Ecological investigations as a basis for integrated management of bean Rhizoctonia root rot

Am Fachbereich Gartenbau
der Universität Hannover
zur Erlangung
des akademischen Grades eines

Doktors der Gartenbauwissenschaften

-Dr. rer. hort.-

genehmigte

Dissertation

von

MSc. Trazilbo José de Paula Júnior
geboren am 23. 05. 1966 in Resplendor (MG), Brasilien

2002

de Paula Júnior, Trazilbo José:
Ecological investigations as a basis for integrated management
of bean Rhizoctonia root rot

Referent:	Prof. Dr. Bernhard Hau
Korreferent:	Prof. Dr. Francisco Xavier Ribeiro do Vale
Tag der Promotion:	3. Juli 2002

Kurzfassung

Im Gewächshaus wurden verschiedene Experimente zur integrierten Kontrolle von Rhizoctonia-Wurzelfäule (*Rhizoctonia solani*) an Phaseolus-Bohnen durchgeführt, wobei folgende Maßnahmen kombiniert wurden: Tiefes Eingraben des Inokulums, Regulierung der Bodenfeuchtigkeit, flache Aussaat von Bohnensamen und Verwendung des Antagonisten *Trichoderma harzianum*. Darüber hinaus wurden Wechselwirkungen zwischen Rhizoctonia-Wurzelfäule und Anthraknose (*Colletotrichum lindemuthianum*) bzw. Bohnenrost *(Uromyces appendiculatus*) untersucht.

Um die Überdauerung von *R. solani* in unterschiedlichen Bodentiefen zu testen, wurde Inokulum des Pathogens, das auf Reissamen produziert wurde, in Bodentiefen von 0-5, 5-10, 10-15 und 15-20 cm eingearbeitet. In den Experimenten, die in Töpfen durchgeführt wurden, erfolgte die Aussaat der Bohnen in die obere Bodenschicht (3 cm tief), während in den Experimenten mit PVC-Zylindern, die aus entfernbaren Ringen gebildet wurden, die Bohnensamen in die infizierte Bodenschicht gelegt werden konnten. Die Aussaat erfolgte in beiden Fällen unmittelbar nach dem Ausbringen des Inokulums bzw. nach 15, 30, 60 und 90 Tagen. Wenn das Inokulum in den Topfexperimenten in die beiden oberen Bodenschichten (bis 10 cm Tiefe) ausgebracht wurde, waren die Befallshäufigkeit und -stärke an allen Erhebungsterminen hoch, die Auflaufrate und das Gewicht der Pflanzen dagegen verringert. Eine tiefere Ausbringung des Inokulums bewirkte geringere Schäden. Wenn das Inokulum tiefer als 15 cm in den Boden eingearbeitet wurde, waren bei allen Tests weder die Auflaufrate noch das Pflanzengewicht reduziert, obwohl häufig Infektionen an den Pflanzen festgestellt wurden. In den Experimenten mit den Zylindern war die Befallsstärke in allen Tests hoch und die Auflaufrate sowie das Pflanzengewicht durch den Schadpilz verringert, und zwar unabhängig von der Tiefe, in die das Inokulum ursprünglich ausgebracht wurde. In beiden Experimenten war nach 90 Tagen die Überlebensfähigkeit des Pathogens bei Abwesenheit von Wirtsgewebe kaum niedriger. Allerdings war eine Isolierung des Erregers aus der Bodenschicht oberhalb des ursprünglich infizierten Zylinderbereichs nur in geringem Maße möglich, wenn das Inokulum in einer tiefen Bodenschicht überdauerte.

