tb, Ae and Fsp, different dose ranges were used in testing SR. For comparison of SR between pathogens care was taken to include densities as high or higher than those occurring in highly infested natural soil (Pfender and Hagedorn, 1983; Blume and Harman, 1979; Burke et al., 1970; Papavizas, 1968; Nash and Snyder, 1965). With Tb, first disease responses became visible at an infestation dose 10 times larger than that used with Fsp (Oyarzun et al., 1994). In experiments with sterile soil, however, Fsp and Tb did not differ in pathogenicity at the same inoculum density (data not presented). Differences in

receptivity of a specific soil to *Fusarium solani* f. sp. *phaseoli* compared to that for Tb in *Phaseolus* bean were observed by Lechappe (1986), who suggested that indigenous non-pathogenic *Fusarium oxysporum* might be responsible for low SR to Tb. This fact points to the great sensitivity of Tb to biological factors in soil, as documented by Sneh et al. (1976).

Temporal changes in IPS. Information about the stability of SR can be obtained by studying the change of IPS over time. In our 1991 samples a period of three or four years had elapsed since the last pea crop so that no further drastic changes in IPS were expected. Mainly due to Ae, IPS in 1991 was significantly higher than before the last pea crop in 1986-1987. This result suggests a high receptivity of soils to this pea pathogen. The large proportion of soil samples from 1987 (a very rainy year) severily infested by Ae in 1991 suggests the association of this pathogen with very wet soil conditions. A period as short as 48 hrs is considered sufficient to incite acute development of soft root rot (Long, 1984). Only soils non-infested or slightly infested with Ae were used to test SR. With few exceptions (Fig. 4), these soils were highly receptive to the pathogen, so that escape from contamination is more likely than disease-limiting factors in these soils.

In contrast to Ae, no significant changes in IPS by dry root rot causing pathogens could be established.

Concluding remarks. We conclude that soil receptivity affects the most important soil-borne pea root rot pathogens but the magnitude differs according to the pathogen.

The high SR for Ae and the resulting change in IPS since the last pea crop in 1986/87 indicate that Ae is the most dangerous among the potential root rot pathogens affecting peas. If soil is or becomes infested by this pathogen, IPS can drastically increase, especially under wet conditions.

IPS, as determined by indigenous Tb or other locally occurring pathogens causing dry root rot, did not significantly change from 1986/87 to 1991, whereas a wide range of responses to artificial infestation with Tb was found. Apparently inoculum density is not a reliable criterion to forecast dry root rot outbreaks in natural soils. This is in accordance with results obtained by many authors (Burke, 1965; Burke et al., 1970; Campbell et al., 1980; Shew et al., 1984; Campbell and Noel, 1985; Chellini et al., 1988; Kraft and Wilkins, 1989; McFadden et al.,1989).

The result also explains the low correlations between pea cropping frequency and root rot disease in pea crops reported earlier (Oyarzun et al., 1993). The dynamics of root rot IPS do not only depend on crop frequency but also on the dominant pathogen in the soil and on the receptivity of the soil.

The results, obtained from laboratory experiments with a limited number of pea root rot pathogens and a relatively small number of samples, do not permit an unequivocal extrapolation about the value of SR in practice. Only further field research will provide such information. To enable management of SR, the causative mechanisms must be clarified.

Acknowledgements

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Chapter 10

Biological control of foot and root rot in pea, caused by *Fusarium solani*, with nonpathogenic *Fusarium oxysporum* isolates

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Abstract

Two nonpathogenic isolates of *Fusarium oxysporum* (Fo) were examined for their ability to antagonize *F. solani* f.sp. *pisi* (Fsp) causing foot and dry root rot in pea. Antagonism was studied *in vitro*, in a sterilized field soil, and in natural field soils. The natural field soils contained, besides native *F. solani*, other typical pea root rot pathogens. Nonpathogenic Fo reduced disease severity and prevented plant weight losses caused by various amounts of Fsp in sterilized soil. Pre-colonization of sterilized soil improved the antagonistic effect. In natural, highly infested field soils, antagonistic effects were variable and more evident in absence than in presence of *Aphanomyces euteiches*. Fo was a fast colonizer of soil organic matter, even when native *F. solani* was present in the soil. The performance of benomyl-resistant mutants of Fo did not differ from wild types.

Additional keywords: antagonism, soil organic matter, colonization, *Pisum sativum*.

Introduction

Fusarium solani (Mart.) Sacc. f.sp. *pisi* (Jones) Snyder & Hansen (Fsp) is an important component of the complex of soil-borne pathogenic fungi causing foot and dry root rot of pea, here indicated as root rot. Severe attacks of *F. solani* cause considerable yield loss (7, 8, 12). Even at low infection levels, losses of 20% were reported (9). In the Netherlands, *F. solani* has been known for a long time to cause problems in dry peas (39), especially in fields with low lime content and poor soil structure (15). The pathogen is established in the traditional pea growing area (24, 31) and is highly specialized on pea (26).

Seed dressing with fungicides provides effective control of the pathogen, but the effect lasts only for a short period (Gerlagh, pers. comm.). Other ways of disease management, such as crop rotation, are often unsatisfactory. Resistance breeding has not yet been fruitful (13). Van der Spek (35, 36) reported considerable reductions of root rot severity due to increased microbial activity, when straw and chitin-rich

material were added to infested soil. Research on biological control of *F. solani* by antagonists was seldom directed to f.sp. *pisi* (10).

Nonpathogenic or low-virulence isolates of *Fusarium* have been used to control wilt caused by several *formae speciales* of *F. oxysporum* (Fo) (4, 17, 20, 22, 28, 30, 34). Foot and crown rot of tomato caused by *F.oxysporum* f.sp. *radicis-lycopersici* was controlled by nonpathogenic *F. oxysporum* (Fo) isolates (4, 18). The few attempts to reduce pathogenic activity of Fsp with avirulent strains of *Fusarium* have been unsuccessful (14, 32), but Lechappe et al. (16) found some decrease of rot severity in bean caused by *F.solani* f.sp. *phaseoli* after the application of nonpathogenic Fo isolates, and Walz-Borgmeier (38) demonstrated that nonpathogenic Fo isolates reduced the symptoms caused by *F. solani* in red clover.

Results of research with suppressive soils (2) stressed the importance of the soil ecosystem, in particular the soil biota, for the inoculum potential of soil-borne pathogens. A similar effect of the soil might be expected when a biological agent is introduced to control a specific soil-borne disease. For a correct application of biological control agents, it is important to know to what extent the antagonist can survive, proliferate and be effective under different soil conditions.

To examine whether nonpathogenic Fo isolates can antagonize *F. solani* in pea, two isolates were chosen: isolate Fo47, originally isolated from suppressive soil in the Châteaurenard region of France (4), and isolate 61812, isolated from a healthy plant of a wilt-resistant carnation line (30). These two nonpathogenic Fo isolates were confronted with *F. solani* in a system of increasing substrate complexity. The experiments were performed under standardized conditions in the laboratory or in a climate room. Six field soils containing different pathogens of the pea root rot complex and sterilized soil were used. Antagonistic activity *in vitro*, disease expression, plant production and colonization of soil organic matter and pea roots were determined with the nonpathogenic Fo isolates and with Fsp. To facilitate colonization studies in soil, benomyl-resistant mutants (11, 29) of both isolates were used.

Materials and methods

Fungal isolates

Three virulent isolates of Fsp (Fs48, Fs04 and Fs14) were used (26). The isolates were kept on Carnation Leaf Agar (CLA) for immediate use or lyophilized. Mixtures of the three isolates in equal proportions were used to infest soils. Detailed information about the nonpathogenic Fo isolates is given by Postma and Luttikholt (29).

In vitro interactions between Fsp and nonpathogenic Fo

Fsp isolates were grown in Petri dishes containing Czapek Dox Agar (CDA) at 25 °C under black light, whereas Fo isolates were grown on Potato Dextrose Agar (PDA) in the dark. After six days of growth, 5 mm discs of each isolate were taken from the edge of the colony and placed on Malt Agar (MA) in two ways, side by side (juxtaposition) or at a distance of 5 cm (opposed). For reference purposes, isolates were incubated separately. Each treatment was replicated 5 times. The incubation was performed at 20 °C under black light. The test was repeated in a sligthy modified

way, according to Mańka et al. (21). Interaction index and angular interaction (16) were calculated after incubation periods of 5 and 8 days.

Biological control in sterilized soil, experiment I

The effect of inoculum density of the pathogen and the effect of pre-colonization of the soil by the antagonists on disease severity and plant weight were studied in sterilized soil. The soil, a light clay (7.7% $CaCO_3$, 2.8% organic matter and pH-KCl 7.4), was obtained from a field with a high inoculum potential of root rot pathogens after 10 years monoculture of field pea. After homogenization and sieving through a 5 mm mesh sieve, the soil was heat treated at 104 °C for 12 hours. Samples were stored in plastic bags at 4 °C until artificial infestation.

Fsp isolates were grown separately in Petri dishes containing CDA for 2 weeks at 25 °C under black light. A mixture of macro- and microconidia of the isolates was suspended in sterilized deionized water, filtered through cheesecloth and adjusted to the proper density.

The nonpathogenic Fo isolates, grown for 2 weeks at 25 °C on PDA, produced mainly microconidia. These were suspended in sterile deionized water, filtered through cheese cloth and adjusted to the proper density.

Suspensions of Fo47 and 61812 (10^5 conidia g^{-1} dry soil) or water were added to test soils 10 days before (i.e. pre-incubation) or just before infestation with Fsp. Due to the first infestation, the gravimetric water content of the soil was raised to 15%. Infestations were performed in plastic bags by spraying the suspension onto the soil under continuous shaking. The treated samples were incubated in the dark at 24 °C. Soil was infested with Fsp so as to obtain 0, 10^2, 10^3, 10^4 and $5*10^4$ conidia g^{-1} dry soil and a soil water content of 20%. The samples were stored 3 days at 4 °C in the dark.

Minipots (4*4*12 cm) were filled with the treated soil. Per minipot four surface-sterilized pea seeds, cv Allround, were sown at 2.0 cm depth. After sowing, the water content of the soil was carefully raised to water holding capacity, approx. 26% (31 vol%, soil density 1.2 g cm^{-3}). Pots were covered with plastic and placed in a growth room at 22 °C in the dark. At emergence, the pots were placed in tanks (Fig. 1) on oasis, randomized over four blocks in a split-plot design, with doses of Fsp as main plots (tanks) and Fo isolates, with or without pre-incubation, as subplots. During the first week, the water table was maintained at 10 cm under sowing depth and in the second and third week lowered to 15 and 20 cm, respectively. During the experiment air temperature was 24/20 °C day/night, with 12 hours light at 90 $W.m^{-2}$, and 80% RH.

Plants were harvested one month after sowing. Fresh and dry plant weight, root rot disease severity, and colonization of roots and soil organic matter were determined (see sampling procedures).

Biological control in sterilized soil, experiment II

Water or the nonpathogenic isolates Fo47 and 61812 and their benomyl-resistant mutants Fo47B10 and 61812B17 were added to sterilized soil 10 days before infestation with Fsp. Soil was infested at a rate of 0, 10^3 or 10^4 Fsp conidia g^{-1} dry soil.

Conditions and experimental design were as in experiment I. Soil samples without pea seeds were used to determine colonization of the soil by Fo and Fsp 4 days after infestation with Fsp. Thirty one days after sowing, plants were harvested to determine fresh and dry weight, root rot disease index, colonization of roots and soil organic matter.

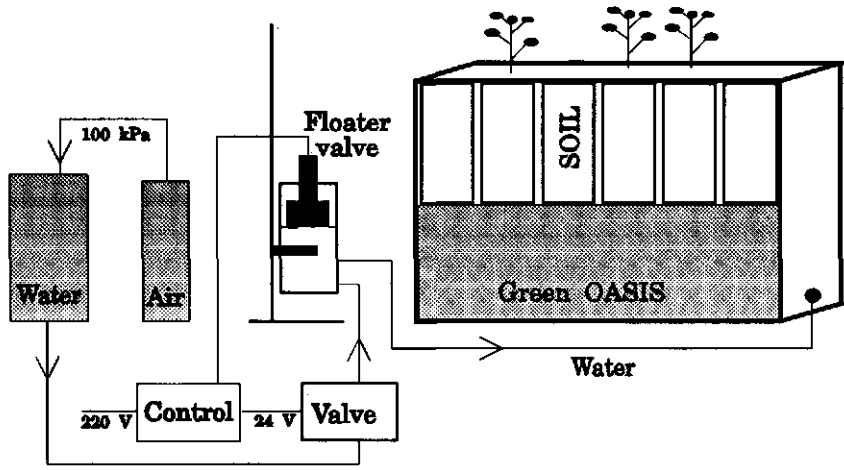

Fig. 1.Semi-automatic experimental device used to test the antagonistic effect of *F. oxysporum* against inoculated or native *F. solani* in field soil.

Biological control in naturally infested soil, experiment III

To test the antagonistic activity of the benomyl-resistant mutants of the two Fo isolates, six naturally infested soils, representing different soil types, were chosen because of their high infestation level and their variation in pea root rot pathogens (Table 8.1/3). Before introduction of the antagonists, part of the soil was gamma sterilized (4 Mrad).

Fourteen days before sowing peas, the sterilized and non-sterilized soil samples were infested with Fo47B10 or 61812B17, 10^6 conidia g^{-1} dry soil. As a control, part of the samples was treated with sterile water only. Incubation temperature was 22 °C and soil water content was adjusted to 5% below water holding capacity (Table 8.2). Conditions and experimental design were as in experiment I, with Fo isolates and sterilized/non-sterilized soils as subplots. Soil samples without pea seeds were used to determine colonization of the soil by Fo and Fsp 15 days after infestation with the nonpathogenic isolates. One month after sowing, plant fresh and dry weight, root rot disease index, and colonization of soil organic matter were determined.

Sampling procedures.

Production of dry matter. Vines were cut at soil level, weighed and dried at 70 °C

for 48 hours to determine dry weight. Roots were placed in a 500 ml glass flask containing fresh tap water and carefully cleaned from soil and organic matter. Root rot disease index (0 = no necroses; 5 = 100% necrotic roots) was assessed. Roots were weighed, part of the roots was sampled to determine colonization (see below) and the remainder was used for dry weight.

Root colonization. Roots were surface sterilized for 3 min in 0.5% sodium hypochlorite with 5% ethanol and rinsed 3 times in sterilized water. These roots were blended for 2 min in a Braun blender containing 100 mL sterile deionized water. The suspension was filtered through cheesecloth and plated in triplicate with a spiral plater (Spiral Systems, Inc., Cincinnati, Ohio, USA) on Fusarium selective agar medium (SFA) containing 20.0 g D-(+)-glucose, 0.5 g KH_2PO_4, 2.0 g $NaNO_3$, 0.5 g $MgSO_4.7H_2O$, 1.0 g yeast-extract (Oxoid), 0.01 g $FeSO_4.7H_2O$, 20.0 g agar, 25 mg dicloran (Allisan, 50% a.i.; Asepta, Delft, The Netherlands), 100 mg streptomycin, 10 mg tetracyclin, 0.21 g fenaminosulf (Bayer 5072, 70% a.i.) and 1 L deionized water (28). In experiments where benomyl-resistant mutants were present, SFA with 10 mg L^{-1} benomyl (Benlate, 50% a.i.) was used. The loss of root material on the cheesecloth was determined by drying and weighing.

Colonization of soil organic matter. The presence of nonpathogenic Fo isolates and of Fsp in organic matter particles of 0.5-1.0 mm was determined. The soil, which was carefully rinsed from the roots, was sieved over 1.0 and 0.5 mm sieves with running tap water. The particles on the 0.5 mm sieve were vibrated three times in 600 ml fresh tap water with a vibromisher and placed on sterile filter paper. From each sample, 25 organic matter particles were plated on SFA with or without benomyl. After 5 days incubation at 25°C in the dark, outgrowth of *Fusarium* was scored. An additional incubation of several days in diffuse daylight (room temperature) facilitated identification of *F. solani*.

Soil colonization. Soil colonization was determined by shaking soil samples (10 g) 30 min at 400 rpm in 90 mL of 0.1% sodium pyrophosphate and 10 g gravel (28). Appropriate dilutions were plated in triplicate on SFA with or without benomyl and the number of cfu g^{-1} dry soil was calculated.

Soil sample properties. Chemical properties of the samples were determined by spectrophotometry (Traact 800) after extraction of soil in 0.01 M $CaCl_2$ for soluble elements, or Flame photometry in other cases. The mineral phase was determined by ignition at 600°C for 12 hours. Other data on physical and biological properties were available from earlier experiments (24). Agronomic data were obtained by compiling information from the farmers' field books. Development of soft rot and presence of *Aphanomyces euteiches* was studied using a Petri dish test (27). The inoculum potential of the sample was examined by bioassay (25).

Statistical analysis

Analyses of variance were carried out with the statistical programme Genstat 5. Least significant differences (LSD) were calculated at $P \leq 0.05$. Treatments with 0% or 100% diseased plants were excluded from the analyses if trended residuals were present. Numbers of micro-organisms in soil or roots were compared by analysis of

variance after logarithmic ($\log_{10}$ n + 1) transformation.

Results

In vitro interactions between Fsp and nonpathogenic Fo

Fo isolates grew faster than Fsp (Table 1). Confrontation experiments indicated that Fo and Fsp did not interfere, since the interaction index was approximately 1 (Table 2). When both *Fusarium* spp. were put side by side (juxtaposition) the interaction significantly favoured Fo, in particular 61812.

Biological control in sterilized soil

In experiment I, infestation of sterilized soil with Fsp resulted in severe infection of the roots, even at the lowest doses (Fig. 2). Fresh (Fig. 3A) and dry (Fig. 3B) weight of the shoots strongly decreased due to infection by the pathogens ($P \leq 0.05$). Simultaneous introduction of Fsp and nonpathogenic Fo significantly ($P \leq 0.05$) reduced root rot severity, in particular at the two lowest doses of Fsp. A 10 day pre-incubation of Fo isolates strongly increased the antagonistic effect of the nonpathogenic isolates ($P \leq 0.05$) and this effect was present at all Fsp doses. The same was found for fresh and dry weight ($P \leq 0.05$). If nonpathogenic Fo isolates were added to soil just before Fsp, the antagonistic effect declined with increasing Fsp dosages. Fo47 without pre-incubation had no effect on dry weight (Fig. 3B). Sowing peas immediately after infestation with the nonpathogenic isolates in absence of the pathogen, resulted in some root rot symptoms and shoot weight loss (Fig. 3 A,B).

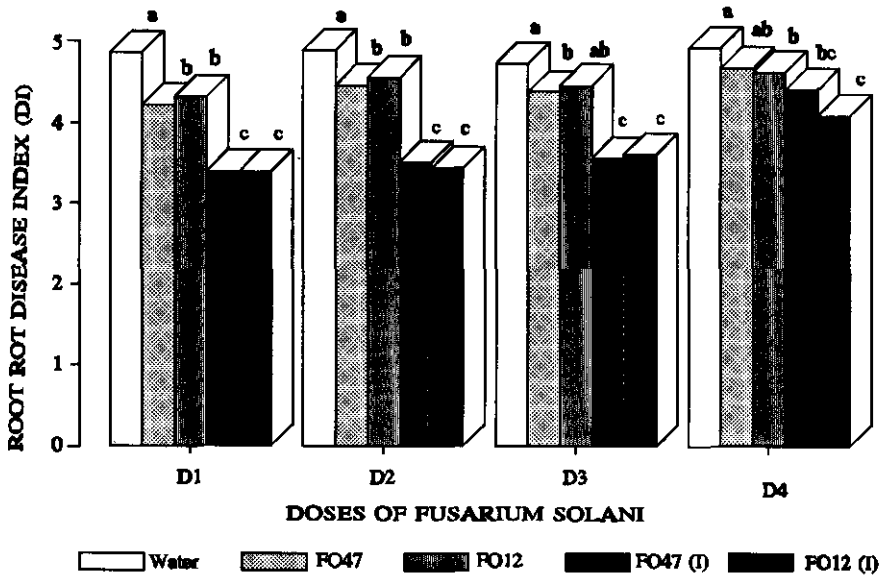

Fig. 2. Root rot disease index after addition of nonpathogenic *F. oxysporum* isolates Fo47 or 61812 (F012), with and without incubation (i), before infestation of soil, at increasing doses of *F. solani*. Characters above the columns indicate statistically reliable differences ($P \leq 0.05$)

Table 1. Radial growth (mm) at 20°C of *F. solani* f.sp. *pisi* isolates Fs48, Fs14 and Fs04, and nonpathogenic *F. oxysporum* isolates Fo47 and 61812. Values with the same letter in the rows are not significantly different at $P \leq 0.05$, according to LSD.

Medium	Fs48	Fs14	Fs04	Fo47	61812	LSD
MA[1)]	16.8^a	15.6^a	15.8^a	21.2^b	23.6^c	1.74
CDA[2)]	30.6^b	28.4^a	28.8^a	36.4^c	39.6^d	1.17

[1)] Growth after 5 days on malt agar at 12/12 hrs light/dark.
[2)] Growth after 8 days on Czapek-Dox agar in the dark.

Table 2. *In vitro* interaction of *F. solani* f.sp. *pisi* isolates Fs48, Fs14 and Fs04 (Fsp) with the nonpathogenic *F. oxysporum* isolates Fo47 and 61812 (Fo).