Der Einfluss von Bodenfeuchte und Aussaattiefe auf die Rhizoctonia-Fäule und deren biologische Bekämpfung mit *T. harzianum* wurden ebenfalls untersucht. Das Pathogen wurde auf Reissamen, der Antagonist auf Weizenkleie angezogen. Das Inokulum wurde kurz vor der Aussaat mit der Erde vermischt. Die Auswertung erfolgte drei Wochen nach Aussaat, wobei die Auflaufrate der Bohnen, die Höhe und das Gewicht der Pflanzen sowie die Befallsstärke der Wurzelfäule bestimmt wurden. Die Auflaufrate und das Wachstum der Pflanzen, die in

mit *R. solani* inokuliertem Boden wuchsen, wurde nicht durch die Bodenfeuchteniveaus zwischen 15 und 42% (v/v) beeinflusst. Bei einer Inokulation von *R. solani* reduzierte eine tiefe Aussaat die Auflaufrate und das Wachstum der Pflanzen signifikant. Dieser Effekt der Aussaattiefe konnte nicht mehr nachgewiesen werden, wenn der Antagonist zusätzlich inokuliert wurde. Bei einer Aussaat 6 cm tief liefen in der Variante ohne *T. harzianum* 6.7% der Pflanzen auf, während unter dem zusätzlichen Einfluss des Antagonisten die Auflaufrate 50% betrug. Der Antagonist schützte die Pflanzen vor der Umfallkrankheit im Vorauflauf, verminderte die Befallsstärke der Wurzelfäule und förderte das Pflanzenwachstum, wobei die Wirkung von *T. harzianum* bei höherer Bodenfeuchte wirksamer war.

Die Überdauerung von *R. solani* und *T. harzianum* sowie die Dynamik der Mikroorganismen im Boden in Abhängigkeit der Bodenfeuchte wurde ebenfalls untersucht. Beide Pilze wurden gleichzeitig inokuliert, und die Aussaat der Bohnen wurde sofort nach der Inokulation bzw. 20, 60, 180 und 360 Tage danach durchgeführt. In einem ergänzenden Versuch erfolgte die Aussaat bereits 3, 6, 12 und 18 Tage nach Inokulation. Die Bodenfeuchte wurde regelmäßig kontrolliert und auf vier Stufen zwischen 20 zu 57% (v/v) eingestellt. Der Erreger überdauerte im Boden und verursachte Wurzelfäule an allen geprüften Zeitpunkten, wobei die Befallsintensitäten und die Schadwirkung ab dem 180 Tag abnahmen und am 360 Tag wesentlich niedriger waren. Der Antagonist verbesserte die Auflaufrate und zeigte antagonistische Wirkungen gegen *R. solani*. Der Erreger konnte bei Anwesenheit von *T. harzianum* nur schwer aus dem Boden wieder isoliert werden. Wenn der Erreger sich gut im Boden etabliert hatte, war die antagonistische Wirkung allerdings niedriger. Nach 360 Tagen konnte die antagonistische Wirkung kaum beobachtet werden. Bei den Tests bis zum 60 Tag förderte der Antagonist das Pflanzenwachstum auch auf den Pflanzen, die nicht mit *R. solani* in Kontakt kamen. Die Bodenfeuchteniveaus zwischen 20 und 57% beeinflussten nicht die Befallsintensität. Jedoch konnte der Erreger aus trockenerem Boden leicht wieder isoliert werden. Die antagonistische Wirkung und die Überlebensfähigkeit von *T. harzianum* waren deutlicher höher in Erde mit mittlerer Bodenfeuchten im Vergleich zu nassen bzw. trockenen Böden, hing aber auch vom Inokulumpotenzial beider Pilze im Boden ab.

Die gemeinsamen Wirkungen von *R. solani* und *C. lindemuthianum* bzw. *U. appendiculatus,* die in unterschiedlichen Konzentrationen inokuliert wurden, auf die Dynamik der Krankheiten und auf das Wachstum der Bohnenpflanzen wurden in weiteren Experimenten untersucht. Dazu wurden Bohnensamen in Erde gesät, die mit auf Reissamen produziertem *R. solani*-Inokulum vermischt worden war. In zusätzlichen Versuchen wurden Pflanzen in infizierte Erde umgepflanzt. Suspensionen von *C. lindemuthianum*-Konidien bzw.