		Fs48		Fs14		Fs04		
Index[1]	Medium	Fo47	61812	Fo47	61812	Fo47	61812	LSD[2]
II	MA	1.02	0.99	1.00	0.94	1.09	1.01	0.07
II	CDA	1.13	0.99	1.17	1.17	1.22	1.10	0.07
AI	MA	0.58	0.31	0.60	0.35	0.64	0.33	0.09

[1)] II = interaction index = (radius Fsp : radius Fo in confrontation) / (radius Fsp : radius Fo growing separately).
AI = angular interaction = α (Fsp) / α (Fo), where α is the angle of a fungal thallus grown in juxtaposition.

[2)] LSD ($P \leq 0.05$), for statistical comparisons within rows.

Table 3. Experiment II. Fresh and dry weight of pea shoots after addition of nonpathogenic *F. oxysporum* isolates Fo47, Fo47B10, 61812, 61812B17, or water to sterilized soil, 10 days before infestation with different doses of *F. solani* f.sp. *pisi* (Fsp). Treatments were additive ($P \leq 0.05$). Values in rows followed by the same character are not significantly different ($P \leq 0.05$, LSD).

Doses of Fsp	Water	Fo47	Fo47B10	61812	61812B17
Fresh weight					
0	8.77	11.57	13.07	10.95	12.51
10^3	3.91	5.30	7.95	7.86	6.49
10^4	1.75	2.26	5.53	8.05	5.87
mean	4.81^a	6.38^b	8.85^c	8.95^c	8.29^c
Dry weight					
0	0.87	1.06	1.47	1.06	1.25
10^3	0.47	0.62	0.79	0.89	0.78
10^4	0.25	0.34	0.63	0.79	0.75
mean	0.53^a	0.67^b	0.86^c	0.92^c	0.93^c

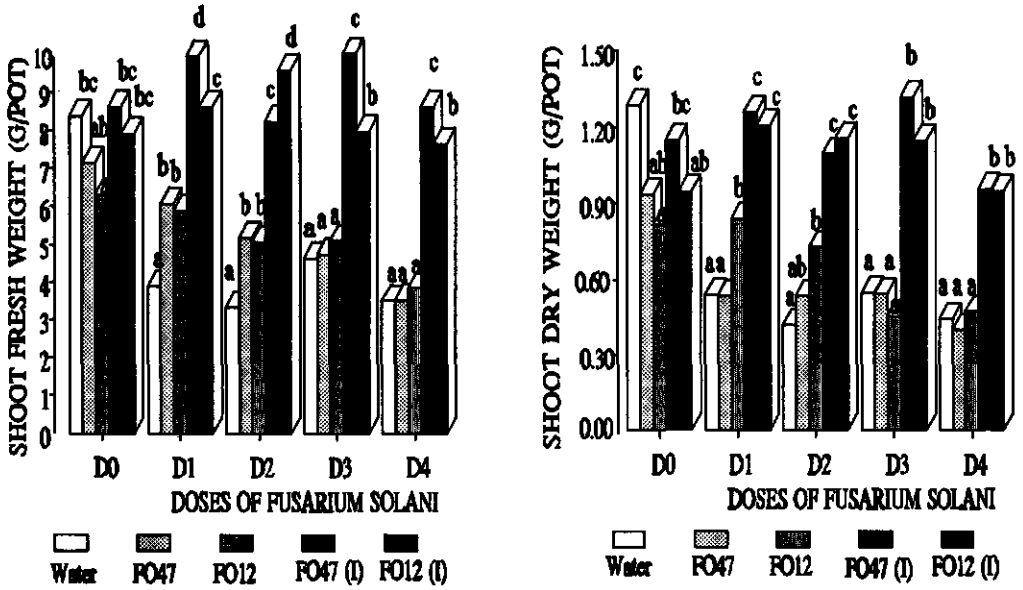

Fig. 3. Fresh (A) and dry (B) weight of pea shoots after addition of nonpathogenic *F. oxysporum* isolates Fo47 or 61812, with and without incubation before infestation of soil, at increasing doses of *F. solani*. Water was used as a control. Significant differences per dose are indicated by different characters above the columns ($P \leq 0.05$).

Results of experiment II confirmed these patterns of antagonism by the two nonpathogenic Fo isolates and their effects on plant weight (Table 3) and root rot severity (Table 4). Analysis of variance showed significant effects of nonpathogenic Fo and of Fsp inoculum dose. Fresh and dry matter production were enhanced by nonpathogenic Fo at all inoculum doses. Benomyl-resistant nonpathogenic Fo isolates were as antagonistic as the wild types, or even better as in the case of Fo47B10 ($P \leq 0.05$).

Table 4. Experiment II. Disease index after addition of nonpathogenic *F. oxysporum* isolates Fo47, Fo47B10, 61812, 61812B17, or water to sterilized soil, 10 days before infestation with different doses of *F. solani* f.sp. *pisi* (Fsp). The effect of the nonpathogenic isolates on root rot disease index is additive for the two Fsp doses ($P \leq 0.01$). Values within row with the same letter are not significantly different ($P \leq 0.05$, LSD).

Doses of Fsp	Water	Fo47	Fo47B10	61812	61812B17
10^3	4.69	4.40	3.50	3.68	3.90
10^4	4.95	5.00	4.05	3.70	3.98
mean	4.82^a	4.70^a	3.77^b	3.69^b	3.91^b

Colonization in experiments with sterilized soil

Colonization of soil organic matter particles by Fo was almost 100%, irrespective of Fsp and pre-incubation (Table 5). Fsp colonized 100% of the organic matter after

single infestation, but its incidence was reduced by 50% when infested together with nonpathogenic Fo and by >70% if the soil was pre-incubated with nonpathogenic Fo (Table 5). Differences in colonization between the two *Fusarium* spp. were similar in experiment II (Table 6). In addition, colonization of organic matter by Fsp was influenced by its infestation doses (Table 6).

Colonization of the roots by Fsp and Fo was more difficult to determine. Only in experiment II, where benomyl-resistant Fo isolates were used, Fo could be detected. The nonpathogenic Fo isolates were present in the roots after surface sterilization, but their numbers were close to the detection limit (10^4 cfu g^{-1} dry root).

In experiment II, the numbers of cfu of the pathogen incorporated and recovered from the soil were comparable. The numbers of cfu of the nonpathogenic isolates had augmented 10 to 30 times during pre-incubation in the sterilized soil, from 10^5 cfu g^{-1} soil in the inoculum to about 3 x 10^6 cfu g^{-1} soil at the beginning of the experiment (Table 7). Benomyl resistance of the mutants was found to be stable, also after multiplication in soil.

Biological control in naturally infested soil

Soil types varied from sandy loam to very heavy clay, but all soils had a pH-$CaCl_2$ between 7.2 and 7.5. Soil BER was poor in lime and had the highest content of organic matter, silt, sodium and soluble potassium. Except for soil DJM, all soils had a low C/N-ratio (Tables 8.1 and 8.2). All soils were infested by *F. solani* (Table 8.3).

Table 5. Experiment I. Percentage of organic matter particles occupied by *F. oxysporum* (Fo) and/or *F. solani* f.sp. *pisi* (Fsp) at two doses of the pathogen after addition of water or nonpathogenic *F. oxysporum* isolates Fo47 or 61812, with or without pre-incubation.

			without pre-incubation				with pre-incubation			
Doses	Water		Fo47		61812		Fo47		61812	
of Fsp	Fo	Fsp	Fo	Fsp	Fo	Fsp	Fo	Fsp	Fo	Fsp
0	7±10	0±0	99±2	9±13	99±2	15±11	99±2	0± 0	100±0	3± 4
10^4	0± 0	100±0	99±2	55±26	99±2	51±32	100±0	15±13	100±0	31±26

Table 6. Experiment II. Percentage of organic matter particles occupied by benomyl-resistant *F. oxysporum* (Fo) or by *F. solani* f.sp. *pisi* (Fsp) at three doses of Fsp, after addition of water or nonpathogenic benomyl-resistant isolates Fo47B10 or 61812B17.

	Water		Fo47B10		61812B17	
Doses of Fsp	Fo	Fsp	Fo	Fsp	Fo	Fsp
0	*	*	99± 2	0± 0	98± 2	0± 0
10^3	0± 0	93± 4	99± 2	32±11	100± 0	37±21
10^4	0± 0	100± 0	98± 4	71±16	100± 0	58±10

* not determined.

Table 7. Experiment II. The number of cfu ($\log_{10}$) *Fusarium* spp. per g dry soil (F) and the percentage of benomyl-resistant *Fusarium* colonies (%B) in the absence of pea seeds, 4 days after infestation with Fsp.

Doses of Fsp	Water		Fo47		61812		Fo47B10		61812B17	
	F	%B	F	%B	F	%B	F	%B	F	%B
0	0	0	6.3	0	6.3	0	6.3	100	6.4	128
10^3	2.9	0	6.4	0	6.2	0	6.1	114	6.5	120
10^4	3.6	0	6.3	0	6.3	0	5.6	147	6.5	112

The inoculum potential of root rot pathogens was high, except for ZU where it was moderate. BER, ZU, and HER represented soils producing dry rot symptoms on pea. HER also produced severe black root rot (*Thielaviopsis basicola*). The other field soils, HIL, HP and DJM, contained *A. euteiches* and in bioassays produced predominantly soft rot. Only 2 or 3 pea crops had been grown in the last 20 years (Table 8.3).

Root rot severity in five of the control treatments with water was very high (Table 9) as predicted by the bioassay performed before the experiments (Table 8.3). After sterilization of the soil, shoot fresh weight increased 2 to 4 times (Table 9). Dry weight data showed the same trend as fresh weight (figures not shown).

Disease index in the naturally infested soils was lower due to infestation with either nonpathogenic Fo isolate in HER, ZU and BER, but only in BER the difference with the control was significant ($P \leq 0.05$). The reduction of root rot severity led to a significant increase in fresh weight of plants, including roots, in naturally infested BER after addition of Fo47B10 or 61812B17. In HER and DJM the yield after application of either nonpathogenic strain was higher than the water control, but this increase was not significant.

In the sterilized soils, plant weight was not significantly affected by the presence of the nonpathogenic Fo isolates. The exception was HP, where plant weight was significantly higher with than without 61812B17 ($P \leq 0.05$). The sterilized soil HER was contaminated and discarded from the analyses.

Colonization in naturally infested soil

The nonpathogenic Fo isolates colonized 40 to 90% of the organic matter particles in the naturally infested soils (Table 10). There was no significant difference between the two isolates. Organic matter in HER was colonized less than in the other soils. Colonization by native *F. solani* was difficult to quantify, since this *F. solani* had no resistance marker and had to be identified by colony morphology, colour and conidiophores. *F. solani* occupied 50 to 75% of the organic matter in all naturally infested soils without Fo. This occupation decreased significantly if one of the nonpathogenic Fo isolates was added.

Colonization of the soil 15 days after infestation with the nonpathogenic Fo isolates is presented in Table 11 for the water control. The number of cfu of *F. solani* g^{-1} dry soil was about 10^3 in the naturally infested soils. ZU had the lowest value for *F.*

Table 8.1. Chemical properties of the field soils used to test antagonistic activity of nonpathogenic *F. oxysporum* against *F. solani* f.sp. *pisi*. Except Ca (mg g^{-1}), the other elements are in mg kg^{-1} dry soil. Total nitrogen (Nt) and carbon (C) are given as percentage of soil dry weight. pH- $CaCl_2$.

Sample code:	Ca	NH_4	NO3	K	P	Mg	Na	Nt%	C%	C/N	pH
BER	16.8	14.1	33.8	217.9	1.7	178.5	46.6	0.5	5.9	12.0	7.4
HIL	10.9	22.1	9.6	63.6	0.0	72.1	21.9	0.1	1.6	13.6	7.3
HER	30.3	73.4	73.4	68.9	1.7	61.3	8.3	0.1	2.0	14.1	7.2
HP	27.5	2.4	23.9	53.9	4.7	76.3	41.5	0.2	2.0	12.8	7.3
DJM	52.0	9.4	7.3	51.6	0.4	61.4	32.2	0.1	3.1	21.5	7.5
ZU	10.4	7.7	5.3	131.6	4.1	166.4	30.1	0.3	2.9	11.5	7.4

Table 8.2. Physical properties of the field soils. Soil type; percentage organic matter (o.m); percentage lime; K.value; Pw.value; For (dra)inage, (dro)ught and (cru)st forming sensitivity: 0 = no limitations, 5 = bad condition or very sensitive. In the last two columns: Gravimetric soil water content at water holding capacity (whc) of the samples at the indicated soil density (s.d).

Sample code	s.t[1)]	o.m	lime	K.val	Pw.val	dra.	dro.	cru.	whc	s.d[2)]
BER	vhcl	9.5	1.5	42	76	1	0	0	45	0.9
HIL	vllo	3.2	1.0	18	39	2	2	2	23	1.2
HER	slo	3.4	8.3	16	26	0	5	4	26	1.2
HP	lcl	2.8	7.4	18	25	2	2	2	30	1.1
DJM	lcl	2.4	12.8	23	30	2	1	1	31	1.0
ZU	vhcl	2.1	11.8	27	24	1	1	1	35	1.0

[1)]. Soil types: vhcl = very heavy clay; lcl = light clay; slo = sandy loam; vllo = very light loam.
[2)]. Soil density ($g.cm^{-3}$) applied in the experiment.

Table 8.3. Root rot characteristics of fields soils. Presence (+) or absence (-) of *F. solani* (Fs), *Aphanomyces euteiches* (Ae), *Pythium* spp. (Py) and *Thielaviopsis basicola* (Tb). Severity of disease determined in 1991 in bioassays (B) or by a specific test for soft rot in petri dishes (P), and root rot severity in the last pea crop (field) with the year of the last pea crop. Frequency of pea and legumes in the field during the last 20 years.

Code	Pathogenic spp.				Root rot disease indices				Crop frequencies	
	Fs	Ae	Py	Tb	B	P	field	year	Pea	Legumes
BER	+	-	+	-	4.3	0.5	2.9	86	2	4
HIL	+	+	+	-	5.0	0.8	1.2	87	2	5
HER	+	-	+	+	5.0	0.0	4.0	86	3	4
HP	+	+	+	-	4.5	1.3	2.5	87	2	5
DJM	+	+	+	-	4.5	1.3	1.7	87	3	3
ZU	+	-	+	-	2.6	0.3	0.7	86	2	4

solani. Values in the soils infested with Fo47B10 and 61812B17 were comparable. The log number of cfu *F. oxysporum* per gram dry soil varied between 5.2 and 5.9 in non-sterilized soils and between 5.3 and 6.7 in sterilized soils. In some of the sterilized soils (BER and ZU), Fo47B10 and 61812B17 had multiplied, since the log number of cfu inoculum was 6.0 cfu.g^{-1} dry soil, for both isolates. The percentage of benomyl resistance in the inoculum, and in the isolates recovered from the sterilized and non-sterilized soils, was always about 100%.

Table 9. Experiment III. Effect of the addition of water, Fo47B10 or 61812B17 on disease index and fresh weight (g) of pea plants including roots in six naturally infested soils, non-sterilized or sterilized.

Code	Disease index non-sterilized soil			Fresh weight non-sterilized soil			Fresh weight sterilized soil		
	Water	Fo47B10	61812B17	Water	Fo47B10	61812B17	Water	Fo47B10	61812B17
BER	4.7	3.4	3.1	6.3	11.9	10.8	17.2	17.7	16.3
HIL	5.0	5.0	5.0	3.5	2.7	3.3	17.8	18.5	19.6
HER	4.7	4.2	4.0	4.6	7.1	6.9	*	*	*
HP	4.9	4.8	4.7	6.2	6.9	7.4	11.3	13.7	17.1
DJM	4.7	4.5	4.6	5.0	6.8	6.7	13.8	17.0	16.2
ZU	2.0	1.3	1.6	11.2	12.2	11.3	19.3	16.9	19.4
LSD[1]	0.7			3.9			4.2		

[1] To compare values in the rows ($P \leq 0.05$).
* contaminated.

Table 10. Experiment III. Percentage of organic matter particles occupied by benomyl-resistant *F. oxysporum* or by *F. solani* in six naturally infested soils after addition of water, Fo47B10 or 61812B17.

Code	*F. oxysporum*			*F. solani*		
	Water	Fo47B10	61812B17	Water	Fo47B10	61812B17
BER	0	70	84	66	32	39
HIL	0	67	67	71	52	37
HER	0	41	42	57	52	46
HP	2	74	70	51	38	30
DJM	0	86	89	75	35	43
ZU	3	82	78	71	28	34
LSD[1]	15*			23		

[1] To compare values in the rows ($P \leq 0.05$).
* values of the water control were excluded since they were almost 0.

Table 11. Experiment III. The number of cfu (Log_{10}) *F. oxysporum* (Fo) and *F. solani* (Fs) per g dry soil 15 days after infestation with the nonpathogenic isolates, in six naturally infested soils, non-sterilized or sterilized.

Code	non-sterilized soil			sterilized soil	
	Water	Fo47B10	61812B17	Fo47B10	61812B17
	Fs	Fo	Fo	Fo	Fo
BER	3.3	5.9	5.7	6.7	6.5
HIL	2.8	5.6	5.5	5.9	5.9
HER	3.0	5.3	5.2	5.8	5.3
HP	3.1	5.6	5.7	5.9	5.9
DJM	3.0	5.6	5.5	6.0	5.6
ZU	2.6	5.4	5.4	6.2	6.4

Discussion

Biological control in sterilized soil

When nonpathogenic Fo isolates had the opportunity to pre-colonize the sterilized soil, their antagonistic activity was evident and consistent. Root rot severity was lower, and fresh and dry shoot weights were significantly higher ($P \leq 0.05$) than in the absence of Fo. Pre-colonization effects suggest that nonpathogenic Fo might have used the carbon present in organic matter to increase their energy status, thus increasing their competitiveness to *F. solani* when nutrients are released in the soil by germinating pea seeds.

In the absence of Fsp, an increase of plant weight by the addition of nonpathogenic Fo was observed in sterilized soil in experiment II and in sterilized HP soil in experiment III. This growth promoting effect of Fo applied to sterilized soil is difficult to explain. In alkaline soils nutrient solubility is low, especially that of micronutrients. Fo might increase their availability to the plants.

When sterilized soil was infested with nonpathogenic Fo isolates shortly before sowing, Fo was somewhat pathogenic provoking light root rot and consequently some plant weight loss (experiment I). This effect disappeared almost completely after pre-incubation (Fig. 3 A,B). The change of microconidia to chlamydospores during incubation probably explains part of this effect.

Biological control in non-sterilized soil

Severe root rot developed on plants in five of the six naturally infested soils and greatly affected plant weight (Table 9). The three main root rot symptoms were present, brown and black dry rot and soft rot. All soils had a high inoculum potential of *F. solani* and therefore can be considered as very conducive to root rot pathogens.

Successful control by Fo was found in a very heavy clay soil, poor in lime and with a relatively high sodium content, BER. Such a soil type was reported to be very conducive to *Fusarium* (15). A good colonization percentage of the organic matter by

Fo was found in BER, but also in the other soils. BER, however, has a high organic matter content. Competition for nutrients and space in soil and rhizosphere plays an important role in the mode of action of nonpathogenic Fo isolates (5, 19, 20, 34).

The organic matter content is important for the introduction of Fo. An indirect function has been ascribed to a high carbon content. Use of organic matter for growth means nitrogen immobilization, which inhibits germination of *F. solani* propagules (6). In our samples with low C/N-ratio, and low use of soil nitrogen by seedlings, it seems rather difficult to attribute the antagonistic effect to a nitrogen deficit. In alkaline soils, Fe can be limiting to the pathogenic activity, especially when Fe-fixing bacteria are present (17).

Interactions between Fo and Fsp isolates

In *vitro* studies on agar media and assessment of colonization of soil organic matter explain some aspects of the competition success of Fo. Isolates of nonpathogenic Fo proved to grow faster than Fsp on agar media. In confrontation experiments no particular influence was found of nonpathogenic Fo isolates on the growth rate of the pathogenic Fsp isolates. Their interaction index was close to 1, indicating an equivalent capacity to use energy for growth if energy and time are available. Apparently, the nonpathogenic Fo isolates *in vitro* do not produce substances inhibitory to growth of Fsp. When competition started immediately, in juxtaposition, significant reduction in growth of the pathogen was achieved, in particular by isolate 61812. The differences in saprophytic growth between the two *Fusarium* species became evident when energy was scarce and less easily accessible, e.g. when colonizing soil organic matter in natural soil. Though the colonization of soil organic matter was affected by the inoculum dose of the pathogen, the colonized fraction was always highest for the nonpathogenic Fo isolates. Amir (1) also detected a better colonization of sterilized soil by *F. oxysporum* than by *F. solani*. In sterile soil, the pathogen colonized the organic matter, but the number of cfu per gram dry soil did not increase after infestation as was the case with *F. oxysporum*.

Benomyl-resistance permitted detection of the nonpathogenic Fo isolates in the presence of other *Fusarium* isolates. Such manipulation of isolates may have deleterious consequences for their ecological fitness. Under the present experimental conditions, the two benomyl-resistant nonpathogenic Fo mutants showed equal or slightly better antagonistic activities than the wild types (Tables 3 and 4). In non-sterilized field soil, benomyl-resistant Fo isolates were not only able to colonize organic matter, but also colonized organic matter which had already been occupied by native *Fusarium* spp. (Table 10). The conclusion is that the benomyl-resistant mutants of Fo are adequate to study biological control and colonization in soil under the conditions of the present experiment.

Conclusion

We conclude that both nonpathogenic Fo isolates were successful in antagonizing Fsp in sterilized soil and in some naturally infested soils. Isolate Fo47 was already shown to be antagonistic against several *formae speciales* of *F. oxysporum* in different crops, f.sp. *dianthi*/carnation, f.sp. *melonis*/muskmelon, f.sp. *lini*/flax, f.sp. *lyco-*

persici/tomato, f.sp. *radicis-lycopersici*/tomato and f.sp. *cyclaminis*/cyclamen (4, 17, 22, 28, 33, 37). Isolate 61812 was shown to be effective against wilt in carnation (28, 30). The control of naturally present Fsp is remarkable, since it is often recommended to apply the nonpathogenic Fo isolates prior to infestation with the pathogen to obtain good biological control effects (3, 23, 30). The antagonistic effect of Fo isolates in naturally infested soils varied with the soils. In our experiments the beneficial effects were restricted to soils not contaminated by *A. euteiches* causing soft rot. When this pathogen was present, soft rot symptoms dominated and disease was acute. The control of one individual pathogen, e.g. *F. solani*, might create an ecological vacuum which possibly stimulates other pathogens. Consequently, it is important to correctly characterize the soil before introducing biocontrol agents.

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Chapter 11

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

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Abstract

Potential antagonists of *Fusarium solani* f. sp. *pisi* (Fsp) were selected from soil samples with different receptivity to this pathogen. They were tested against Fsp isolate 48 (Fs48), in systems of increasing complexity.
Most species tested *in vitro* were able to antagonize Fs48. *In vitro*, no relation could be established between the receptivity of the soil from which an isolate originated and its antagonism to Fs48. Three *Gliocladium roseum* isolates (Gr1,Gr2,Gr3) significantly reduced disease severity and prevented root weight losses caused by Fs48 in sterilized soils. A density of *G. roseum* of 10^5 conidia g^{-1} dry soil was sufficient in sterilized soil infested with Fs48 at 10^4 conidia g^{-1} dry soil. In naturally infested soils, in which soil biota were activated by growing pea before the assays, doses of 10^6 and 10^7 of *G. roseum* were required to reduce root rot. The antagonistic effects of *Pseudomonas fluorescens* (Pf) strains were variable and more evident in the absence than in the presence of *Aphanomyces euteiches* (Ae) in the root rot pathogen complex. No relation was found between the antagonistic activity of Pf and the receptivity of the soil from which they originated.

Additionalkey words: Soil micro-organisms, *Aphanomyces euteiches*, *Gliocladium roseum*, *Pseudomonas fluorescens*.

Introduction

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

The effect of the 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 pathogen, soils may be ranged from suppressive to conducive. Many characteristics of the soil have been reported to influence the level of SR (Rouxel, 1991). The cause of low SR often was 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 were made to identify micro-organisms involved and to exploit them in biocontrol (Baker, 1990).

Recently, conspicuous differences in SR to Fsp were found (Oyarzun et al.,1994 a). The possibility was open to investigate antagonistic activity of microflora associated with soils of low receptivity - synonymous to suppressive - in natural, highly infested soils considered of high receptivity or conducive to Fsp.

Common soil fungi and bacteria, including *Actinomycetes*, might have antagonistic potential towards Fsp. Some species pertaining to these groups have already been documented for control of root rot in peas or in others 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 *Fusarium roseum* (Lin and Cook, 1979). Controll of root rot caused by *F. solani* by non-pathogenic *Fusarium oxysporum* isolates was reported in bean (Lechappe et al., 1988), in red clover (Walz-Borgmeier, 1991) and in peas (Oyarzun et al., 1994 b). Anderson and Guerra (1985) reported protection of bean against *F. solani* f. sp. *phaseoli* by *Pseudomonas putida*. Actinomycetes are well known to antagonize pathogens with chitinous cell walls like *Fusarium* spp. (Campbell, 1989). Van der Spek (1968) reported successful reduction of root rot severity in soils highly infested with *Fusarium 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 *Fusarium solani* f. sp. *pisi*; (ii) to select potential antagonists, and (iii) to assess their effects on disease suppression in naturally infested soil where Fsp was occurring.

Materials and methods

The pathogen. In experiments with *Fusarium solani* f. sp. *pisi*, a monosporic culture of the virulent isolate Fs48 was used. The culture was conserved 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). The presence of Fsp, the varying composition of pea root rot pathogens, and their difference in receptivity to Fsp were used as criteria for selection. 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.

Microbial populations in rhizosphere and roots of peas grown in soils with different receptivity. Fungi, bacteria and actinomycetes were enumerated in pea plant rhizosphere soil (rs) and in and on the roots (rhizoplane: rp), respectively. Soil samples with low, SLR, (CTF, ROC, MA1) and high, SHR, (CTE, HA1, JON, BER, HER, DHO, HUL, HIL, HOE) receptivity to Fsp were examined.

Four minipots (4x4x12 cm) were filled with a known amount of the sieved soil samples.

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 characteristcs of soils, and soil type.

sample code	SR	Pathogens[a]			Root-rot DI[b]		Frequency[c]		[d]				Soil[e]
		Fs	Ae	Tb	B	F	Pea	Leg.	C/N	pH	Or.m%	Lime%	type
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
MAl	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
HAl	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.

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 hours light at 90 $W.m^{-2}$, and 80% RH. Three weeks after sowing, the root system had almost completely invaded the soil, which was therefore considered as 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. Then roots were 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, 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 hours.

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, random selections were made. Fungal isolates originated from SLR only.

Various isolates of *Actinomycetes* and pseudomonads, originating from soil samples with different degrees 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 in Petri dishes against Fs48. 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 just beside each other in the middle of the Petri dish ('juxta position'), (ii) the two fungi placed 2.5 cm apart from each other ('dual culture'). All combinations were replicated four times. An angular interaction and inhibition of mycelial growth were 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 *Pseudomonas fluorescens* were subcultured on King´s B medium, KBM (King et al., 1954), and individually tested for their ability to inhibit 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 hours 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 $5x10^4$ spores ml^{-1}, was evenly sprayed on 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 obtained, we did not further test actinomycete isolates and some fungal species, like *Mortierella*, 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 and filtered through cheese cloth. The spore suspension was 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. Soil samples used in this experiment were stored humid at 4° C for a period 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 either 10^5, $4x10^5$, or 10^6 spores g^{-1} of dry soil of *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 (2x4x12 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 experimental device (Oyarzun et al., 1994 b) under the same experimental conditions as described above.

Plants were harvested by cutting them at the soil level. All roots were cleaned with running tap water and assessed for symptoms of root rot (disease index) with the 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. Before this experiment the soil biota were activated by growing pea in containers with 25 kg of soil during 45 days. The resulting condition of the soil is called '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 in soils CTE, HA1, JON, BER and HER at 10^6 spores g^{-1} of dry soil.

Experiment 3. Experiment two was repeated with soils HER, HIL and HOE, at 10^7 spores g^{-1} of 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 wavelength. 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 $2x10^9$ cfu ml^{-1} was diluted with 1% methylcellulose (1:1 v/v) to obtain a final inoculum density of $1x10^9$. 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. 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 both tests, *Penicillium* spp. strongly reduced the growth of Fs48. In dual culture a pre-contact growth reduction of Fs48 occurred. The most effective species was *P. aurantiogriseum.*

In juxtaposition, but not in dual culture, all *Fusarium* isolates were able to reduce the growth of Fs48. The fast grower *F. culmorum*, however, made contact with Fs48 within a few hours, reducing drastically the growth of the pathogen.

In dual culture, isolates of *G. roseum* presented very slight inhibitory effects on the mycelial growth of Fs48. After direct contact the mycelium of *G. roseum* overgrew the established colonies of the pathogen producing discoloration and disturbance of the colony. No effects were found in juxta position.

Bacteria. Nineteen of the 23 fluorescent pseudomonad isolates significantly inhibited mycelial growth of Fo48 (Fig. 1). The largest effects were found with the isolates 2, 6 and 9 which reduced the growth by 60, 51 and 45% respectively. 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. Fs48 was inhibited by the different fluorescent pseudomonad isolates irrespective of the receptivity of the soil from which they were isolated.

Actinomycetes. Thirty of the 31 isolates, significantly reduced the spore germination of Fs48 ($P \leq 0.05$; data not shown). No relation was established between the receptivity of the soil from which the isolates originated and their antagonistic activity.

Fungal antagonism in soil. The choice of natural field soils to assay antagonists covered the diversity of soil types and pathogens producing a high level of root rot disease in peas (Table 1).

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									
Isolates	Low receptivity (SLR)			high receptivity (SHR)						
	CTF	ROC	MA1	CTE	HA1	JON	BER	HER	DBO	HUL	HIL	HOE
F. solani	91 abcd	50 bcde	0	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 ($P \leq 0.05$, based on LSD). 0 values were ignored.

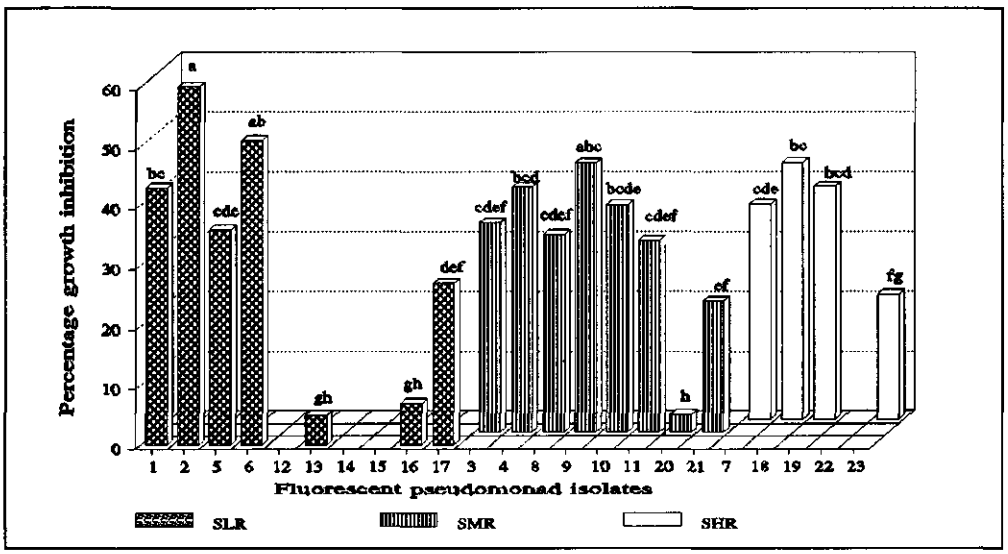

Fig 1. *In vitro* inhibition of mycelial growth (as percentage of the control) of *Fusarium solani* f.sp. *pisi*, Fs48, by fluorescent pseudomonad isolates originating from soils with low (SLR), moderate (SMR), and high (SHR) receptivity to Fs48. Significant differences are indicated by different letters 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]								
A	Sample code	Soil condition	Gr1	Gr2	Gr3	Pa	Pg	Fo1	Fo2	Fo3	Ref
	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 comparisons of DI; LSD^2 valid for all comparisons of dry weight. ($P \leq 0.05$).

Experiment 1. Infestation of the sterilized soils CTE, HA1 and JON with Fs48 led to a severe root rot disease level similar to that caused by Fsp and other naturally occurring root-rot pathogens in the 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 did work in soil JON 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 a poor development of 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).

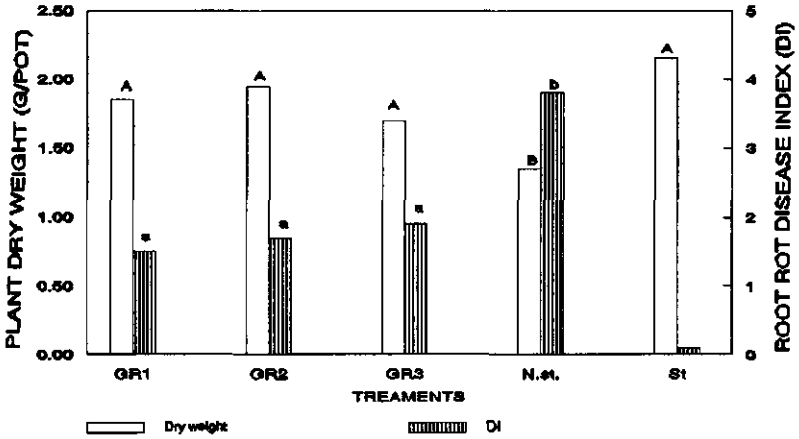

Fig 2. Antagonistic effect of three *Gliocladium roseum* isolates (Gr1,Gr2,Gr3) applied to a highly infested soil (JON) at a rate of 10^6 spores g^{-1} dry soil on the root rot severity (DI, 0 = healthy, 5 = 100% necrotic roots) and plant dry weight of pea. N.st. = control, non-sterilized without *Gliocladium*;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

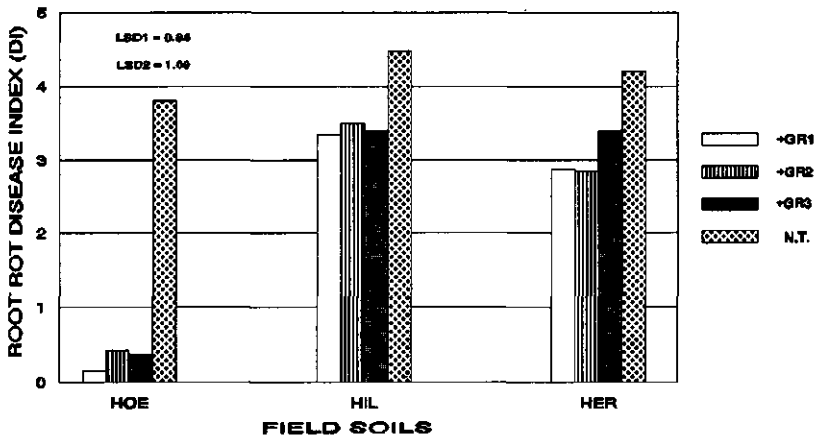

Fig 3. Effect of three *Gliocladium roseum* isolates (Gr1,Gr2,Gr3), applied to three highly infested soils (HOE, HIL and HER) at 10^7 spores g^{-1} dry soil, on root rot disease index (DI, 0 = healthy, 5 = 100% necrotic roots). NT = control, without *Gliocladium*. LSD1 for comparison within a soil, LSD2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

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 significantly less root rot than the

control, was found (Fig. 2). The dry weight of the 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} of 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 Figure 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.

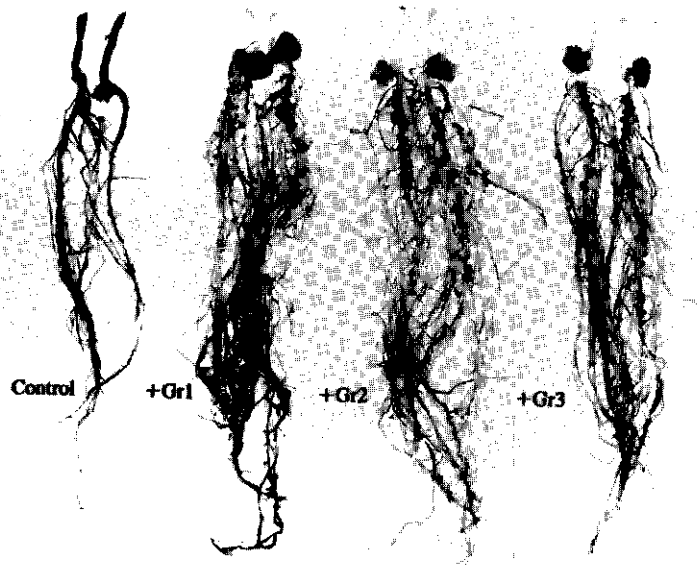

Fig 4. Root rot symptoms of pea plants growing in a highly infested soil (G17 = HOE) without (left plant) and with incorporation of *Gliocladium* isolates (+Gr1, +Gr2, +Gr3) to the soil.

Bacterial antagonism in soil

Seed coating. When applied as a seed coating, none of the 23 bacterial isolates revealed a reduction of the disease severity in the soils HER and BER. However, in soil JON the 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, isolate 8 only 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 the disease severity, but in soil DHO some isolates were effective. Some of these latter 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 gave 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.

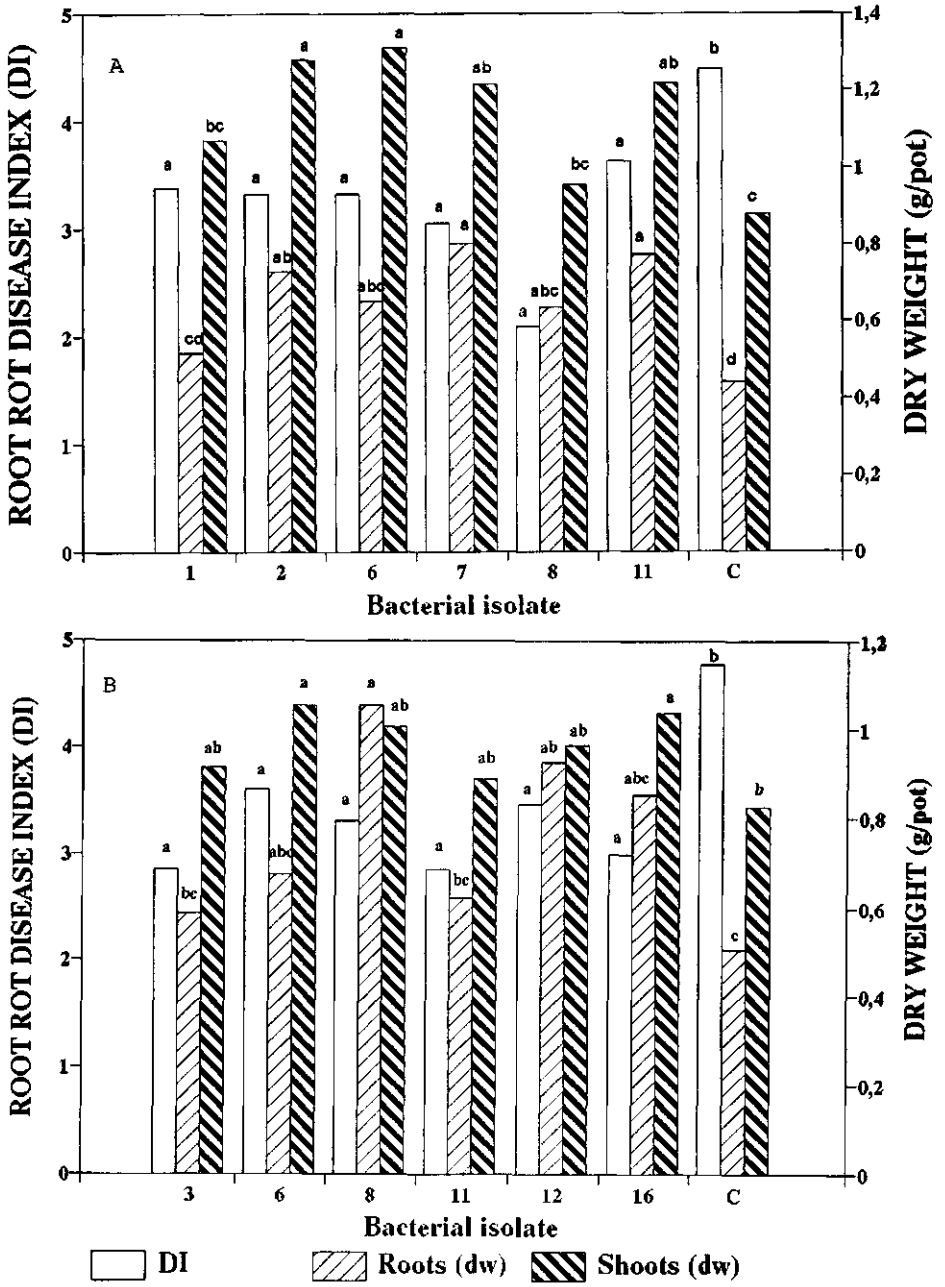

Fig 5. A. Antagonistic effects of fluorescent pseudomonad isolates, applied by seed-coating, on disease index (DI) and dry weight of roots and shoots of pea grown in a highly infested soil (JON). B. Antagonistic effects of fluorescent pseudomonad isolates, added to the soil, on disease index (DI) and 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).

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 provoke 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 were 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.

In the pea rhizosphere of SLR the highest number of *Penicillium* and *Actinomycetes* was found, but their abundance was soil dependent. In the pea rhizoplane of SLR the actinomycete populations were consistently high, in contrast to the virtual absence of *Pseudomonas fluorescens* and *Gliocladium* spp. In fact, the last two species were often present in SHR examined, 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. On agar, all fungal isolates selected from SLR affected the growth of Fs48, but in different ways. *F. culmorum* overgrew it. This occurred specially in juxtaposition tests where competition for nutrients starts immediately after putting both fungi on the artificial substrate. *Penicillium* spp. induced pre-contact growth reduction of the pathogen suggesting production of antibiotics. With *G. roseum* isolates, in dual culture a narrow zone of inhibited mycelial growth of Fs48 was observed. The zone could 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. This antagonistic behaviour 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 found between the receptivity of the soils from which fluorescent pseudomonads and *Actinomycetes* were isolated and their antagonistic ability in the *in vitro* tests, suggest that in the SHR some factor(s) impede or invalidate their antagonism. The relatively large amount of isolates of these species showing antagonistic activity stressed 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 natural, 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 black rot causing pathogen *T. basicola* 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. (1994 b) 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 nor by soil applications. This is at variance with 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 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 of antagonists *in vitro*, does not necessarily predict performance in natural conditions. Biocontrol agents are living entities with particular needs and ecological requirements. In our study various antagonists selected were effective in more complicated conditions. Several species were very antagonistic *in vitro*, particularly species of *Actinomycetes* and *Mortierella*. Insufficient inoculum production of these 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 More 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 be obtained also from highly receptive soils. Ineffective isolates obtained from SLR are not or not singly responsible for low receptivity. Therefore, to successfully control soil-borne 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.