U. appendiculatus-Uredospores wurden in den Pflanzenentwicklungsstadien V2 bzw. V3 auf die Blätter gesprüht. Die Wechselwirkungen zwischen der Wurzelfäule und den Blattkrankheiten waren von der Inokulumkonzentration und dem Inokulationszeitpunkt von *R. solani* abhängig. Die Befallsstärke der Anthraknose war tendenziell auf den Pflanzen höher, die mit *R. solani* befallen waren. Andererseits verringerte der Befall durch *R. solani* die Befallsstärke des Rosts sowie den Pusteldurchmesser deutlich. Bei niedrigen *R. solani*-Inokulumkonzentrationen wurden der Wurzelfäulebefall und die Dichte des Erregers im Boden bei den höchsten Konzentrationen von *C. lindemuthianum* bzw. *U. appendiculatus* erhöht, wenn die Pflanzen in infizierte Erde umgepflanzt wurden. In diesen Experimenten mit kombiniertem Befall durch Wurzelfäule und Anthraknose wurde eine synergistische Wechselwirkung auf das Pflanzengewicht beobachtet. Wenn die Bohnensamen in Erde gesät wurden, ergab sich für die Kombination Wurzelfäule-Rost dagegen eine antagonistische Wechselwirkung.

Schlagwörter: Phaseolus Bohnen, *Rhizoctonia solani*, integrierte Kontrolle

Abstract

Different experiments were conducted under greenhouse conditions to investigate the control of Rhizoctonia root rot caused by *Rhizoctonia solani* on Phaseolus beans in an integrated way through burial of the inoculum, water management, shallow sowing and use of the antagonist *Trichoderma harzianum*. Moreover, interactions between root rot and anthracnose (*Colletotrichum lindemuthianum*) or rust (*Uromyces appendiculatus*) were investigated.

To study the effect of different depths on the *R. solani* survival, inoculum of the pathogen produced on rice grains was buried in the soil at depth of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm. The experiments were carried out in pots, where bean seeds were sown in the top layer (3 cm deep), and in PVC cylinders with removable rings, where the sowing was performed directly on infested soil. Bean seeds were sown immediately after the soil infestation, as well as at 15, 30, 60 and 90 days after the soil infestation. In the pots, disease incidence and severity were high, and emergence of seedlings and plant weight were consistently reduced at all evaluation dates when the inoculum was confined in the upper 10 cm of soil. Damaging effects of *R. solani* on emergence and plant growth were reduced with deeper placement of inoculum. Emergence and plant weight were not reduced at any evaluation date when the inoculum was buried deeper than 15 cm, but infected plants were frequently observed. In the cylinders, high disease severities were observed at all evaluation dates; emergence and plant weight were reduced by *R. solani* independently of the depth in which the inoculum was originally buried. Pathogen survival was not consistently reduced after 90 days in absence of host tissue in both experiments, although effects of *R. solani* on emergence and plant growth decreased over time. Moreover, *R. solani* could very hardly be recovered from soil samples collected above the layer originally infested in the cylinders when it was deeply buried.

The effect of soil moisture and sowing depth on the disease and its control by *T. harzianum* was investigated. The pathogen was grown on rice grains and the antagonist on wheat bran. Both fungi were inoculated before sowing. Root rot severity, percentage of plants emerged, plant height and dry weight were evaluated three weeks after sowing. Emergence rate and growth of plants inoculated only with *R. solani* were not affected by soil moisture varying from 15 to 42% (v/v). Sowing deeper than 1.5 cm significantly reduced the emergence rate and growth of plants inoculated only with *R. solani*. However, in the presence of the antagonist, the effect of sowing depth was not significant. At a sowing depth of 6.0 cm, the percentage of plants emerged was 50% in the presence of *T. harzianum*, but only 6.7% when the pathogen was alone. The antagonist protected seedlings from preemergence

damping-off, reduced disease severity and increased plant growth in the presence of *R. solani*, especially in moist soil.