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Chapter 12

Exploring factors associated with soil receptivity to some fungal root rot pathogens of peas

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Abstract

The relationship between various abiotic and biotic soil factors and the soil receptivity (SR) to three major pea root rot pathogenic fungi was investigated. Several relations were established. SR to *Thielaviopsis basicola* (Tb) showed to be positively associated with abundance of pseudomonads, soil reaction (pH), organic matter content and C/N coefficient. The number of saprophytic *Fusarium solani* in the rhizoplane was higher in soil less conducive to *Fusarium solani* f. sp. *pisi* (Fsp), whereas the abundance of indigenous Fs in the rhizosphere was unrelated to the receptivity. The amounts of soluble K, P, Mg and total C and N in soil, individually, were higher with increasing conduciveness to Fsp. The SR to *Aphanomyces euteiches* (Ae) was not well associated with any investigated soil aspect, except *Gliocladium* in the rhizoplane and *Acremonium* in the rhizosphere, of the which populations were higher in soil less receptive to this pathogen. Experiments with soil sterilization showed that the soil biota were the main factor responsible for the inhibition of the investigated pathogens. Therefore, despite its correlation with chemical and physical factors, SR to the investigated pathogens can be considered essentially of microbiological nature.

Additional keywords: *Fusarium solani* f. sp. *pisi*, *Thielaviopsis basicola*, *Aphanomyces euteiches*, *Pisum sativum*, multivariate analysis, soil micro-organisms.

Introduction

Soil is a complex habitat. Interactions between diseases caused by soil-borne pathogens and soils have been recognized for a long time. Walker and Snyder (1934) noted that *Fusarium* wilt of peas was common in silty loam soils, and that once established in a field, it persisted indefinitely, even in the absence of peas. Heavier soils did not become severely infested even with intensive pea growing. After inoculation experiments in pots with these soils they concluded that the wilt pathogen does not become equally destructive in all soils. In the Netherlands, Van Emden (1967) found soils where, despite the high frequency of potato cropping, *Rhizoctonia* seldom achieved a high incidence ('suppressive ' soils), whereas in other soils the disease incidence always reached high values ('conducive ' soils). The differences were related to marked differences in fungal flora of the soils.

Alabouvette et al.(1982) coined the term 'soil receptivity ' (SR) to indicate the effect

of the soil habitat on establishment, survival and capacity to produce disease by soil-borne pathogens. SR is a soil characteristic, assuming a value within a range between suppressive and conducive.

Most studies on soil receptivity to soil-borne pathogens point to the microflora (Louvet et al., 1976; Bouhot, 1979; Lemanceau, 1988), or to soil constituents affecting the microflora, as responsible for receptivity (Défago et al., 1990; Simon and Sivasithamparam, 1990; Sarniguet et al., 1992a,b).

In this study, we explore the association of SRs to three major pea pathogens with factors characterizing their habitat in field soils. The exploration was made at high integration level, making use of SR data from earlier work (Oyarzun et al., 1994a; Oyarzun and Maas, 1994). SR data resulting from ordination and classification analyses were combined with soil habitat data (Ludwig and Reynolds, 1988). Data on physical, chemical and biological variables were analyzed for association by means of ordination techniques, and simple and multiple regression analysis. Our ultimate aim was to identify variables which may explain SR.

Materials and Methods

Target pathogens. This study is targeted to three major pea root rot fungi, *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyder & Hansen (Fsp), *Thielaviopsis basicola* (Tb = *Chalara elegans* Nag Raj & B.Kendr., Boerema et al., 1993) and *Aphanomyces euteiches* Drechs. (Ae), investigated separately (Oyarzun et al., 1994a; Oyarzun and Maas, 1994).

The soils. The soils originated from a survey of a total of 51 commercial fields and 6 experimental plots, sampled in 1991. Two soils were sterilized and used as references in the SR tests.

SR data sets. SR information consisted of soil scores on the first Principal Component axis of a Principal Components Analysis, PCA, of root rot disease average (PG) or a PCA on fitted values obtained by adjusting the Weibull model to root rot disease responses (PW), as discussed in Oyarzun et al. (1994a). In addition, we use the Weibull scale parameter BE, representing for each pathogen-soil combination the dose necessary to cause 63% root necrosis. Soils with high, moderate and low receptivity will be indicated as SHR, SMR and SLR, respectively. Mainly due to high natural inoculum potential, several soils were discarded from analysis. Data sets on SRs consisted of 31 soil scores for Fsp; 33 for Tb and 27 for Ae, plus the references.

Abiotic characteristics. Concentrations of nitrate, ammonium, calcium, and phosphate were determined by spectrophotometry after extraction in 0.01 M $CaCl_2$. Sodium and potassium were quantified by flame spectrometry. pH was determined in $CaCl_2$. The determinations were made at the IPO laboratories of chemistry and soil ecology. The percentage of ashes, water content at field capacity (WHC: water holding capacity), carbon and total nitrogen were determined and complemented with farmers' informations originating from determinations made at the Laboratory for Soil and Crop Testing at

Oosterbeek. Data on soil structure and related factors like crust formation and drought tolerance, scored according to Boekel (1968, 1986), were available from our field survey on pea root rot (Oyarzun, 1991). The ranges and mean values of available data on physical and chemical soil characteristics are presented in Table 1.

Table 1. Ranges and means of some physical and chemical properties of soils tested for soil receptivity to each of the pathogens *Thielaviopsis basicola*, *Fusarium solani* f.sp. *pisi* and *Aphanomyces euteiches*.

Physical				Chemical				
Variable		min.	mean	max.	variable		min.	mean	max.
Ashes%		75.1	90.4	96.6	Calcium	mg/g	2.8	28.2	70.8
Silt%		0.1	32.5	55.0	pH-$CaCl_2$		4.6	7.3	7.8
OM%[1)]		1.6	2.8	10.2	Ammonium	mg/kg	1.7	10.7	35.5
Carbon%		0.9	2.4	12.7	Nitrate	mg/kg	1.2	15.6	73.4
Lime%		1.0	7.6	20.3	Nitrogen%		0.1	0.1	0.4
WHC %[2)]		18.0	31.6	60.0	Potassium	mg/kg	10.3	80.7	190.0
C/N		11.3	17.5	32.0	Phosphate	mg/kg	0.0	1.5	12.2
Structure		0.7	1.6	3.1[i]	Magnesium	mg/kg	28.4	57.3	200.0
Crust[3)]		0.0	1.4	5.0[i]	Sodium	mg/kg	4.6	29.1	60.3
Drought[4)]		0.0	0.8	3.0[i]					
Drainage		0.0	1.3	5.0[i]					

[i] = indexed values; 0 = good, not sensitive, 5 = very bad or highly sensitive.
[1)] organic matter; [2)] Water holding capacity = gravimetric soil water content at field capacity;
[3)] sensitivity to crust formation; [4)] sensitivity to drought.

Biotic soil characteristics

Cropping history. Data on cropping sequences of the fields were supplied by farmers. The frequency of crops grown on the fields during the last six years are shown in table 2. Soils from a continuous cropping experiment at the PAGV in Lelystad, included in all SR tests, were examined in more detail. They originated from a 10-year-old monoculture experiment, with maize (*Zea mais*), broad bean (*Vicia faba*), flax (*Linum usitatissimum*), dwarf bean (*Phaseolus vulgaris*) and onion (*Allium cepa*). Since all samples originated from one homogeneous site, they were discarded from the analysis which correlated physical properties to SR. They were separately analyzed for cropping effects on SR.

Table 2. Frequency of crops, expressed as a percentage, grown on the sampled fields over the last six years.

	Crops*									
	Po	Sb	Ww	Sw	Ma	Gr	Le	On	Mfc	Oc
Frq	11	18	27	4	2	6	20	3	7	2

* Po = potatoes; Sb = sugar beet; Ww = winter wheat; Sw = summer wheat; Ma = maize; Gr = grasses; Le = legumes; On = onions; Mfc = minor field crops; Oc = other crops.

Soil micro-organisms. Fungi, actinomycetes and bacteria were determined in and on pea roots and in the rhizosphere of 21-day-old seedlings for a subset of 40 samples. Details of the isolation procedures are presented elsewhere (Castejón-Muñoz and Oyarzun, 1994). Colonies were enumerated at 10^{-4} for fungi, 10^{-6} for pseudomonads and 10^{-8} for total bacteria and actinomycetes. Complementary, all available samples were bioassayed for soft rot in Petri dishes (Oyarzun, 1991) and for black rot using carrot discs (Lloyd and Lockwood, 1962). The $\log_{10}(x+1)$ transformed mean values of the numbers of micro-organisms per gramme in rhizosphere and rhizoplane are presented in Table 3. Of the 40 samples, SR to Fsp data were available in 26 cases, to Ae in 25 and to Tb in 27 cases.

Table 3. Abundances of micro-organisms determined in samples tested for SR to *Thielaviopsis basicola*, *Fusarium solani* f.sp. *pisi* and *Aphanomyces euteiches* expressed as mean values of $\log_{10}(x + 1)$ transformed numbers (cfu) of the species isolated from rhizosphere (rs) and rhizoplane (rp) of peas.

Species of micro-organisms	rs	rp	dilution factor	Isolation medium
P. fluorescens	0.26	0.34	10^6	Gould's S1. Counted under NUV (λ = 365 nm).
Pseudomonas total	0.33	0.43	10^6	Gould's S1 non-fluorescent.
Bacteria total	0.50	0.88	10^8	TSA. Tryptone Soya Agar.
Actinomycetes	0.55	0.55	10^8	COA. Chitin Oat agar.
F. solani	0.40	0.90	10^4	SFA. Selective Fusarium Agar.
F. oxysporum	0.22	0.63	"	"
Fusarium spp	0.42	0.58	"	"
Gliocladium spp	0.53	0.52	"	Selective solidified Medium.
Acremonium spp	0.53	0.81	"	WA+. Water Agar + tetracycline.
Pythium spp	0.41	0.26	"	Pythium medium.
Mortierella spp	0.24	0.10	"	WA+.
Penicillium spp	0.59	0.44	"	WA+.
Cylindrocarpon spp	0.11	0.13	"	SFA.
Paecilomyces spp	0.18	0.08	"	WA+.
T. basicola	0.08	0.10	"	VDYA_PCNB. Thielaviopsis medium.
Ascochyta spp	0.07	*	"	Phoma selective medium.
Rhizoctonia spp	0.07	*	"	WA+.
Alternaria spp	0.06	*	"	WA+.

*: non-determined.

Procedural steps in data analysis. To explore the association between SR and soil sample characteristics, each variable was first plotted against SR. When necessary the data were transformed in several ways, including normal standardization, ranks, logarithms, indexing or simply presence-absence. As criteria for transformation the width of the range (scale) and the skewness of each variable were used. Second, simple correlations were determined.

Ordination. Using ordination procedures, Correspondence Analysis (CA), Principal

Components Analysis (PCA), Redundance Analysis (RDA) and Canonical Correspondence Analysis (CCA), described by Ter Braak (1987a) and available in CANOCO (Ter Braak, 1987b), multiple relations between soil variables and SR were studied.

Data on abiotic factors. In order to visualize main features of the data set, relations between abiotic variables were investigated by a Principal Components Analysis, PCA, on a covariance matrix. Ordination scores were scaled to produce a correlation biplot, the angle between variables representing their correlation.
Constrained ordinations. Relations between SR and abiotic environmental variables were investigated by Redundancy Analysis (axes constrained to represent abiotic variables) on a covariance matrix. A correlation biplot was constructed to visualize reciprocal relations between variables in the analysis.

Data on micro-organisms. Abundance of species of micro-organisms was ordinated, with the axes constrained to represent SR, in a Canonical Correspondance Analysis, CCA.

Data on cropping. Data on cropping sequencies were transformed to crop species presence/absence per sample and ordinated together with the classified receptivity of the sample for each pathogen individually in correspondance analysis.

Multiple regression. Best Subset Selection procedures available in GENSTAT5 (RSelect, Goedhart, 1992) and STATISTIX (Anonymous, 1985) were used to asses simultaneous associations of chosen sets of variables and SR. As criteria of selections were used: the adjusted and non-adjusted coefficients of determination, the CP-Mallow coefficient, a criterium for tolerance used to limit the maximum degree of association between criterium variables in the set, and finally the value of the t-statistics for the significance of the regression coefficients. The relations found were graphically summarized by using results obtained in ordination analyses.

Effect of soil sterilization on SR. In the winter of 1992, a subset of 15 fields was sampled and tested again, this time with a mixture of the three pathogens. To estimate the overall contribution of soil biota to the receptivity level, subsamples of the soil samples were sterilized by γ-irradiation (4 Mrad). Production of inoculum, inoculation and test methods used to determine SR to Fsp, Tb and Ae, were published elsewhere (Oyarzun et al., 1994 a; Oyarzun and Maas, 1994). A mixture of equal numbers of spores of Tb, Fsp and Ae was added at 10, 100 and 1000 spores in total per g dry soil. In two higher concentrations, 10.000 and 50.000 spores.g^{-1} dry soil, the Ae dose was reduced to 10% and 4% of the total number, respectively.

Test procedures, experimental conditions and disease assessments were as described for Fsp (Oyarzun et al., 1994a). Results were analysed by ANOVA and disease responses per soil clustered for graphical representation.

Results

Soil abiotic characteristics. The relationships between variables representing chemical fertility of the samples tested are summarized in a PCA diagram in figure 1. The diagram, representing 55% of the total variance between variables, was obtained from a PCA on a variance-covariance matrix, after centring and standardizing data. On the first PC axis ($\lambda 1 = 0.37$) C, total nitrogen (N_t), Na, Mg, K and P are contrasted with pH and Ca, indicating that the axis represents a soil reaction gradient and that richness in macronutrients increases with increasing acidity. On the second PC-axis ($\lambda 2 = 0.18$) NO_3 is negatively correlated with K and Na. Figure 2 shows a similar analysis for some physical variables. Variables expressed as percentages were transformed to $\log_{10}(x+1)$ but indexed variables were not transformed. Several wellknown facts (Boekel, 1986) become apparent. On the first axis ($\lambda 1 = 0.37$), fields with high silt (fraction ≤ 16 μm) content tend to have a bad structure and a high water holding capacity (WHC). The latter is correlated with the organic matter content (O.M%). In contrast, light soils were more liable to drought and crust formation. The second axis ($\lambda 2 = 0.30$) shows that soils having a high lime content tend to have a lower organic matter content. Heavy soils were better drained than the light ones.

Factors associated with SR

Abiotic characteristics

Chemical properties. **Tb.** Using simple correlations of untransformed data, increased suppressiveness to Tb, as given by parameter BE, showed significant ($P \leq 0.05$) positive correlations with NH_4 ($r = 0.36$), P ($r = 0.62$), Mg ($r = 0.48$) total nitrogen ($r = 0.41$) and total carbon ($r = 0.54$). The pH ($r = 0.64$) and $N0_3$ ($r = 0.42$) were negatively correlated with suppressiveness to Tb. The ordination in figure 3, resulting from a RDA of SR variables (BE and PG) and chemical variables, illustrates the above presented relationships. In RDA, the SR variables were ordered with respect to the axes which are linear combinations of the chemical variables ($P \leq 0.05$, I-axis, based on Monte Carlo permutation test). In multiple regression ammonium and nitrate together explained at least 60% of the variance of BE (R^2-adj = 0.60; R = 0.81). After transformation (ranking or normalization) the simple correlations were maintained for $N0_3$, Mg and C (Table 4). The set of $N0_3$, P and C was significant in multiple regression analysis (Table 5).

Fsp. Raw or transformed data of K, P, Mg, total N and C each produced significant negative correlations ($P \leq 0.05$) with increasing suppressiveness to Fsp, as given by PCA axes (Table 4). Correlations with BE were not significant. Significant multiple correlations were found with the subset Ca, NH_4, NO_3 and C (R^2-adj = 0.33 and 0.39 for PG and PW, respectively). With $R = 0.72$ ($P \leq 0.05$), BE presented a significant correlation with Ca, NH_4, K, P, Na and C (Table 5).

Ae. No significant simple correlations were found between SR to Ae and soil nutrients. P and total nitrogen (N_t) values, transformed or untransformed, accounted together for about a quarter of the variance in the SR data (Table 5).

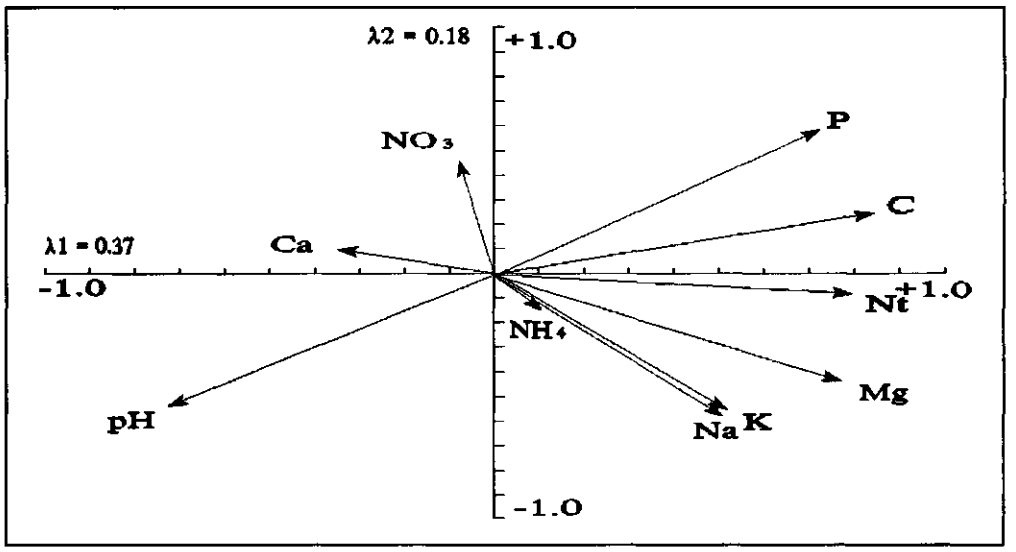

Figure 1. Scatter of soil chemical properties of a sample of field soils used in researching receptivity to root rot pathogens of pea. The figure was obtained from a PCA on a variance/covariance matrix with standardized data. The correlations between variables are represented by the angles between the vectors.

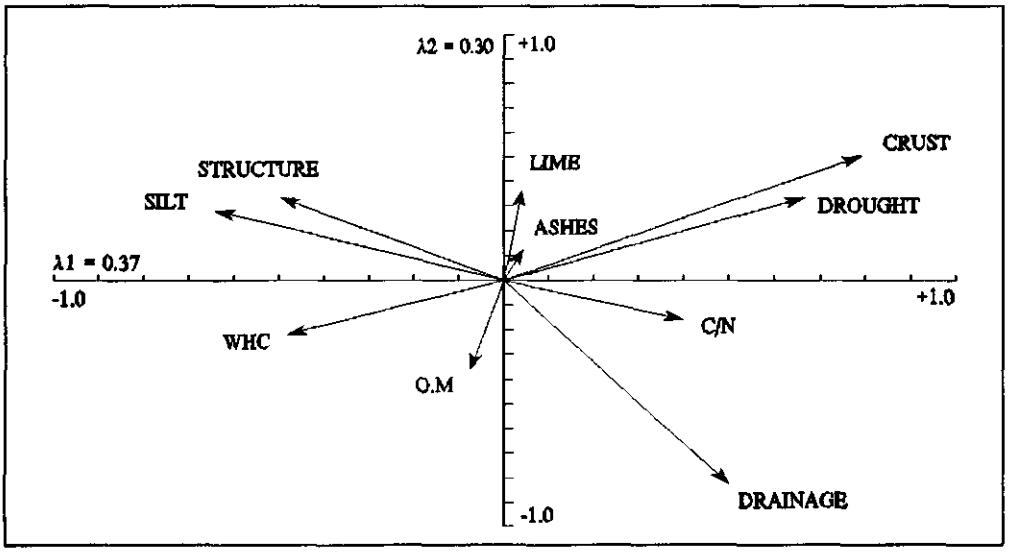

Figure 2. Scatter of some physical properties of a sample of field soils used in studying receptivity to root rot pathogens of pea. The figure was obtained from a PCA on a variance/covariance matrix. Data in percentage were transformed to $\log_{10}(x+1)$, indexed values were not transformed. The correlations between variables are represented by the angle between their vectors. In percentage: WHC = water content at field capacity; silt; ashes; C/N = carbon/nitrogen quotient; lime and O. matter. Indexed (index 0 = good; 5 = bad condition): structure; crust formation; drainage and drought sensitivity.

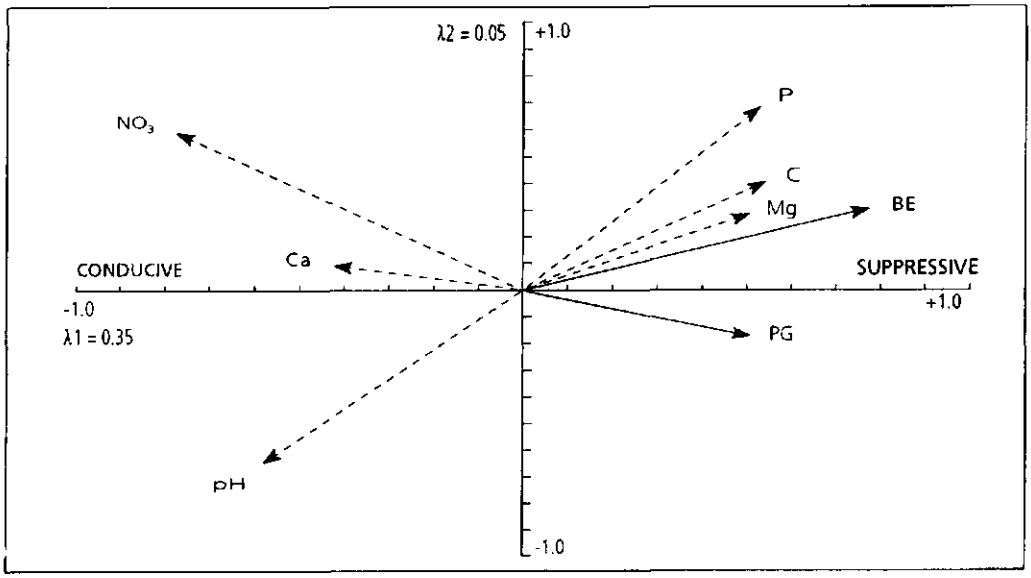

Figure 3. Results of Redundancy Analysis (RDA) of soil receptivity to *T. basicola*, represented by BE and PG, and six chemical environmental variables. The variables increase in the direction of the arrows. The angle between two arrows (broken = chemical; full pattern = SR) indicates the degree of correlation. $\lambda 1 = 0.35$, $\lambda 2 = 0.05$. The variance accounted for by the first axis = 88% of the SR-chemical relations. (Monte Carlo permutation test for the first axis, $P \leq 0.05$).

Table 4. Simple correlations between soil receptivity (SR) to *Thielaviopsis basicola* (n=34; R(0.05, 32) = 0.34); *Fusarium solani* (n=32; R(0.05, 30) = 0.35) and some chemical properties of tested soil samples. Data of criterium variables were normalized. BE, PW and PG = SR data.

	Pathogens and receptivity criteria					
	Tb			Fsp		
Variables	BE	PW	PG	BE	PW	PG
Magnesium mg/kg	0.34*	-0.22	0.23	-0.07	0.37*	-0.41*
Potassium mg/kg	0.25	0.00	0.01	-0.26	0.36*	-0.40*
Phosphate mg/kg	0.30	0.01	0.01	-0.29	0.36*	-0.38*
Nitrate mg/kg	-0.39*	0.32	-0.30	0.08	0.06	-0.07
Nitrogen %	0.24	-0.01	0.01	-0.19	0.38*	-0.44*
Carbon %	0.44*	-0.26	0.25	-0.28	0.39*	-0.42*

* = significant at $P \leq 0.05$.

Physical properties. **Tb.** Tb was the only pathogen for which SR showed significant simple correlations with soil physical variables (Table 6). For raw data, SR, as represented by BE, significantly correlated with ashes % (r = -0.58), silt% (r = -0.41), C/N (r = 0.62), WHC (r = 0.42) and organic matter% (r = 0.77). Organic matter,

WHC, Ashes and C/N were also significantly correlated with SR variables after normalization (Table 6). In multiple regression analysis, organic matter(%) and the liability to crust formation explained together 69% of the adjusted variance of BE (Table 7).

The relation between the physical properties and PCA axes was slight and complicated. The (linear) contribution of several variables was required to explain part of the data (Table 7). Soils with high organic matter, low carbonate content and good drainage tended to be less receptive ($P \leq 0.05$).

Fsp. No simple correlations were established. In multiple regression analysis (Table 7), WHC and drought sensitivity accounted for more than a quarter (R^2-adj = 0.27 for raw data; R^2-adj = 0.33 after transformation) of the variance in SR, represented by the PCA axes ($P \leq 0.05$). BE did not correlate.

Ae. Drought sensitivity and liability to crust formation yielded a significant R^2 of about 0.29 with the PC-axes. The adjusted R^2 did not reach significance, indicating an instable contribution of one of these variables to explain SR variance.

Table 5. Multiple regression analysis of soil receptivity (SR) variables to *T. basicola*, *F. solani* f. sp. *pisi* and *A. euteiches* and soil macronutrients. *t-values* of the regression coefficients, non-adjusted (R^2) and adjusted coefficient of determination.

	Ca	NH4	NO3	K	P	Na	Nt	C	R^2	R^2-adj	SR
Tb (n = 34)											
			-3.38		3.14			2.60	0.47^{***}	0.49^{***}	BE
Fsp(n = 32)											
	2.29	2.38		-1.32	-1.46	1.97		-3.42	0.51^{**}	0.40^{*}	BE
	-3.47	-3.34	2.61					4.48	0.47^{***}	0.39^{***}	PW
	3.22	2.29	-2.43					-4.16	0.42^{***}	0.33^{*}	PG
Ae (n = 28)											
									-	-	
					-3.16		-3.16		0.32^{*}	0.26^{*}	PW
					-3.16		-3.23		0.32^{*}	0.27^{*}	PG

R significant at $P \leq 0.05$ (*); 0.025 (**); 0.01 (***).

Table 6. Simple correlations between soil receptivity (SR) to *Thielaviopsis basicola* (r(0.05, 26)= 0.37) and some physical properties of soil samples (independent variables normalized). Samples of continuously cropped fields omitted from analysis. BE and PG = receptivity data.

SR	Soil variables[1)]			
	Ashes%	O.m.%	WHC	C/N
BE	-0.46*	0.43*	0.34	0.42*
PG	-0.26	0.41*	0.39*	-0.09

[1)]. see table 1.

Biotic characteristics

Cropping history and SR. **Tb.** No interesting relation was found between SR to Tb and cropping history.

Results of Correspondence Analysis (CA), based on presence/absence of the crop species on the field in the past 6 years and the SR, are summarized in figure 4 A,B for Fsp and Ae respectively.

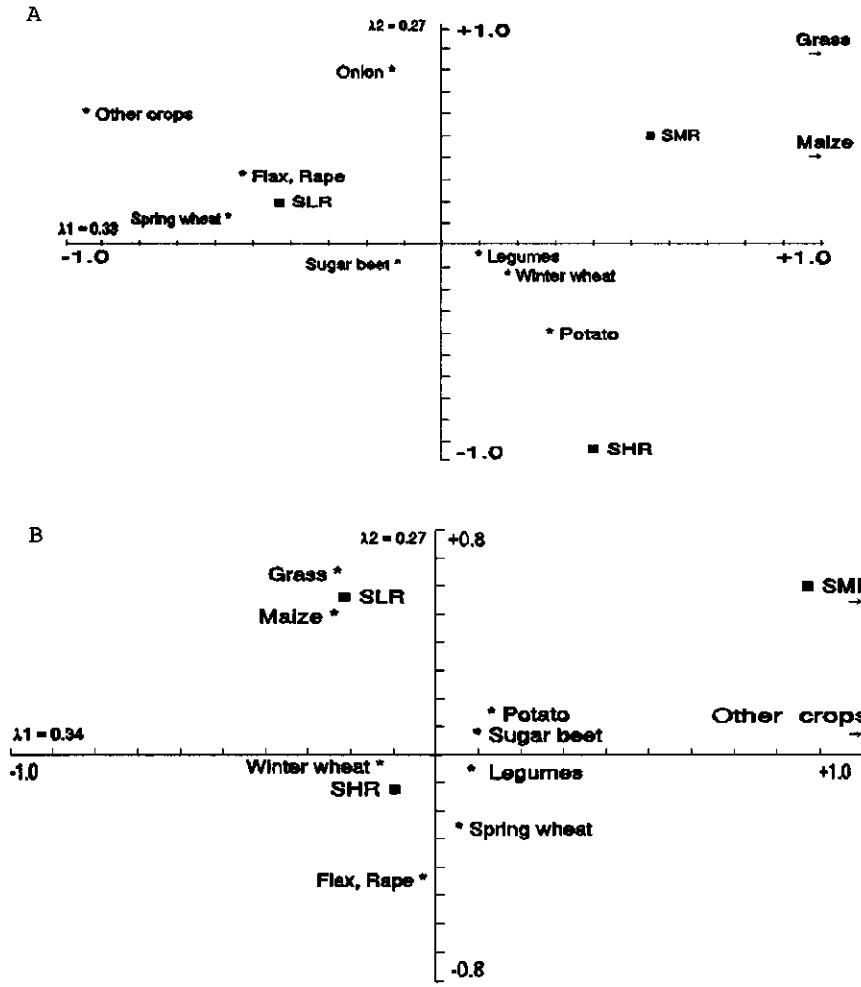

Figure 4 A,B. Ordination diagrams resulting from a Correspondence Analysis (CA), based on a table of samples by presence/absence of crops on fields in the last six years and data of soil receptivity according to a classification in Low (SLR), intermediate (SMR) and high receptivity (SHR) to (A) *Fusarium solani* f.sp. *pisi* (var% = 44) and (B) *Aphanomyces euteiches* (var% = 45).

Table 7. Multiple regression analysis of soil receptivity (SR) on some physical soil properties. t-values of the multiple regression coefficients, non-adjusted and adjusted coefficients of determination for the regression of SR data of *T. basicola* (Tb) and *F. solani* f. sp. *pisi* (Fsp) on some soil variables. SR data as BE, PW and PG.

Pathogens		Variables									
	SR	Silt%	O.m%	Lime%	Strc.	WHC	Crust	Drought	Drain	R^2	R^2-adj
Tb											
	BE		7.5				-3.2			71.3	69.0*
	PW	-3.4	-4.1	3.5	2.2		-3.0			59.0	47.3*
	PG	3.4	4.1	-3.6	-2.0		2.9		-3.5	60.4	49.1*
Fsp											
	BE									-	-
	PW					2.9		3.1		33.2	27.4*
	PG					-2.9		-3.1		33.3	27.5*

See table 1 for abbreviations of variables.
* R significant at $P \leq 0.05$; - = not significant according to F-test.

Table 8. Simple correlations between the $\log_{10}(x+1)$ number of micro-organisms in the pea rhizoplane and rhizosphere and the receptivity of the soil (SR) to the pea root rot pathogens *Thielaviopsis basicola* (Tb; n = 27; R(0.05, 25) = 0.38), *Fusarium solani* f.sp. *pisi* (Fsp: n = 26; R(0.05, 24) = 0.39); *Aphanomyces euteiches* (Ae; n = 25; R(0.05, 23) = 0.40). SR data as BE, PW, PG.

Site and Species	Pathogens and SR criteria								
	Tb			Fsp			Ae		
	BE	PW	PG	BE	PW	PG	BE	PW	PG
Rhizoplane:									
P. fluorescens	.41*	-.17	.15	-.04	-.13	.11	.24	-.16	.18
Pseudomonas total	.44*	-.24	.22	-.05	-.17	.14	.21	-.15	.18
Bacteria total	.08	.43*	-.44*	-.28	.25	-.32	.03	.02	.00
F. solani	-.42*	.29	-.31	.24	-.45*	.51*	-.04	.01	-.02
Gliocladium spp	-.32	.25	-.24	.04	-.24	.20	.40*	-.32	.34
Acremonium spp	-.02	.10	-.11	.41*	-.40*	.37	.28	-.28	.31
T. basicola	-.42*	.66*	-.66*	-.19	.20	-.23	-.07	.06	-.06
Rhizosphere									
Acremonium spp	03	-.13	.11	.22	-.23	.22	.37	-40*	.36
Ascochyta spp	-.25	.34	-.34	-.48*	.58*	-.52*	-.08	.07	-.14
Mortierella spp	.25	-.41*	.42*	-.10	.07	.03	-.23	.14	-.22
Penicillium spp	.41*	-.36	.38*	.06	.08	-.10	.09	-.12	.12

* R significant at $P \leq 0.05$

Fsp. For Fsp, soils presenting low receptivity were more frequently cropped with minor agricultural crops among which flax and oilseed rape. On soils with high receptivity, potatoes were more frequently present (Fig. 4A). Winter wheat, sugar beet and legumes have a neutral position near the origin.

Ae. Soils with low receptivity, SLR, occurred in fields were grasses and maize were frequently present, whereas soils with moderate receptivity (SMR) were associated with other minor crops such as poppy and chicory (Fig. 4B).

In both figures, the percentage of variance accounted for by the two first CA-axes was about 45%, but the variables were not well separated ($\lambda 1 = 0.3$; $\lambda 2 = 0.3$). For this reason we did not engage into a detailed analysis of the relationship of SR and cropping history.

Continuous cropping. The disease responses obtained in testing SR to the three pathogens with soils from the fields in continuous cropping are given in figures 5 A-C. These figures indicate an unfavourable environment for the pea pathogens Fsp and Tb with continuous growing of flax. In contrast, a very conducive soil environment was developed by growing *Vicia* beans continuously. These different soil environments did not affect the pathogenic activity of Ae to the same extent as of the other two pathogens (Figure 5c).

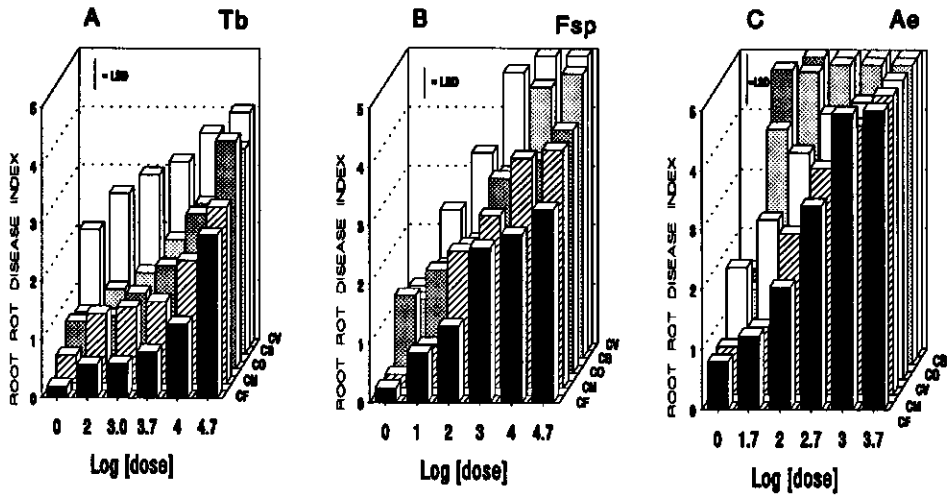

Figure 5. Root rot disease index (0 = healthy; 5 = 100% necrosis) resulting from infestation of the soils after continuous cultivation of flax (CF), maize (CM), *Phaseolus* beans (CB); onion (CO) and *Vicia* beans (CV) with increasing quantities of inoculum of *T. basicola* (A), *F. solani* f.sp. *pisi* (B) and *A. euteiches* (C). Bar represents LSD to distinguish mean disease responses between soils at each inoculum level.

Soil micro-organisms. Analysis of log transformed numbers of individual micro-organisms from rhizoplane and rhizosphere indicated a few species showing significant and differential correlations with SR to Tb, Fsp and Ae. In the rhizoplane, more individual species of micro-organisms showed significant correlations with SR than in the rhizosphere (Table 8).

Tb. *Pseudomonas* spp were associated with soils of low receptivity to Tb whereas total abundance of bacteria correlated with high receptivity. With increasing receptivity to Tb, abundance in the rhizoplane of indigenous Tb and Fs tended to be high, whereas SR was inversely proportional to the abundances of *Mortierella* and *Penicillium* in the rhizosphere. When the joined linear contribution of micro-organisms to explain SR data is considered (Table 9) it becomes apparent that bacteria (including fluorescent and non-fluorescent pseudomonads and total bacteria), *Pythium* spp and indigenous *Thielaviopsis* in the rhizoplane (rp), largely account for the variance in SR to Tb ($R = 0.84$; R^2-adj $= 0.65$; $P \leq 0.001$). In rhizosphere soil (rs) the total bacteria, *F. solani*, *F. oxysporum* and *Mortierella* spp. realized the largest contribution (R^2-Adj $= 0.55$ and 0.49 for PW and PG respectively) to explain SR to Tb. Bacteria and *F. solani* were negatively loaded, *F. oxysporum* and *Mortierella* spp. positively.

Table 9. Multiple correlation coefficient and the adjusted coefficients of determination (R^2-adj.) for the regression of SR to *T. basicola* (Tb), *F. solani* f. sp. *pisi* (Fsp) and *A. euteiches* (Ae) on micro-organism abundance, $\log_{10}(x+1)$, in the rhizoplane (rp)and the rhizosphere (rs) of pea. The receptivity of each soil is represented by its score on the first-PCA axis determined from fitted values obtained with the Weibull model, PW.

Pathogen	site	R	R^2-adj.	Species in the regression
Tb	rp	0.84^{***}	0.65^{***}	*Pseudomonas fluorescens*, Pseudomonads, Bacteria, *Pythium* spp., *T. basicola*.
	rs	0.78^{***}	0.55^{***}	Bacteria, *F. solani*, *F. oxysporum*, *Mortierella* spp.
Fsp	rp	0.56^{*}	0.26^{*}	Bacteria, *F. solani*, *Acremonium* spp.
	rs	0.73^{**}	0.45^{**}	Bacteria, Actinomycetes, *F.oxysporum*, *Acremonium* spp., *Ascochyta* spp.
Ae	rp	0.54^{*}	0.20	*Gliocladium* spp., *Paecilomyces* spp.
	rs	0.52^{*}	0.24^{*}	*Fusarium* spp., *Acremonium* spp.

R significant at: $P \leq 0.05$ (*); $P \leq 0.025$ (**); $P \leq 0.01$ (***).

Fsp. *Acremonium* spp and indigenous *F.solani* (Fs) were abundant ($P \leq 0.05$) in the rhizoplane of peas in soil with low receptivity. SR was uncorrelated with the abundance of indigenous Fs and negatively correlated with *Ascochyta* spp. in the rhizosphere (Table 8). The contribution of indigenous Fs, *Acremonium* and total bacteria in the rhizoplane to explain variance in SR was modest (R^2-Adj = 0.37; $P \leq 0.05$) for PG, and even less (0.26) for PW (Table 9). In figure 6, an ordination diagram is presented resulting from a Canonical Correspondence Analysis of micro-organism abundance in the rhizosphere on receptivity. The two first axes account for 81% of the variance of the species-SR relations. This figure illustrates the higher abundances of *Actinomycetes*, *F. oxysporum* and *Acremonium* in suppressive than in conducive soils. In multiple regression analysis, the log of the number of the same micro-organisms increased whereas the number of *Ascochyta* and bacteria decreased (R^2-Adj = 0.45 and 0.47 for PW and PG, respectively; $P \leq 0.025$) with increasing suppressiveness (Table 9).

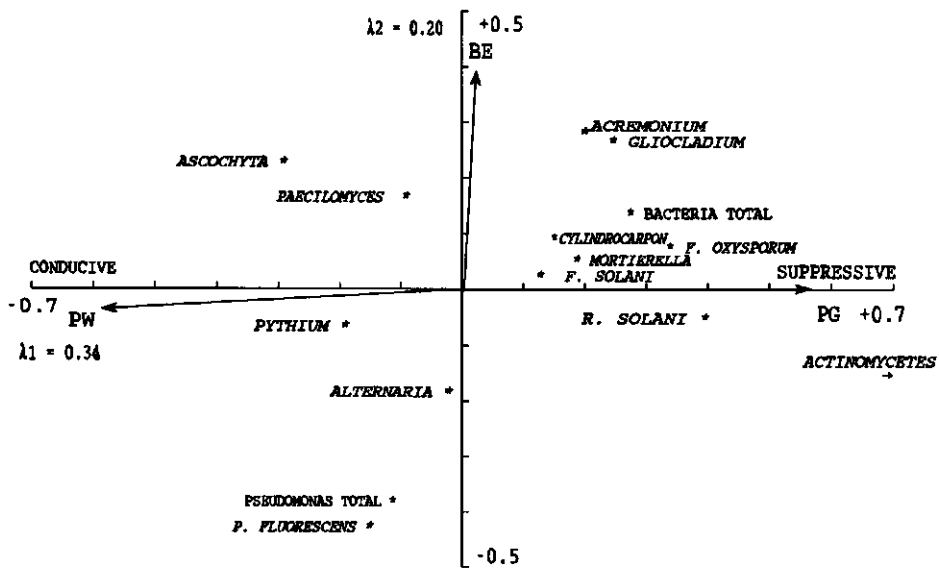

Figure 6. Results of Canonical Correspondence Analysis (CCA) of micro-organism species abundance and three variables BE, PG and PW, representing SR to *F. solani* f.sp. *pisi*. $\lambda 1 = 0.34$, $\lambda 2 = 0.20$. The biplot, micro-organism species (asterisks) and SR (arrows), represents a var% = 81 of the species-SR relation.

Ae. In the rhizoplane, the number of *Gliocladium* increased with decreasing conduciveness (Table 8). In the rhizosphere, the same occurred with *Acremonium* spp. Multiple regression of SR to Ae on micro-organism abundance both in rhizoplane and

rhizosphere showed few significant associations (Table 9). In the rhizosphere the relation with *Acremonium* and *Fusarium* spp, reached statistical significance but to a modest level (R^2-Adj = 0.24). In the rhizoplane the relation of *Gliocladium* and *Paecilomyces* with SR, as BE, was just significant (R^2-Adj = 0.25; $P \leq 0.05$) but for the PCA axes the R^2-adj was not significant.