The survival of *R. solani* and *T. harzianum* and the dynamics of these microorganisms in the soil were also affected by soil moisture. Both fungi were inoculated at the same time, and the sowing was carried out immediately after soil infestation and 20, 60, 180 and 360 days after soil infestation (DAI), and 3, 6, 12 and 18 DAI in a complementary experiment. Soil moisture was periodically monitored and kept at levels varying from 20 to 57% (v/v). The pathogen survived in the soil and caused disease at all tested dates. However, in the first survival tests (0, 20 and 60 DAI), severity of root rot initially increased, but decreased later (180 and 360 DAI). On the other hand, dry weight of *R. solani*-infected plants was reduced in the initial tests, but increased later so that at 360 DAI values as in the treatment control were reached. Soil moisture did not affect severity of root rot. However, the pathogen could easily be recovered from dryer soil. It could hardly be recovered from soil in the presence of *T. harzianum*. Consistent antagonistic effects were observed until 180 DAI, but at 360 DAI they were very slight. When the pathogen was well established in the soil, antagonistic protection was lower. The antagonist improved plant growth even on plants not inoculated with *R. solani* until 60 DAI. The antagonistic ability and survival of *T. harzianum* were greater in soils with an intermediate moisture level than in wet or dry soils, but depended on the inoculum potential of both fungi in the soil.

The co-inoculation of *R. solani* and *C. lindemuthianum* or *U. appendiculatus* at different inoculum levels was investigated on the disease dynamics and on the growth of bean plants. Bean seeds were sown in *R. solani*-infested soil. Additional experiments in which bean seedlings were transplanted to infested soil were also carried out. Conidial suspensions of *C. lindemuthianum* and uredospores of *U. appendiculatus* were inoculated onto leaves at plant developmental stages V2 and V3, respectively. Interactions between root rot and the aerial diseases were observed depending on the inoculum levels and on the timing of *R. solani* inoculation. Anthracnose severity tended to be higher on *R. solani*-infected plants. On the other hand, *R. solani* infection significantly reduced diameter of pustules and rust severity. At *R. solani* low levels, root rot severity and density of *R. solani* in the soil were magnified at the highest levels of *C. lindemuthianum* or *U. appendiculatus* when seedlings were transplanted to infested soil. In these experiments, a synergistic interaction between root rot and anthracnose was observed to affect the plant weight. Antagonistic effects on the plant weight were seen for the combination root rot/rust only when bean seeds were sown in infested soil.

Keywords: Phaseolus beans, *Rhizoctonia solani*, integrated control

List of main symbols

Symbol	Description
AG	Anastomose Group
ANOVA	Analysis of variance
AUPEC	Area under plant emergence curve
Cfu	Colony forming units
CL	*Colletotrichum lindemuthianum*
CL0-CL3	*C. lindemuthianum* levels 0-3
Cm	Centimeter
D	Soil depth
DAI	Days after inoculation (or soil infestation)
DAS	Days after sowing
DW	Dry weight
G	Gram
L	Liter
Ml	millilitre
ML	Moisture level
Mm	Millimeter
N	Number of observations
P	Probability
PH	Plant height
R	Coefficient of correlation
R^2	Coefficient of determination
RDW	Relative dry weight
RRS	Root rot severity (Chap. 4)
RS	*Rhizoctonia solani*
RS0-RS3	*R. solani* levels 0-3
Rt	With *R. solani*/without *T. harzianum*
RT	Without *R. solani*/with *T. harzianum*
RT	With *R. solani*/with *T. harzianum*
Rt	Without *R. solani*/without *T. harzianum*
SAS	Statistical Analysis System
SD	Sowing depth
T	Time
T1...T4; T1-4	Treatments 1…4; additional treatment (soil layers 1-4)
T_C	Non-infested control
UA	*Uromyces appendiculatus*
UA0-UA3	*U. appendiculatus* levels 0-3
v/v	Volume/volume
V2, V3	Bean plant developmental stages (V = vegetative)
w/w	Weight/weight
WHC	Water holding capacity
y_{CL}	Anthracnose severity
y_{RS}	Root rot severity (Chap. 5)
y_{UA}	Rust severity

Contents

1. Introduction

Rhizoctonia root rot is an important disease of beans occurring in all bean-production areas around the world, especially in Latin America and Africa (ABAWI, 1994; HALL, 1991). The disease is caused by *Rhizoctonia solani* Kühn that is the asexual state of *Thanathephorus cucumeris* (Frank) Donk. Losses occur in the form of seed rot and seedling damping-off, resulting in reduced plant densities. Plants severely infected become less vigorous in later stages and their yield is reduced (ABAWI and PASTOR-CORRALES, 1990).