Effect of soil sterilization on SR. Sterilization of the soil led to a drastic increase of receptivity to the mixture of Fsp, Tb and Ae ($P \leq 0.01$, based on LSD). In sterile soils the first dose (10 spores.g^{-1} dry soil) produced maximum differences in disease response to inoculation between soils and led to a drastic reduction in dry weight of the test plants in all soils ($P \leq 0.01$; data not shown). In 12 sterilized soils out of 15 examined, a dose of 100 spores g^{-1} and higher produced a severe to very severe root rot (DI $\geq$ 4: 0 = healthy roots; 5 = 100% necrotic roots). To summarize the effects, soil samples were analyzed for their similarity in disease responses at each infestation level and clustered (Digby et al., 1989). Figure 7 visualizes the great difference between the sterile and natural soil groups and the high degree of similarity in disease response of all samples after sterilization.

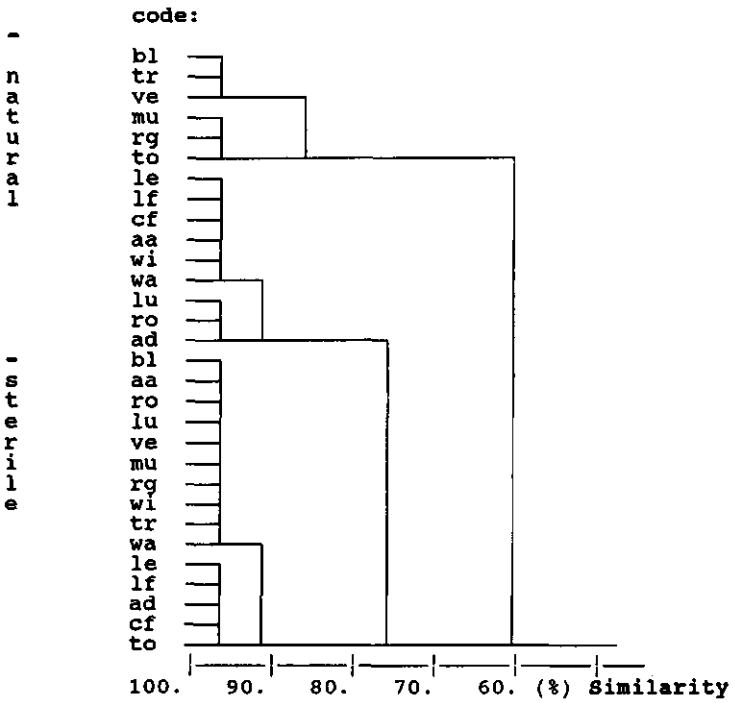

Figure 7. Dendrogram, obtained after an average linkage Cluster Analysis, based on similarity coefficients of field soil samples, natural and sterilized, calculated from root rot disease responses to increasing soil infestation level of *A.euteiches*, *F. solani* f.sp. *pisi* and *T. basicola*.

Discussion

Exploring SR for associations. Preliminary remarks. The results, which are of descriptive nature, mostly originated from observational research. The environmental variables are often redundant and highly intercorrelated. Lack of significant correlation of individual variables with the response should not be interpreted as lack of association. Explanatory variables can be complementary. The noise in the environmental data confers a weakness to the statistics resulting from multiple regression or multivariate analysis. James and McCulloch (1990) suggest, when a battery of explanatory variables is available, to combine them into biologically meaningful groups before exploring the best subset in regression. We used multiple regression only to look for association between SR and selected soil variables. We intended to identify those variables which accounted significantly, individually or in combination, for the variance in SR, in order to direct future investigations. Considering the nature of the problem, the number of fields investigated can be considered as low. The limited sample set represented a configuration with characteristic relations between chemical and physical variables, and with the crop rotations in which pea crops occur in the Netherlands. For technical reasons many other soil factors, such as micronutrients or micro- and mesofauna including nematodes, protozoa and collembola, were not included though they are known to have important effects on soil receptivity (Hofman, 1988; Campbell, 1989; England et al., 1992).

Since the data represent many variables of the same object (soil), multivariate techniques provide an efficient tool for analysis (Morrison, 1990). They have often been used in eco-phytopathological research (Stynes and Veitch, 1983; Doublet, 1986; Lechappe, 1986; Savary, 1987; Lucas and Nignon, 1987; Sarniguet et al., 1992b). Each environmental data set can be reduced to a few factors, and new variables can be created from a combination of original variables. In principal component regression (Goedhart and Ter Braak, 1990) a continuous response variable is regressed on such a factor. It is also possible to ordinate dependent variables with the restriction that sample scores are the best combination, linear or not, of the environmental variables. Redundancy analysis (RDA) and canonical correspondence analysis (CCA) were developed for this purpose (Ter Braak, 1987a,b). We used these techniques in accordance with the nature of the data available (James and McCulloch, 1990).

Abiotic characteristics. Soil factors such as soil water availability, pH, available Ca in soil solution and soil type have important implications for soil-borne diseases (Colhoun, 1973) and SR to micro-organisms (Rovira et al., 1992). In fact, each micro-organism has a characteristic ecological range and optimum for growth and function. The pH range of most soil bacteria extends from 4 to 8≡1539Xwhereas the soil-inhabiting fungi is 1 or 2 pH points lower (Myrold and Nason, 1992). The growth and activity of a micro-organism in its environment can be very different from that found in an isolated situation (Davey and Danielson, 1968), and therefore responses attributed

to micro-organisms have to be interpreted cautiously.

Chemical properties. P and K are commonly the only fertilizers used on peas in the Netherlands (Timmer et al., 1989). Both nutrients had a wide range (Table 1). With the exception of 2 samples consisting of peat and a mixture of peat and sand, which were acid, the soils examined were neutral to alkaline (pH 6.8 - 7.8). However, the symbiotic N-fixation liberates the cation H^+ and causes a decrease of 1 to 2 pH points in the rhizosphere of peas (Nye, 1981, 1984).

Tb. Of the three investigated root rot pathogens of pea, pathogenic activity of Tb on pea was most affected. Most soils substantially reduced black root rot (Oyarzun and Maas, 1994). Several variables were strongly related to SR for this pathogen. The expression of its pathogenicity was positively related to increasing alkalinity of the soil and to elements indicative of alkalinity, such as total Ca and NO_3, whereas in soils with increasing carbon, and high soluble magnesium and P content the receptivity tended to be low (Fig. 3). The high association between parameter BE and the (linear) combination of NH_4 and NO_3 fits in this context. The importance of both nitrogen forms for microbial activity was demonstrated often (Papavizas, 1970; Huber and Watson, 1974; Papavizas and Ayers, 1974; Baker and Scher, 1987).

Fsp. The correlations found between SR to Fsp and nutrients in soil were curious. Increasing the concentration of several nutrients seems to favour disease caused by Fsp. No common contribution of variables was found in multiple regression of raw or standardized data, indicating redundancy. However, ranked values of C, Ca, NO_3, and NH_4, of which the last three were not correlated with SR individually, gave a significant multiple correlation ($P \leq 0.01$). The partitioning of the variance of chemical data indicated that variables favoured by acid soil reaction were associated with increasing conduciveness to Fsp. Garret (1970) showed that several cortical root rots caused by *formae speciales* of *F. solani* and *F. oxysporum* were more severe in acid soils. Tu (1990, 1992) reported a significant decrease of root rot of peas by liming acid soils.

Physical properties. **Tb.** Heavy soils, with higher content of ash and silt were more conducive to Tb, but the high positive correlation of organic matter content and C/N coefficient with suppressiveness points to inhospitality to Tb of a soil environment favourable to microbial activity. Incorporation of high C/N plant residues in soil gave partial control of root rot caused by *F. solani* f.sp. *phaseoli*, *Thielaviopsis basicola* and *Rhizoctonia solani*. C/N coefficients higher than 25 or more were reported to reduce pathogenic activity of Fs f.sp. *phaseoli* (Maurer and Baker, 1965). Papavizas (1970) found a C/N = 32 (alfalfa hay) to drastically inhibit Tb, but such a coefficient does not correspond to values of C and N naturally present in soils.

Fsp. No associations were found between physical variables individually and SR to Fsp. Higher water content of soils at field capacity associated with an increased drought sensitivity, indicative of heavy soils, significantly correlated with a greater conduciveness to Fsp. It is known that some heavy soils have a high water holding

capacity but a small fraction available water, so that they are very sensitive to drought (Thompson, 1962). The main trend in our data was, however, that drought sensitivity was higher in light soils (Fig. 2).

Ae. The lack of association of SR to Ae with individual chemical or physical properties was remarkable. Even after combining variables in multiple regression analysis no or a poor association with SR was found, and the latter was difficult to interpret. For example, increasing conduciveness was associated with the combined effects of the indexed values of crust formation (negative load) and drought sensitivity (positive). The positive correlation of crust formation and drought sensitivity of lighter soils, demonstrated in figure 2, leaves as the only explanation that the association with SR was explained by a few heavy soils scored as drought sensitive.

Biological characteristics

Cropping history and SR. The relation between cropping history and SR has to be interpreted cautiously. Winter wheat was dominant in the fields sampled. At least one legume crop occurred in each field in the past six years. Therefore the presence of cereals and legumes would not contribute to differentiate SR and their score is placed in the centre of the diagrams in figures 4 A,B.

Tb. No meaningful configuration was found for Tb. Reddy and Patrick (1989) found a reduction in the number and activity of Tb in soil during and after the cultivation of rye (*Secale cereale*), which they attributed to an increased number of antagonistic bacteria.

Fsp. Low receptivity to Fsp, SLR, converged with minor field crops, mainly rape, flax, and spring wheat, in contrast to SHR which was associated with potato growing. Soil with intermediate receptivity (SMR) tended to be associated with grasses and maize (Fig. 4A). No literature about effects of crops on receptivity to Fsp was found, though rape and other cruciferous crops were shown to suppress Ae root rot of pea (Chan and Close, 1987).

Ae. The association of grasses and maize with SLR to Ae (Fig 4B) was remarkable. Grasses are known to induce a microflora which is very inhospitable to soil-borne plant pathogenic fungi of dicotyledonous crops (Butterfields et al., 1978; Speakman et al., 1978; Cook, 1981). Members of the grass family used as green manure increased bacterial populations, particularly those of *Bacillus* spp and fluorescent *Pseudomonas* spp. (Tu, 1992).

Continuous cropping. The samples representing continuous cropping (CC) all originated from the same site on a relatively young soil. In this context no conclusion can be drawn as to the effect of continuous cultivation in general. The soil environment after continuously growing *Vicia faba* was highly conducive to Fsp. The response of peas to soil infestation with Fsp on this CC variant did not differ from the response to Fsp infestation on sterilized reference soil. After continuous *Vicia faba* the inoculum potential of the soil (IPS) for root rot of peas was low or nil, however.

Of all variants, continuous flax produced the most unfavourable environment (low SR) to the tested pathogens. This observation supports the association of low SR to Fsp

and flax discussed above. Flax growing also resulted in a remarkably low SR to Tb. Peas remained healthy whereas the soil was highly contaminated with Tb and other pathogens such as *Verticillium dahliae* and *Cylindrocarpon* spp. The soil with continuous maize was intermediately receptive to Fsp and poorly conducive to Ae.

Soil micro-organisms. Of all investigated variables, microbial abundance gave the highest simple or multiple correlations with SR. The absence of *Trichoderma* spp. in the soils was remarkable. Their absence probably indicates that they were out of the tolerance range of alkalinity. The germination of propagules of *Trichoderma* spp is inhibited at neutral or higher pH (Baker and Scher, 1987). The results of Chet and Baker (1980), that *Pseudomonas* spp in an alkaline soil environment inhibited the activity of *Trichoderma*, forms an additional explanation why we did not find this genus.

Tb. The higher number of fluorescent pseudomonads in the rhizoplane associated with suppressiveness to Tb (Tables 8, 9), agreed with results reported in the literature. Bacteria, in particular fluorescent pseudomonads in an alkaline environment are claimed to be implied in natural disease suppressiveness (Baker, 1990; Schippers, 1992). Total bacteria in the rhizoplane of pea in SHR were abundant, most probably indicating that saprophytes are stimulated by root decomposition.

Considering the low frequency of Tb in soils, the positive correlation between Tb abundance in the rhizoplane with increasing receptivity, indicated that its presence is indicative of soil conduciveness, a result contrasting with the pattern of Fsp described below. *Mortierella* and *Penicillium* in the rhizosphere were significantly higher in soils with higher suppressiveness to Tb. These spp. have not been reported before to be directly related to SR to Tb.

Fsp. The rhizosphere population number of indigenous Fs was not correlated with SR and IPS. In addition, the population of Fs on and in roots of pea was higher in suppressive soils. The result points to an indigenous saprophytic population, having the same niche as Fsp and therefore possibly related to disease reduction. Competition between pathogenic and saprophytic isolates both for nutrients and occupation of niches in soil is inherent to the antagonism between organisms closely related from the systematic point of view (Louvet et al., 1981). Already in 1943, Van Koot recommended the use of non-pathogenic forms of Fs to control *F. oxysporum*. Nonpathogenic *F. oxysporum* was reported to suppress several *formae speciales* of *F. solani* in different legumes (Lechappe, 1986; Walz-Borgmeier, 1991; Oyarzun, et al., 1994b). The populations of *Acremonium* spp and *Actinomyces* spp also seem to be higher in soil of low receptivity. *Actinomyces* spp., are known for their ability to degrade cellulose and chitin (MacCarthy and Williams, 1990) and seem to be of potential importance in the control of Fsp (Van der Spek., 1967), a fungus containing chitin in its cell wall. Most of the *Actinomyces* spp. isolated from soils with varying receptivity to Fsp were found to inhibit the growth and germination of Fsp *in vitro* (Castejón-Muñoz and Oyarzun, 1994). The number of *Ascochyta* spp. in the rhizosphere was associated with conduciveness. Their frequency in soils was rather

low, however, which renders the relevance of *Ascochyta* doubtful.

Pseudomonas spp. were able to reduce wilt and rot diseases in some crops (Alabouvette, 1990; Wüthrich and Défago, 1991) and to reduce root rot in pea in natural field soils independent of whether they originated from SLR or SHR. Our results showed that their abundance was not or negatively correlated with SR to Fsp (Fig. 6). Similar results were found by Lemanceau (1988) working with *Fusarium* wilts.

Ae. The abundance of *Gliocladium* in the rhizoplane and of *Acremonium* in the rhizosphere was related to decreasing receptivity to Ae. Such an association was not reported before. In experiments with natural soils, highly contaminated with Ae, soft rot was reduced by *Gliocladium roseum* if doses were appropriately adjusted (Castejón-Muñoz and Oyarzun, 1994). *Acremonium* spp and *Fusarium* spp in the rhizoplane together significantly correlated with SR, but the explained variance in the SR data was low (Table 9).

Effect of soil sterilization on SR. The drastic change in root rot severity after elimination of soil biota by sterilization implies unmistakably that the receptivity of a soil to pea pathogens is dominated by factors of biological nature. This necessarily results in a pattern of receptivity changing in time.

Final considerations. The validity of the soil receptivity concept was illustrated with root rot of pea in various agricultural soils. However, since the same soil may be conducive to one pathogen and repressive to another, SR is not an absolute phenomenon; it is pathogen specific.

Our exploration of the soil environment, in which root rot disease pathogens of peas operate, indicated an overlap of multiple factors in reducing disease caused by individual pathogens, in agreement with Rouxel (1991). Our results demonstrated that SR was almost completely determined by the soil biota. The association between physical or chemical properties of the soil and SR therefore operates through their effects on composition or activity of the microbiota of the soil.

Several micro-organisms seem to be related to receptivity for each pathogen investigated. *Pseudomonas fluorescens* was well associated with SLR to Tb, whereas conduciveness increased with increasing alkalinity. This indicates, if causality is present, the capacity of *P. fluorescens* to operate effectively in more neutral conditions, despite its well-known preference for alkaline soils.

T. basicola from continuous flax, which seriously affects flax, does not attack pea. On the contrary, this soil was one of the most hostile to pea root pathogens. Physiological specialization of this pathogen was described (Oyarzun et al., 1993). The SRs of the continuous cropping variants can be attributed to differences in microbial composition, which certainly was more favourable to pea root rot pathogens in soils cropped with pea related crops.

A. euteiches seems an obscure pathogen, of which the presence in soils and the epidemiology depend on accidental factors. As reported before (Oyarzun & Maas, 1994) most natural infestations occurred in the field in the rainy year 1987. For a

correct interpretation of our results, it should be remembered that samples highly contaminated by Ae were discarded from the analysis. On such soils severe root rot occurred without extra inoculation, making the assessment of disease caused by artificial inoculum impossible.

The relation between *Gliocladium*, which was found to control root rot of pea in highly infested field soils, and SR to Ae deserves more attention.

For a correct use of antagonistic micro-organisms in integrated control of disease caused by soil-borne pathogens, it seems necessary to analyze the dynamics of pathogens and antagonists in soil, taking into account the soil properties which affect the microbial balance. The cropping system, including fertilization and rotations, and with special attention to grasses and crucifers, should be investigated for its effect on microbial evolution. Classification of soils according to IPS and receptivity permits a better long-term management strategy of soil-borne pea diseases, and avoids costs and deceptions.

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Chapter 13

General discussion

The chapters presented so far report ecological studies on root rot pathogens of pea. They intend to develop a methodology for integrated management of soil-borne pathogenic fungi.

Root rot of pea. Our results demonstrated that root rot is a generic name referring to various root diseases of pea, caused by different pathogenic fungi (Chapter 2). It only depends on the inoculum potential of the soil, IPS, whether the same fungus provokes seed rot, damping off, or cortical rot of foot and root. In addition, some of these fungi cause tracheomycosis and blight. Their common feature is survival in soil and ability to infect the plant from the soil. Most species investigated showed a high degree of pathogenicity on pea. In the field, root rot is rarely caused by just one pathogen only. In the complex environment of the soil all kinds of interactions occur, leading to disease with a characteristic symptom if for a specific pathogen the conditions are stable and near its ecological optimum. During the growing season, changes in the plant and the soil habitat occur and more pathogens will infect the roots.

Information about the pathogenic fungi present in the soil is important for a correct interpretation of experimental research in the field as illustrated by the following experiences. In testing fungicides to control *Phoma* and *Fusarium*, contradictory results were found between experiments with isolates of these pathogens and with soil in which these pathogens were naturally present (Oyarzun et al., 1990). The same was found when testing pea cultivars bred for resistance against Fsp in greenhouses and naturally infested soil. Part of the contradiction was solved when *Aphanomyces euteiches* was demonstrated to be present in these soils (Chapter 3).

Control of root rot. No curative methods are available permitting the eradication or an adequate control of pea pathogens in the soil. Disease management is therefore mainly based on prevention. To secure economical continuity of the crop, highly infested soils have to be avoided. Cultural practices should try to prevent high pathogen populations in soil. Information on the degree of disease pressure in soil, here called inoculum potential of the soil, IPS, may contribute to optimal allocation of fields in a farm's cropping plan.

Determination of IPS. A bioassay (Chapter 6) is an adequate instrument to obtain information on the level of IPS. The disease score, IPS, obtained by the bioassay of a soil sample before growing the crop, was a reliable estimate of the level at which pea root rot occurred in the field. Apparently IPS dominates all other factors affecting the development of root rot in the field. IPS is a good short term predictor. It can be used to calculate the risks of economic loss and to help in determining appropriate cultural practices.

Factors determining field IPS. Rotation. Growing peas or other leguminous crops is an important factor in determining the soil infestation level with root rot pathogens. We did not find any noticeable infection in plants growing in soil where the same crop had never been grown. However, a large variation characterized the relation between cropping frequency and soil infectivity level (Chapter 4). Similar results with peas (Zerlik, 1979)

and other crops (Lechappe, 1986; Larsson, 1992) were reported elsewhere.

Several factors seem important in refuting the generally accepted rule of the crop frequency effect. Most root rot pathogens of pea are polyphagous and can be seed-borne (Chapter 5). Especially the latter factor causes unexplained variation. Even the *formae speciales* of these pathogens need not be restricted to one plant species (Chapter 2). Several root rot fungi develop persistent survival structures and are able to grow saprophytically on roots of non-host plants. The recommendation of a crop rotation with pea once in 6 years seems too general and insufficient to avoid the risk of crop losses.

Soil water. Of all soil-environmental factors affecting outbreaks of root rot in pea, soil water status seems the most relevant. It influences not only plant production, but also many factors affecting root growth and functioning. High water potential favours attack by oomycetes. Zoospores of *A. euteiches* are produced and infect roots within 24 hours if free water is available in the soil (Burke et al., 1969). Free water favours *F. solani* and *T. basicola* by hampering oxygen diffusion to the root.

Cultural practices. Cultural practices contributing to low predisposition and vigorous growth of pea will, at least partially, alleviate the rot or delay the soil infestation. Management of pea root rot was discussed by several authors (Temp and Hagedorn, 1967; Hagedorn, 1986; Sherf and Macnab, 1986; Tu, 1992). Avoidance of highly infested fields is the most reliable measure against crop failure (Sherwood and Hagedorn, 1958; Olofson, 1967;Biddle, 1984; Hagedorn, 1986).

Soil receptivity. Cultural practices could not satisfactorily explain why some fields with an intensive regime of legumes remained at a low level of infectivity. Soil suppressiveness or, more general, soil receptivity, SR (Chapters 8 and 9), could provide an explanation. For successful colonization and establishment a plant pathogen has to overcome the hostile environment which the soil ecosystem represents for alien micro-organisms. If pathogen failure is caused by intrinsic properties of the soil, the knowledge about these properties would add a new dimension, stability, to the IPS. This opens the possibility of **long term recommendation.**

Research on soil receptivity. Methodological difficulties. During the study of SR to root rot pathogens of pea we faced several difficulties. The choice of fields to be investigated, the pathogen, the way to study infectivity, form of inoculum, inoculation methods, enumeration of soil population, conservation of soil and inoculum, etc., were only some of the topics needing attention beforehand.

Choosing former commercial pea fields on the basis of their low IPS, a prerequisite for SR tests, had the advantage to increase the probability of identifying factors operating in disease reduction. But this choice certainly did not favour the discrimination between environmental variables and range of SR.

The restriction in the choice of pathosystems can be criticized. Major pea root rot pathogens, such as *Phoma medicaginis* and *Mycosphaerella pinodes*, were disregarded because their dependence on seed infection makes the effect of IPS erratic and SR meaningless. The problem of dealing with a disease complex remains unsolved until new techniques become available, permitting a relevant characterization of the species relationships in soil, the reciprocal relationships of within-species populations, and their interactions with plant and soil.

Whereas IPS, as determined by a bioassay, represents an instantaneous value, SR must

be derived from the range of the IPS-values achieved when the population density or the energy status of the pathogen is changed in the soil. To this purpose, several methodological approaches were proposed. We rejected the use of selective substances activating a specific pathogen and techniques making use of mixtures of substrates, as proposed by Bouhot (1979), because these change more than only the status of the target pathogen. The time necessary with different soils to express a fixed amount of disease was a parameter used by Louvet (1976). This method is appropriate if disease is evaluated as incidence. In our approach, the degree of SR is derived from the behaviour of IPS after artificial increase of inoculum. The determination of the degree of SR is equivalent to distinguishing between disease response curves caused by an artificial increase of soil infestation level.

Determination of IPS is a time consuming activity (Chapter 6). In SR-tests the number of objects can be increased considerably. Test results have to be precise and reproducible. Much time was spent to develop equipment (Chapter 7) to meet such exigencies.

The test results are curves. The problem is then to take a decision about the importance of individual curve parameters which are intercorrelated. Multivariate methods help to face these difficulties (Madden and Pennypacker, 1979). Principal components analysis performed with the values of IPS at each level of soil infestation (intercorrelated variables) produced a new variable representing an ordination of soils. Subsequently, this variable was used in studies on association with environmental factors.

SR results. Our results indicate the existence of different levels of soil receptivity to the major pathogens causing root rot of pea (Chapters 8 and 9). This indicates that SR is based on an equilibrium differentially affecting individual pathogens.

A. euteiches. SR to *A. euteiches* tended to conduciveness. Only a few soils clearly reduced the disease. To explain the lack of ecological resistance of the soil we hypothesize that the presence of Ae in Dutch soils might be a recent phenomenon and that not enough time has passed for the build-up of antagonism to the pathogen. A support to this hypothesis is the experience with 23 soils where soft rot IPS drastically increased with the last pea crop and remained high over next three or four years. Unfortunately no reports are available about soils suppressing this disease in countries where the pathogen has been known for a long time.

The high receptivity to soft rot, combined with the intrinsic severity of this disease under climatic conditions which frequently occur in The Netherlands, indicates that *A. euteiches* is the most dangerous among the root rot pathogens affecting peas. If a field becomes contaminated, the disease can reach high expression levels, especially under wet conditions (Chapter 8).

Thielaviopsis basicola was most affected by habitat in its capacity to provoke disease. Its frequency in the plants and in soil was low, and after artificial infestation Tb was most pathogenic in alkaline soils. Probably its survival strategy is more directed to polyphagy and dormancy than to parasitism if conditions are not clearly conducive.

Fusarium solani f. sp. *pisi*. Most of the soils were classified as intermediately receptive to Fsp but shifts of a soil in either direction were not exceptional. *F. solani* was the most widely disseminated and frequent fungus found in roots of pea plants in our studies. The existence in soil of mixtures of saprophytic and strongly specialized pathogenic forms partially explains why no clear relationship between population density and disease

existed. Fs was even most abundant in the roots of peas growing in soil were fusarium root rot was most suppressed (Chapters 8 and 12). The pathogenic strains within the population will increase on pea, but antagonism, even within the genus *Fusarium*, seems possible. Therefore, inoculum density is not a reliable criterium to predict disease outbreaks in natural soils.

SR and biological control. SR research opens the possibility to identify suppressive soils, isolate antagonists and use them to induce suppressiveness. The considerations about SR as discussed for pathogenic fungi, also apply when a biological control agent, BCA, is introduced to a soil. The suitability of micro-organisms for biocontrol depends on their capacity to persist, be active and successful in the competition with other micro-organisms in the peculiar habitat of the soil. A soil-dependent control effect on Fsp was found with nonpathogenic *F. oxysporum* isolates (Chapter 10) and with *Pseudomonas* and *Gliocladium* in highly infested natural soils (Chapter 11). These substrate-specific differences in antagonistic activity of a BCA are a warning against the indiscriminate use of such organisms. Unlike chemicals against foliar diseases, BCA's have their ecological optimum and their typical tolerance.

The expectation that screening of suppressive soils increases the probability to find efficient biological control agents had to be moderated. Most of the isolates obtained from highly infested soil were able to significantly reduce IPS. The quality 'highly infective soil' points to an equilibrium of antagonists and pathogens in which the latter dominate, and not to the absence of antagonists. Research on ranges of ecological conditions suitable for a BCA may be as complicated as the study of the conditions operating shifts in an ecosystem to conduciveness or suppressiveness.

SR related factors. Different groups of micro-organisms could be related to SR. Types of crop in the rotation, soil reaction, content of some nutrients, were associated with the magnitude of the disease reduction (Chapter 12). After elimination of soil biota by sterilization, differences in SR disappeared, leading to the conclusion that SR is mainly caused by soil biota. We attribute the association of SR with physical and chemical factors to mediation by microbial activity. Extensive information was published linking microbial activity and disease severity directly to abiotic properties (Schneider, 1982; Engelhard, 1989). Most of the investigated soils were neutral to alkaline. Under such soil conditions, suppressiveness to Tb might be attributed to competition for iron, which is necessary for fungal growth. Pseudomonads, which are protected by montmorillonite clay (England et al., 1992) in certain soils, are known for their Fe siderophores (Bakker et al., 1991). The investigated soils were formed from sea clay, which contains mostly illite, a fraction to which no protective properties have been attributed. Suppressiveness to Tb is thus not necessarily associated with a protected site for antagonistic bacteria.

Final considerations. SR with respect to the major pathogenic fungi causing root rot of pea is a measurable characteristic of arable fields. Suppressiveness is not a characteristic of some specific soils only, but a position on a scale on which every natural soil can be located, a SR scale. SR research opens the possibility to identify suppressive soils.

SR powerfully affects individual pathogens and pathogen complexes. Thus, SR must be considered when studying epidemiology of and resistance breeding against soil-borne pathogenic fungi in field crops.

A more comprehensive determination of SR to soil-borne pathogens, and an analysis of the major factors responsible, may allow field-specific predictions to be made about the effect of crop rotation and cultural practices in root rot management. We expect that identification of factors underlying low receptivity and their mode of action will reveal additional possibilities to enhance resident and to introduce new disease suppressing soil organisms, thus reducing the need for chemicals. Much research is still needed before a practical contribution to a better disease management can be made in this area. In particular, we expect that extending the range of chemical and physical properties of the soil beyond that examined in this study, will expose more of the structure of the factors determining soil receptivity.

Validation of our results is a long term enterprise and, therefore, beyond the scope of this study. SR information as an isolated element is of limited utility. A long road has to be gone in evaluating SR for other pathosystems and in integrating this information in a disease management strategy.

For practical application of SR a simple parameter, a receptivity index, RI, is needed. The RI should contain additional information about the actual level of IPS and the average effect of soil biota.

$$RI(do_i) = 1 - [(1-DI_n/DI_{max})(1-IPS/DI_{max})] \text{ or}$$

$$= (DI_n + IPS)/DI_{max} - DI_n.IPS/(DI_{max})^2$$

with: do_i = minimum inoculum dose of the pathogen needed to force consistent complete necrosis of roots (DImax) on a reference sterilized substrate.
DI_n = DI at do_i in natural soil
IPS = DI by natural infestation of the soil.

The $RI(do_i)$ proposed is proportional to the difference in disease severity between a biologically buffered and unbuffered condition. By weighting this difference by a factor for the importance of natural soil infection, RI varies from 0 for suppressive soils to 1 for conducive ones. The use of $RI(do_i)$ saves time and work, but sacrifices useful biological information.

The current situation. With a total of 6000 ha in 1992, pea is again disappearing from Dutch agriculture. The trend is not the consequence of disease problems or of the difficulties of harvesting peas in the rainy seasons of recent years, but the result of diminishing EC price support.

In the future, with the development of new farming systems (Goewie, 1993), in which soil fertility is maintained through a more balanced alternation of different crops, as intended in ecological agriculture (Zadoks, 1993), the role of legumes, in particular peas, will become more important. The trend could be reversed.

The results presented in this report deal with research projects which aimed at anticipating responses to avoid the difficulties faced by pea growers in the past. The disappearance of the crop invalidated these worries, but not the methods and procedures

developed. They can be applied to other diseases. SR concerns all soil-bound pathosystems. Understanding the way it operates can contribute to integrated disease management, a necessary condition for sustainable agriculture.

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Summary

Root rot of pea is caused by several species of soil-borne pathogenic fungi. Soil factors influence their success in the microflora community, and the expression of their inoculum potential.

Chapter 2. The present study on root rot pathogens of peas in the Netherlands confirmed the prevalence of *Fusarium solani*, *F. oxysporum*, *Pythium* spp., *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella*. Other pathogens such as *Rhizoctonia solani* and *Cylindrocarpon destructans* were also found on diseased parts of roots. *Thielaviopsis basicola* was identified for the first time as a cause of root rot in pea in the Netherlands. *F. solani* (Fs) existed in different degrees of pathogenicity, and was sometimes highly specific to pea (*Pisum sativum* L.), dwarf bean (*Phaseolus vulgaris* L.) or field bean (*Vicia faba* L.), depending on the cropping history of the field. *T. basicola* (Tb) showed some degree of physiological specialization, whereas *Aphanomyces euteiches* (Ae) was specific to peas. These data were discussed in relation to the *forma specialis* concept.

Chapter 3. The occurrence of Ae in Dutch soils was reported for the first time. A bioassay was used in which seedlings of a highly susceptible pea cultivar served to bait the fungus from the soil. The pathogen could subsequently be isolated on a semi-selective medium. Screening of soils from 56 fields demonstrated the presence of Ae in 26 cases.

Chapter 4. Pea-free periods and legume frequencies had a poor predictive value for crop management purposes. Root rot severity correlated with the frequency of peas or legumes over a period of 18 years, but the frequency explained only a minor fraction of the variation in disease index ($R^2 = 0.12$; $P \leq 0.01$; $n = 169$). Some experimental data pointed to the occurrence of a highly specific pathogen microflora in soil with continuous cropping of only one legume species, but this phenomenon probably does not occur in farmers' fields. In field soils, root disease indices of pea and field beans correlated well.

Chapter 5. Several fungal species causing root rot of pea also occurred as seed infection or seed contaminants. These infections constituted a source of primary inoculum and facilitated the introduction of pathogens in non-infested fields. Seed dressing with fungicides killed or repressed the pathogens, but often only temporarily inhibited infection of the roots by the pathogens. The impact of the quality standards valid for commercialization of certified pea seed in the Netherlands on infestation of new fields, epidemiology and pea production were discussed.

Chapter 6. A bioassay in pots was developed to determine inoculum potential of the soil (IPS). IPS is used to predict the root rot likely to occur in prospective pea fields. In the bioassay, under standardized conditions in the greenhouse, the pea cultivar Finale was sown in pots with a composite soil sample from a field. The plants were removed at the green bud stage and the severity of root rot recorded. Between 1985 and 1988 approximately 200 field pea crops were monitored for root rot development. Each year, root rot readings in the bioassay and disease severity readings of sampled field plants at flowering and green pod were linearly correlated ($P \leq 0.001$). As the degree of root rot in the field crop increased, yield was proportionally lower. In heavily infested fields, up to a 50% yield reduction occurred. The bioassay proved to be a reliable method for predicting root rot severity in pea fields.

Chapter 7. In order to facilitate IPS determinations, a computerized equipment to

control soil temperature and soil water matric potential, ψ_m, at high soil water level was developed and evaluated. The equipment consists of double walled tanks, permitting the adjustment of soil temperature. It is provided with sensors, control software and valves for automatic operation. In a growth chamber at 24°C, with RH 70% and a radiation of 90 $W.m^{-2}$, ψ_m could be maintained in dynamic equilibrium for pF values ranging from 1 (-1 kPa) to 2 (-10 kPa) in various arable soil samples during the four to five weeks period of the bioassays. During the fourth week of pea or iris growth at pF = 2, the system controlled ψ_m within a maximum amplitude of 0.4 pF-units. Soil temperature in the system could be maintained at a constant level with a variance below 0.1 and an amplitude of 0.3° C at most.

Chapter 8. A procedure was developed to differentiate field soils according to their receptivity, ranging from suppressive to conducive, to *Fusarium solani* f. sp. *pisi* (Fsp). Experiments were carried out using samples of natural soil collected from commercial fields which had a low inoculum potential of root rot in peas in previous years. In bioassays, with computer-controlled soil water potential, light intensity, air temperature and relative humidity, dry root rot severity responses were determined to a range of inoculum levels. Disease severity on different soil samples, at the same infestation level, showed that soil as a substrate strongly affected the inoculum potential of Fsp. Univariate and multivariate models were examined for their adequacy to describe and compare disease response data. Two Principal Component Analyses were applied to a matrix, one for raw data and one for Weibull fitted data, versus soil samples. The two analyses produced a similar receptivity order ($P \leq 0.01$) of the soil samples, but a PCA on fitted values increased the amount of variance accounted for by the first axis. Cluster analysis followed by a canonical variate analysis permitted to classify the tested soil samples in groups which significantly differed ($P=0.05$, based on χ^2) in soil receptivity (SR) for Fsp. In selected samples which only produced dry root rot, the amount of native *Fusarium solani* in pea rhizosphere soil was uncorrelated with root rot severity.

Chapter 9. Next, SR to Tb and Ae was examined. SR patterns, resulting from a Principal Components Analysis (PCA) on a variance-covariance matrix, differed for both pathogens. Most disease responses to soil infestations were conducive to Ae and suppressive to Tb. A more comprehensive comparison of SR, including that for Fsp, showed that in the same soils disease caused by Tb was strongly reduced, Fsp intermediately reduced, whereas in the majority of the cases soils were conducive to Ae. IPS, as determined by a bioassay before the last pea crop in 1986 or 1987, and IPS determined during SR tests to individual pathogens in non-artificially infested samples in 1991, showed a significant increase due to the activity of soft root rot pathogens. No change in IPS occurred when disregarding this disease. The value of SR determinations for further ecological research was discussed.

Chapter 10. The effect of soil on the antagonistic activity of two nonpathogenic isolates of *Fusarium oxysporum* (Fo) on Fsp was examined. Antagonism was studied *in vitro*, in a sterilized field soil, and in natural field soils. The natural field soils contained, besides native Fs, other typical pea root rot pathogens. Nonpathogenic Fo reduced disease severity and prevented plant weight losses caused by increasing amounts of Fsp in sterilized soil. In natural, highly infested field soils, antagonistic effects were variable and more evident in the absence than in the presence of Ae. Fo was a fast colonizer of soil organic matter, even when native Fs was present in the soil.

Chapter 11. Next, potential antagonists to Fsp were selected from soil samples with different receptivity to this pathogen. They were tested against Fsp isolate 48 (Fs48). Most species tested *in vitro* were able to antagonize Fs48. *In vitro*, no relation could be established between the receptivity of the soil from which an isolate originated and its antagonism to Fs48. Three *Gliocladium roseum* isolates (Gr1,Gr2,Gr3) significantly reduced disease severity and prevented root weight losses caused by Fs48 in sterilized soils. In naturally infested soils, in which soil biota were activated by growing pea previous to the start of the assays, doses of 10^6 and 10^7 of *G. roseum* were required to reduce root rot. In heavily infested soils, the antagonistic effects of *Pseudomonas fluorescens* (Pf) strains were variable and more evident in the absence than in the presence of Ae in the root rot pathogen complex. In soil, no relation was found between the antagonistic activity of Pf and the receptivity of the soil from which they originated.

Chapter 12. The relationship between various abiotic and biotic soil factors and the SR to Tb, Fsp and Ae, was investigated. Several relations were established. SR to Tb showed association with abundance of pseudomonads, soil reaction (pH), organic matter content and C/N coefficient. The number of saprophytic *Fusarium solani* in the rhizoplane was higher in soil less conducive to Fsp, whereas the abundance of indigenous Fs in the rhizosphere was independent of the receptivity. The amounts of soluble K, P, Mg and total C and N in soil, individually, were higher with increasing conduciveness to Fsp. The SR to Ae was not well associated with any investigated soil aspect, except *Gliocladium* in the rhizoplane and *Acremonium* in the rhizosphere, of which populations were higher in soil less receptive to this pathogen. Experiments with soil sterilization showed that the soil biota were the main factor responsible for the inhibited activity of the investigated pathogens. Therefore, despite its correlation with chemical and physical factors, SR to the investigated pathogens can be considered essentially of microbiological nature.

Chapter 13. The general discussion describes the complexity of the pea root rot disease. The traditional view on root rot pathogens was modified, new pathogens were described and a bioassay for practical disease prevention developed. We discuss data of the habitat effect on some of the most important root rot pathogens. The procedures presented permit the visualization of the structure and state of relevant variables for each degree of soil receptivity. We place emphasis on the necessity to meet the ecological requirements for optimal functioning of biological control agents. The described procedures permit more specific investigation into receptivity mechanisms. In the future, this will facilitate the formulation of disease risks in an integrated management strategy and estimation of the effects of cultural practices. This insight lifts the present research to a level of importance far beyond the pea root rot problem.

Samenvatting

Voet- en wortelrot van erwt wordt veroorzaakt door verschillende soorten pathogene bodemschimmels. Bodemfaktoren bepalen hun succes in de microbiële gemeenschap en de expressie van hun smetstofpotentieel.

In hoofdstuk 2 wordt beschreven dat *Fusarium solani, F. oxysporum, Pythium* spp., *Mycosphaerella pinodes* en *Phoma medicaginis* var *pinodella* frekwent voorkomen als wortelrot veroorzakers bij erwt. Ook andere pathogenen, zoals *Rhizoctonia solani* en *Cylindrocarpon destructans* werden op zieke erwtewortels aangetroffen. *Thielaviopsis basicola* werd voor het eerst als veroorzaker van erwte-wortelrot in Nederland geïdentificeerd. *F. solani* (Fs) kwam voor in verschillende graden van pathogeniteit, en was soms hoogst specifiek voor erwt, slaboon of veldboon, naar gelang de teeltgeschiedenis van het veld. *T. basicola* (Tb) toonde een zekere mate van fysiologische specialisatie, terwijl *Aphanomyces euteiches* (Ae) specifiek was voor erwt. Deze gegevens worden besproken in relatie tot het *forma specialis* concept.

In hoofdstuk 3 wordt het voorkomen van Ae in nederlandse gronden voor het eerst beschreven. In een biotoets dienen zaailingen van een zeer vatbaar erwteras als vangplant voor Ae. Op een semi-selectief medium kan het pathogeen vervolgens worden geïsoleerd. Bij onderzoek van monsters van 56 velden werd Ae in 26 gevallen aangetroffen.

Hoofdstuk 4 beschrijft de geringe voorspellende waarde van erwte-vrije periodes en peulvrucht-frekwentie in de rotatie voor de kans op wortelrot van erwt. De ernst van wortelrot correleerde met de erwte-frekwentie over een periode van 18 jaar, maar de frekwentie verklaarde slechts een minimale fractie van de variatie van de ziekte-index ($R^2 = 0{,}12$; $P \leq 0{,}01$; $n = 169$). Enkele gegevens wezen op het voorkomen van een hoogst specifieke pathogene microflora in gronden met een monokultuur van slechts één soort peulvrucht, maar dit verschijnsel komt waarschijnlijk niet voor in praktijkpercelen. De ziekte-indices van wortels van erwt en veldboon van hetzelfde grondmonster correleerden goed.

Hoofdstuk 5 beschrijft het voorkomen van verschillende wortelrot veroorzakende schimmels als zaad-besmetting of -infectie. Deze vormen een bron van primair inoculum en bevorderen de introductie van pathogenen in pathogeen-vrije velden. Zaad-behandeling met fungiciden doodde of remde de pathogenen, maar had vaak slechts een vertragend effect op de infectie van de wortels door de pathogenen. Het effect van de kwaliteits-eisen bij verhandeling van gecertificeerd zaaizaad in Nederland op besmetting van verse grond, de epidemiologie en de produktie van erwten wordt besproken.

Hoofdstuk 6 beschrijft de ontwikkeling van een biotoets in potten ter bepaling van de smetstofpotentieel van de grond (inoculum potential of the soil, IPS). IPS wordt gebruikt om de meest waarschijnlijke mate van wortelrot te voorspellen bij teelt van erwten op een bepaald veld. In de biotoets werd het ras Finale onder gestandaardiseerde condities in de kas in potten geteeld op een samengesteld grondmonster van een veld. De planten werden gerooid in het stadium van groene bloemknoppen, en de ernst van het wortelrot bepaald. Tussen 1985 en 1988 werd wortelrot van ongeveer 200 erwte-percelen gemeten. Elk jaar bleken wortelrot bepalingen van de biotoets en van bemonsterde planten uit het veld bij bloei of groenrijpe peul lineair gecorreleerd ($P \leq 0{,}001$). Opbrengst was negatief gecorreleerd met wortelrot in het veld. In ernstig zieke percelen kwam tot 50% opbrengst-derving voor. De biotoets bleek een betrouwbare voorspeller van wortelrot te velde.