The pathogen occurs in the soil in many strains that differ in physiology, pathogenicity and appearance in cultures. A common characteristic of all isolates of *R. solani* is the sterile mycelium that is colourless when young and light brown when older. Its cells are long and multinucleate. Branch hyphae arise at right angles, are constricted at their base, and contain a cross-wall near the branch point (HALL, 1991). Most of *R. solani* isolates produce loose sclerotia that, along with thick-walled hyphae in host tissues, are the survival structures in soil. The inoculum of *R. solani* that causes root rot consists of sclerotia and mycelia. The pathogen can penetrate intact plant tissue or through natural openings and wounds (ABAWI and PASTOR-CORRALES, 1990).

Many anastomosis groups (AG) have been described for *R. solani* and they vary in their host range and the diseases they cause (SWEETINGHAM, 1996). The isolates that cause root rot on beans usually belong to AG 2 or AG 4 (ABAWI, 1994).

Initial symptoms of Rhizoctonia root rot on bean plants appear on roots or hypocotyls as small, elongate, sunken, reddish-brown lesions. The cankers enlarge with age, become darker. Hypocotyls are often girdled by the coalescence of several cankers, resulting in pre- or post-emergence damping off. Severe infections cause plant stunting and premature death (HALL, 1991).

The disease is most severe at 15-18°C. At 21°C, the number of cankers is substantially reduced, perhaps because plants emerge rapidly and can escape infection (HALL, 1991; ABAWI, 1994). Seedlings and young plants are highly susceptible to infection, whereas the disease is rarely a problem on older plants (HALL, 1991). The pathogen survives in the soil for a long time and disseminates to new areas through the sowing of contaminated seeds or the movement of infected host tissues, infested soil, or colonized debris by irrigation water, wind, and animals (ABAWI and PASTOR-CORRALES, 1990).

Management strategies to control *R. solani* include seed and soil treatment with fungicides and many cultural practices such as sowing of high quality seeds, shallow sowing,

sowing on raised beds, crop rotation with grain crops, deep plowing and incorporation of decomposable organic residues into the soil (ABAWI, 1994).

The incorporation of residue, deeply and early enough to promote complete decomposition, is effective against *R. solani*, because it reduces inoculum density near the soil surface, where the pathogen is most active (ABAWI and PASTOR-CORRALES, 1990). The pathogen is rarely found deeper than 10 cm (PAPAVIZAS et al., 1975). Different inoculum depths and their effects on disease progress and survival of *R. solani* is the subject of Chapter 2.

Adjustment of soil water content has been recommended as a practice to control root rot by permitting a rapid emergence of seedlings (ABAWI, 1994), although different results have been observed concerning the effects of soil moisture on disease severity and on pathogen survival (VAN BRUGGEN et al., 1986; FENILLE and SOUZA, 1999). Seeds planted at shallow depths emerge faster and escape the root rot (ABAWI and PASTOR-CORRALES, 1990). However, as usually observed for diseases caused by soilborne pathogens, no single treatment provides a satisfactory control of Rhizoctonia root rot. Integrated management systems for disease control are receiving increased attention for economic and environmental reasons. More than 20 years ago, the use of combined strategies to manage *R. solani* in the field was practically non-existent (PAPAVIZAS and LEWIS, 1979), but today several integrated systems have been investigated and some are being implemented at the farm level (SWEETINGHAM, 1996). It has been demonstrated that combined strategies are often additive and sometimes synergistic in their efficiency, leading to a more effective and reliable disease control (ELAD et al., 1980b; LEWIS and PAPAVIZAS, 1980; CHET et al., 1982; SWEETINGHAM, 1996).

The use of microbial antagonists, for instance *Trichoderma* spp., as a component of an integrated disease management program is effective to control Rhizoctonia root rot (ABAWI and PASTOR-CORRALES, 1990; SWEETINGHAM, 1996). The genus *Trichoderma* is cosmopolitan in soils and on decaying wood and vegetable matter. Species of this fungus are frequently dominant components of the soil microflora in widely varying habitats (GAMS and BISSETT, 1998). The high degree of ecological adaptability makes these fungi attractive for biocontrol applications.