In hoofdstuk 7 wordt een gecomputeriseerde apparatuur beschreven om IPS bepalingen te vergemakkelijken. Bodemtemperatuur en de matrix-potentiaal, ψ_m, bij een hoog niveau van bodemvocht werden geregeld, en de werking van het systeeem werd geëvalueerd. De apparatuur bestaat uit dubbelwandige tanks die het mogelijk maken de bodemtemperatuur te beheersen, sensoren, software, en kleppen voor automatische regeling. In een klimaatkamer bij 24°C met een relatieve vochtigheid van 70% en een instraling van 90 $W.m^{-2}$, kon ψ_m voor pF waarden in het gebied van 1 (-1 kPa) tot 2 (-10 kPa) in zeer verschillende landbouwgronden gedurende de vier tot vijf weken van de biotoets in een dynamisch evenwicht worden beheerst. Gedurende de vierde groei-week van erwt of iris bij pF = 2 hield het systeem de ingestelde waarde aan met een maximale amplitude van 0,4 pF eenheden. De grondtemperatuur in het systeem kon konstant gehouden worden met een variantie van minder dan 0,1 en een amplitude van hoogstens 0,3°C.

In hoofdstuk 8 wordt de ontwikkeling van een methode beschreven om gronden te differentiëren naar hun receptiviteit, die verloopt van ziektewerend (onderdrukkend) tot ziektegevoelig (bevorderend). Experimenten worden beschreven met *Fusarium solani* f.sp. *pisi* (Fsp). Grondmonsters werden genomen van velden met een laag niveau van erwtewortelrot in voorgaande jaren. De ernst van droog wortelrot, veroorzaakt door Fsp, werd gemeten in een biotoets bij een reeks van kunstmatige besmettingsniveaux en bij computer gestuurde bodemvocht potentiaal, licht-intensiteit, lucht temperatuur en relatieve vochtigheid. De ziekte-index bij verschillende grondmonsters en met dezelfde inoculum dosis liet zien, dat de bodem als substraat in hoge mate bepalend is voor de expressie van het smetstofpotentieel van Fsp. Modellen met één of meer variabelen werden getoets op het adequaat beschrijven en vergelijken van de gegevens over de ziekte-respons. Principale Componenten Analyses werden toegepast op een matrix, één voor de ruwe gegevens, en één voor "Weibull gefitte" gegevens, tegen de bodemmonsters. De twee analyses gaven dezelfde volgorde van receptiviteit ($P \leq 0,01$) van de bodemmonsters, maar een PCA op "gefitte" waarden leidde tot toename van de door de eerste PCA-as verklaarde variantie. Cluster analyse gevolgd door een "canonical variate" analyse maakte het mogelijk de gronden te classificeren in groepen met significante verschillen ($P = 0,05$, gebaseerd op χ^2) in bodemreceptiviteit (soil receptivity, SR) voor Fsp. In geselekteerde bodemmonsters, die slechts droog wortelrot vertoonden, was de hoeveelheid van nature aanwezige *Fusarium solani* in de erwte-rhizosfeer niet gecorreleerd met de ernst van het wortelrot.

In hoofdstuk 9 worden vervolgens de bodemreceptiviteit voor Tb en Ae onderzocht. De SR patronen, volgend uit een PCA-analyse op een variantie-covariantie matrix, verschilden per pathogeen. De meeste gronden waren bevorderend voor Ae en suppressief voor Tb. Een meer omvattende vergelijking van SR, met inbegrip van die voor Fsp, toonde dat de meeste gronden ziekte door Tb sterk onderdrukten; Fsp werd matig onderdrukt, terwijl de meeste gronden bevorderend waren voor Ae. IPS, gemeten via een biotoets vóór de laatste erwteteelt in 1986 of 1987, en IPS bepaald bij de SR toets tegen individuele pathogenen in niet kunstmatig besmette monsters in 1991, toonde een significante toename van nat wortelrot, veroorzaakt door Ae. Behalve voor deze ziekte trad geen toename van IPS op. Het belang van SR bepalingen voor verdere ecologische studie wordt besproken.

In hoofdstuk 10 wordt het effect van de bodem op de antagonistische aktiviteit van twee niet pathogene isolaten van *Fusarium oxysporum* (Fo) op Fsp bestudeerd. Het antagonisme werd bestudeerd *in vitro*, in gesteriliseerde grond, en in natuurlijke grond.

De natuurlijke gronden bevatten naast Fs ook andere typische voetrotpathogenen. Niet pathogene Fo reduceeerde de ernst van het wortelrot en voorkwam gewichtsverlies van de zieke planten bij toenemende hoeveelheden Fsp toegediend aan gesteriliseerde grond. In natuurlijke, sterk besmette grond waren de antagonistische effecten variabel, en duidelijker in afwezigheid dan in aanwezigheid van Ae. Fo koloniseerde organisch materiaal in de grond snel, zelfs indien Fs van nature in de grond aanwezig was.

Hoofdstuk 11 beschrijft de selectie van potentiële antagonisten van Fsp uit gronden met verschillende receptiviteit voor dit pathogeen. Zij werden getoetst tegen isolaat 48 (Fs48). *In vitro* waren de meeste soorten antagonistisch tegen Fs48, en kon geen relatie gevonden worden tussen het antagonisme en de receptiviteit van de grond waaruit de antagonist was verkregen. Drie isolaten van *Gliocladium roseum* (Gr1, Gr2, Gr3) reduceerden de ernst van wortelrot significant, en voorkwamen reductie van wortelgewicht door Fs48 in steriele grond. In van nature besmette grond, waarin de bodembiota werden geaktiveerd door de teelt van erwt vóór de toets, waren doses van 10^6 en 10^7 van *G. roseum* nodig om wortelrot te beperken. In zwaar besmette grond was het antagonistische effect van *Pseudomonas fluorescens* (Pf) stammen variabel, en duidelijker in afwezigheid dan in aanwezigheid van Ae in het voetziekte complex. In grond werd geen relatie gevonden tussen het antagonistisch effekt van Pf en de receptiviteit van de grond van oorsprong.

In hoofdstuk 12 wordt de relatie onderzocht tussen verschillende abiotische en biotische bodemfaktoren en de SR tegen Tb, Fsp en Ae. Er werden verschillende relaties vastgesteld. Bodemreceptiviteit voor Tb toonde een positieve relatie met de hoeveelheid pseudomonaden, bodem-reaktie (pH), gehalte aan organische stof en C/N quotient. Het aantal saprofytische *Fusarium solani* in de "rhizoplane" was groter in grond die minder bevorderend was voor Fsp, terwijl de hoeveelheid van nature aanwezige Fs in de rhizosfeer onafhankelijk was van de receptiviteit. De hoeveelheid oplosbaar K, P, Mg and totaal C en N in de grond, elk apart, was groter met toenemende ziekte-bevordering voor Fsp. De SR tegen Ae was niet duidelijk gecorreleerd met enig onderzocht bodemaspekt, uitgezonderd *Gliocladium* in de "rhizoplane" en *Acremonium* in de rhizosfeer, waarvan de populaties groter waren in minder receptieve gronden. Sterilisatie-experimenten toonden aan, dat bodembiota de belangrijkste factor waren bij de geremde aktiviteit van de onderzochte pathogenen in de bodem. Derhalve kan SR tegen de onderzochte pathogenen, ondanks zijn correlatie met chemische en fysische faktoren, toch beschouwd worden als in hoofdzaak van biologische aard.

Hoofdstuk 13, de algemene discussie, beschrijft de complexiteit van wortelrot van erwt. De traditionele kijk op wortelrot pathogenen werd aangepast, nieuwe pathogenen beschreven, en een biotoets ter vermijding van voetziekten voor de praktijk ontwikkeld. Wij slaagden in het verkrijgen van gegevens over het effect van de habitat op enkele van de belangrijkste voetrot pathogenen. Er werden procedures ontwikkeld om SR te kwantificeren en de struktuur en toestand van relevante variabelen zichtbaar te maken bij verschillende graden van receptiviteit. Nadruk werd gelegd op de noodzaak de ecologische vereisten van biologische bestrijdings-organismen te respecteren ter verkrijging van een optimaal effect. De beschreven procedures maken een doelmatiger onderzoek van receptiviteits-mechanismen mogelijk. In de toekomst kan dit leiden tot het identificeren van ziekte-risiko's bij een geïntegreerde teelt-strategie en inzicht verschaffen in het effect van kultuurmaatregelen. Dit inzicht tilt het onderhavige onderzoek op tot een niveau dat het belang voor het voetziekteprobleem bij erwt ver te boven gaat.

Resúmen

La podredumbre de raíz y pie en arveja, *Pisum sativum* L., es causada por un complejo parasitario de hongos de suelo. El éxito de estos patógenos respecto a la microflora telúrica, así como la expresión de su potencial de inóculo, es afectada por las propiedades del suelo.

Capítulo 2. Este informe confirma la prevalencia en arvejas de los patógenos radiculares *Fusarium solani*, *Fusarium oxysporum*, *Pythium* spp., *Mycosphaerella pinodes* y *Phoma medicaginis* var. *pinodella*. Muchas otras especies, tales como *Rhizoctonia solani*, *Cylindrocarpon destructans*, fueron encontradas. *Thielaviopsis basicola*, Tb, fue identificado por primera vez como causante de podredumbre radicular en arvejas en Los Países Bajos (Holanda). *Fusarium solani*, Fs, presenta una patogenicidad variable. Aislados de Fs mostraron alta especificidad en arvejas, porotos o habas dependiendo del historial de cultivo del campo de origen. *Aphanomyces euteiches* fue especifico en arvejas mientras que *Thielaviopsis basicola* mostró un grado menor de especialización fisiológica. Se discute el uso del término *forma specialis*.

Capítulo 3. *Aphanomyces euteiches*. Su presencia fue demostrada en suelo agricola en Holanda. Para ello, plántulas de un cultivar suceptible fueron usadas de trampa para forzar al hongo a la infección. A continuación el patógeno fue aislado en medio semi-selectivo. La presencia del patógeno fue demostrada en 26 de un total de 56 campos investigados.

Capítulo 4. Una prospección de la enfermedad a campo fue realizada sobre aproximadamente 200 parcelas. La enfermedad se presentó en parcelas con marcadas diferencias en su historial de cultivo y propiedades agronómicas. Se encontró una baja correlación entre la longitud del período sin cultivo de arveja, o la frecuencia de leguminosas, y la intensidad de la enfermedad en cultivos ($R^2 = 0.12$; $n = 169$; $P \leq 0.05$). Datos obtenidos en parcelas experimentales indican la existencia de una microflora patógena altamente especializada, sin embargo, este fenómeno probablemente no sucede en campos con uso comercial. En experimentos en macetas, usando muestras de suelos infestados en varios grados con patógenos radiculares de arveja, se observó una infección radicular en habas proporcional al grado de infestación del suelo.

Capítulo 5. *Mycosphaerella pinodes* y *Phoma medicaginis* var. *pinodella* fueron corriente en raíces de plantas tomadas a campo. La epidemiología de estas especies esta muy ligada a la infección de semillas. Fungicidas usados en desinfección de semillas pueden eliminar los patógenos albergados en los cotiledones, pero en general solo inhiben temporalmente la infección radicular. Se discute el impacto de los estandares de calidad, validos para la comercialización de semilla certificada, sobre la introdución de patógenos en campos aun no infestados, la epidemiología y la produción del cultivo.

Capítulo 6. Se puso a punto un bioensayo en macetas, con el fin de estimar el potencial de inóculo de hongos patógenos del suelo, PIS. Bajo condiciones ambientales estandarizadas, semillas de cultivar Finale fueron sembradas en muestras compuestas del suelo a ensayar. La severidad de la podredumbre radicular fue determinada al inicio del estado de formación del botón floral. Los valores de enfermedad a campo, obtenidos durante la floración y en el estado de grano verde, mostraron un alta correlación ($P \leq 0.01$) con los valores del bioensayo. Los rendimientos del cultivo decrecieron proporcionalmente al aumento del grado de infestación del campo. Reducciones de más

de un 50% fueron estimadas en campos altamente infestados. Se demostró que el PIS tiene un poder predictivo mayor que cualquier otro factor afectando la epidemiología de la enfermedad a campo.

Capítulo 7. Con el fin de facilitar las determinación del PIS, se desarrollo y evaluó un equipo computarizado con el cual es posible durante un bioensayo, controlar el potencial de agua Ψ_m y la temperatura del suelo. El equipo esta formado de tanques de doble pared, a través de las cuales circula líquido refrigerante. Está equipado con sensores, programas de control y válvulas para operar automáticamente. En ensayos, en una cámara de crecimiento, a 24° C, 70% Hr del aire y 90 W m^{-2}, Ψ_m pudo ser mantenido en un equilibrio dinámico para valores de pF entre 1 (-1kPa) y 2 (-10 kPa) por un período de 4 a 5 semanas, en varios tipos de suelos. Durante la cuarta semana de crecimiento de arvejas o iris ocurrieron variaciones de pF con una amplitud máxima de 0.4 unidades. La temperatura de suelo pudo ser mantenida a un nivel constante con amplitud máxima de 0.3° C.

Capítulo 8. Se desarrolló un procedimiento para diferenciar suelos de acuerdo a su receptividad, SR, a el *Fusarium solani* f.sp. *pisi*, Fsp. Respecto a un patógeno el suelo puede variar de conducivo a supresivo. Los experimentos se realizaron con muestras de suelo recolectadas en campos comerciales en los cuales se constató, durante el último cultivo de arveja, un PIS bajo. Respuestas de enfermedad causada por Fsp fueron determinadas a varios niveles de infestación artificial de los suelos a ensayar. Análisis estadísticos mostraron la dependencia de la respuesta con suelo ($P \leq 0.01$) usado. En suelos, generando en condiciones naturales sólo podredumbre seca de raíz, se constato que las cantidades de *Fusarium solani* nativo, en la rizósfera, no estaba correlacionado con la severidad de la podredumbre radicular. Varios métodos fueron examinados en su adecuacidad para describir y comparar valores de receptividad. Analisis en Componentes Principales, ACP, entregó un resultado satisfactorio. ACPs, realizados sobre una matriz de varianza-covarianza, derivada de curvas respuestas de enfermedad por suelo, ajustadas (usando el modelo Weibull) o no, produjeron un orden muy similar para las muestras. Un Análisis de Cluster seguido de un análisis discriminante múltiple permitió la clasificación de los suelos ensayados en grupos, diferiendo significativamente ($P \leq 0.05$; en base a χ^2) en receptividad a el Fsp.

Capítulo 9. A continuación, la receptividad de suelo fue ensayada para Tb y Ae usando materiales y procedimientos establecidos para el Fsp. Una comparación de la receptividad de suelo, con muestras ensayadas para los tres patógenos, indica en la mayoría de las muestras un ambiente conducivo al Ae, intermediario para el Fsp y supresivo respecto al Tb. Adicionalmente, se analizó el cambio operado en el PIS, tomando de referencia los valores determinados en bioensayos previo al cultivo de arvejas en 1986 o 1987 y los valores encontrados en 1991. Los valores PIS en 1991 mostraron un incremento significante ($P \leq 0.05$; prueba de signos) debido a la actividadad de patógenos causando podredumbre blanda de raíz. No hubo cambio en PIS, juzgado por la severidad de la podredumbre seca de raíz.

Capítulo 10. Se examinó el efecto suelo-habitat sobre la actividad antagónica de 2 aislados no patógenos de *F. oxysporum*, np Fo, conocidos por su efecto supresivo en varios patosistemas, sobre el Fsp en Arvejas. Los enfrentamientos se realizaron *in vitro*, suelo estéril y en suelos no esterilizados de alto PIS conteniendo formas indígenas de Fsp y otros patógenos. En suelo estéril ambos aislados no patógenos de Fo redujeron la

severidad de la podredumbre radicular y previnieron pérdidas en peso de las plantas. En suelos naturales el efecto fue variable y más efectivo en ausencia de Ae. Fo demostró ser un buen colonizador de la materia orgánica del suelo, aún cuando Fs nativo estaba presente con anterioridad.

Capítulo 11. Se ensayó el potencial antagónico sobre el Fs48 y patógenos radiculares nativos, de algunas especies de microorganismos aisladas de suelos diferiendo en receptividad al Fsp. *In vitro*, la mayoría de las especies ensayadas fueron antagónicas al Fs48 y no hubo una relación entre la magnitud del efecto y el grado de receptivi- dad, al Fsp, del suelo del cual las especies fueron aisladas. Tres aislados de *Gliocladium roseum*, obtenidos de suelos de baja receptividad al Fsp, redujeron significativamente la severidad de la enfermedad y la pérdida de peso de las plantas causada por la infestación de Fs48 en suelos estériles. En suelos naturalmente infestados, cuya bióta fue activada por un cultivo adicional de arvejas previo a los experimentos, dosis de *Gliocladium* de 10^6 a 10^7 conidia g^{-1} suelo seco fueron requeridas para obtener efecto. En estos suelos el efecto antagónico de cepas de *Pseudomonas fluorescens* fue variable y más efectivo en ausencia de Ae. No se constató correlación entre la actividad antagónica de estas cepas y la receptividad del suelo del cual fueron originadas.

Capítulo 12. Se analizó la relación entre RS y varios factores abióticos y bióticos de suelo. Varias relaciones fueron establecidas. La receptividad al Tb mostró estar asociada con la abundancia de pseudomonads, reacción del suelo (pH), contenido en materia orgánica y el cuociente C/N. La cantidad de Fs saprófito en la raíces de arvejas fue alta en suelos menos receptivos al Fsp, mientras que la abundancia de Fs nativo en la rizósfera fue independiente de la receptividad. Las cantidades individuales de K, P, Mg y los contenidos totales de N y C, tendieron a aumentar en suelos más conducivos a Fsp. Con excepción de *Gliocladium* en la rizóplana y de *Acremonium* en la rizósfera, cuyas poblaciones fueron más numerosas en suelo de baja receptividad, la receptividad de suelo al Ae no presentó una relación clara con ninguno de los factores en análisis. Ensayos de receptividad, esterilizando una parte del suelo, mostró que la bióta del suelo es el principal responsable de la inhibición de la actividad patógena de los hongos investigados. Se concluye que las relaciones entre SR y factores abióticos deben ser mediatizadas por la actividad microbial.

Capítulo 13. Se discute las complejas relaciones que caracterizan las enfermedades radiculares en arveja. La visión tradicional en Holanda sobre los agentes etiológicos del podredumbre del pie y raíz fue modificada, nuevos patógenos en arveja fueron demostrados y un bioensayo fue puesto a punto para su uso práctico. Se discute la influencia del hábitat sobre algunos de los principales patógenos radiculares en arveja. Los procedimientos elaborados permiten visualizar la estructura y estado de las variables para cada condición de receptividad de suelo. Se enfatiza que para el éxito del control biológico de estas enfermedades, se debe necesariamente tener en cuenta la exigencias o requerimientos ecológicas del organismo de control. Los procedimientos descritos permiten una mejor orientación en la investigación sobre mecanismos de receptividad, dan una visión de conjunto sobre el efecto de prácticas culturales y en un futuro mejorar la predicción de riesgos al formular una estrategia de manejo integrado de enfermedades. Estos logros confieren la investigación presentada un caracter mas amplio que el area de enfermedades radiculares de arvejas.

Curriculum vitae

Pedro Juvenal Oyarzun Miranda werd geboren op 28 februari 1952 te Coyhaique, Chili. Na het behalen van het diploma aan het Lyceum San Felipe Benicio te Coyhaique in 1970 begon hij met de studie Agronomie aan de Universidad de Concepcion, Chili. Eind 1973 onderbrak hij zijn studie in Chili. In 1977 werd hij assistent-onderzoeker by de Vakgroep Landbouwplantenteelt en later dat jaar begon hij aan zijn studie aan de voormalige Landbouwhogeschool te Wageningen in de richting Landbouwplantenteelt. Na het behalen van het kandidaats-examen werden praktijk-stages van elk drie maanden doorgebracht bij het Centrum voor Agrobiologisch Onderzoek en het Instituut voor Planteziektenkundig Onderzoek. De doctoraal-studie die hierop volgde omvatte naast het hoofdvak Landbouwplantenteelt, de bijvakken Fytopathologie, Entomologie en Grondbewerking. Gedurende deze tijd verrichtte hij o.a. studies aan mycorrhiza schimmels van landbouwgewassen en bestudeerde hij processen in de bodem die de kieming van zaden beïnvloeden. In een verzwaarde doctoraal-studie onderzocht hij waterhuishoudkundige relaties in de aardappel. Het doctoraal-examen werd afgelegd in 1984. Van mei 1985 tot december 1988 was hij, in dienst van het Nederlands Graan-Centrum, werkzaam als wetenschappelijk onderzoeker bij het Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond te Lelystad, waar hij onderzoek deed naar de aard en het belang van wortelrot- pathogenen bij erwt. In Januari 1989 werd hij gedetacheerd bij het IPO, waar hij methodologisch onderzoek verrichtte ter bepaling van bodemreceptiviteit t.a.v. wortel-pathogene schimmels bij erwt.

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