Typical characteristics of *Trichoderma* include rapid growth, bright green or white conidial pigments, and a repetitively branched, but otherwise poorly defined conidiophore structure (GAMS and BISSETT, 1998). *Trichoderma harzianum* Rifai comprises the strains often used in biological control of plant pathogenic fungi (GAMS and BISSETT, 1998). It has

been frequently demonstrated that it may reduce activities of *R. solani* (HENIS et al., 1978; HADAR et al., 1979; ELAD et al., 1980a; CHET et al., 1982; MARSHALL, 1982). The fungus grows rapidly and its temperature optimum for growth is at 30°C, which means it is characteristic of warm climates (GAMS and BISSETT, 1998; KLEIN and EVELEIGH, 1998).

Several environmental and edaphic factors influence the control and survival of *R. solani* and the antagonistic ability and survival of *T. harzianum*. *Trichoderma* spp. grow fast enough to maintain a high population density in the rhizosphere. However, some cultural practices used to manage the disease may affect growth and antagonistic ability of *Trichoderma* (SWEETINGHAM, 1996). The integration of *T. harzianum* with water management offers a great challenge, since moisture influences the natural distribution of *Trichoderma* in the soil (DANIELSON and DAVEY, 1973). However, little is known about how shallow sowing could affect the biological control. Questions related to how soil moisture and sowing depth can affect Rhizoctonia root rot and its biological control by *T. harzianum* are addressed in Chapter 3. Moreover, effects of soil moisture on the survival of both fungi are discussed in Chapter 4.

Synergistic effects are frequently observed in interactions involving *R. solani* and other soilborne pathogens (ABAWI and PASTOR-CORRALES, 1990; HALL, 1991). However, little is known about interactions involving *R. solani* and aerial pathogens of beans, although multiple infections may frequently occur in the field. Many examples in the literature show that soilborne pathogens can alter the susceptibility of the host to infection by aerial pathogens and vice versa (WALLER and BRIDGE, 1984). The occurrence and implications for disease management of interactions involving *R. solani* and two aerial pathogens of beans - *Colletotrichum lindemuthianum*, causal agent of anthracnose, and *Uromyces appendiculatus*, causal agent of rust - are discussed in Chapter 5.

2. Effects of inoculum depth on survival of *Rhizoctonia solani* and on bean root rot development

Abstract

The effects of different depths of *Rhizoctonia solani* inoculum on the survival of the pathogen and the development of Rhizoctonia root rot on beans were studied under greenhouse conditions. Inoculum was grown on rice grains and buried in the soil at depths of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm. The experiments were carried out in pots, where bean seeds were sown in the top layer (3 cm deep), and in PVC cylinders with removable rings, where the sowing was performed directly on infested soil. Bean seeds were sown immediately after the soil infestation, as well as at 15, 30, 60 and 90 days after the soil infestation. In the pots, disease incidence and severity were high, and emergence of seedlings and plant weight were consistently reduced at all evaluation dates when the inoculum was confined in the upper 10 cm of soil. Damaging effects of *R. solani* on emergence and plant growth were reduced with deeper inoculum placement. Emergence and plant weight were not reduced at any evaluation date when the inoculum was buried deeper than 15 cm, but infected plants were frequently observed. In the cylinders, high disease severities were observed at all evaluation dates; emergence and plant weight were reduced by *R. solani* independently of the depth in which the inoculum was originally buried. Pathogen survival was not consistently reduced after 90 days in absence of host tissue in both experiments, although effects of *R. solani* on emergence and plant growth decreased over time. Moreover, the pathogen could very hardly be recovered from soil samples collected above the layer originally infested in the cylinders when it was deeply buried.

2.1 Introduction

Root rot caused by *Rhizoctonia solani* Kühn is a limiting factor in the commercial production of beans in Latin America and Africa. Seed and seedling infection results in severe pre- and postemergence damping-off or root rots, which reduce plant stands (ABAWI, 1994). The pathogen survives in the soil as sclerotia and melanized mycelium, free or embedded in soil organic debris (BOOSALIS and SCHAREN, 1959), originating from parasitized crops and weed hosts and/or from saprophytically colonized crop residues, comprising sources of inoculum for the following crop (HERR, 1976; SUMNER, 1996).

The vertical distribution of the pathogen in the soil as well as the disease development are affected by several environmental and edaphic factors (SUMNER, 1996). In the field, inoculum of *R. solani* seems to be confined to the upper 10 cm of soil (PAPAVIZAS et al., 1975). For this reason, deep plowing of bean plant residues infected by *R. solani* to a depth of 20-25 cm has been recommended to reduce inoculum density and survival of the pathogen in the soil (PAPAVIZAS et al., 1975; DE PAULA and ZAMBOLIM, 1998). Also on other crops than beans, effects of this practice in reducing population density, incidence and severity of root rot have been reported (HUSSEY and RONCADORI, 1977; BAIRD, 1993; LEACH et al., 1993). Apparently *R. solani* is not able to extensively colonize plant residues at depths of 20-25 cm (PAPAVIZAS et al., 1975). Moreover, a faster and more complete decomposition of infected organic debris takes places after deep plowing of infected residues (ABAWI and PASTOR-CORRALES, 1990).

However, it cannot not be expected that deep plowing completely inhibits the activities of *R. solani* (SUMNER et al., 1986). In the absence of host tissue, *R. solani* can survive more than one year in the soil when the inoculum is homogenously distributed in pots, although disease severity and plant damage are reduced (see Chapter 4). After deep plowing in the field, contact between roots and inoculum of *R. solani* can occur in the next season if roots grow deep enough into the soil. Moreover, cultural practices involving soil movement can bring viable inoculum to the soil surface. These practices may have serious epidemiological implications, since infected plants increase the inoculum in the soil and become an inoculum source for the following crops.

The purpose of the present study was to investigate how different inoculum depths can affect the survival of *R. solani* in the soil and the development of root rot on beans.

2.2 Material and methods

Two complementary experiments were conducted under greenhouse conditions at Hanover, Germany. The purpose of the first experiment was to observe the ability of the pathogen to survive and to cause disease when the inoculum was confined in different depths in the soil and bean seeds were sown in the top layer. The objective of the second experiment was the same, but here a direct contact between inoculum previously buried in the soil and emerging seedlings was established by sowing the seeds in the infested layer after the removal of upper inoculum free layers.

The isolate of *R. solani* (AG-4) was taken from the fungi collection of the Institute of Plant Diseases and Plant Protection, University of Hanover. It was originally isolated from

bean and maintained at 4°C. Two 5 mm diameter mycelial-agar disks produced on PDA were transferred from the margin of growing colonies to 200 ml-Erlenmeyer flasks containing rice grains. After six days of incubation at 25°C in darkness, they were totally colonized by *R. solani*. The mass of rice grains was manually separated and the grains were dried for 24 hours on trays.

Soil-sand (2:1) was sterilized at approximately 100°C for 24 hours. Besides the non-infested control (TC), the inoculum was incorporated at a concentration of 0.05% (w/w) to the soil layers in different depths: 0-5 cm (T1), 5-10 cm (T2), 10-15 cm (T3) and 15-20 cm (T4). In an additional treatment, the inoculum was placed in the 0-20 cm layer (T1-4). The initial population density of *R. solani*, expressed as number of colony-forming units (cfu)/g of soil, was determined according to KO and HORA (1971). Seeds of bean cultivar 'Dufrix' were sown 3 cm deep. The experiments consisted of six treatments in combination with five different sowing dates: immediately after soil infestation and at 15, 30, 60 and 90 days after the soil infestation (DAI). In the first experiment, pots containing 10 l of soil-sand were used. Each replication consisted of a pot where 10 seeds were sown. In the second experiment, PVC cylinders with a diameter and height of 11 and 30 cm, respectively, consisting of removable rings and containing 3 l of soil-sand were used. The rings were removed and the sowing was performed directly on the layer containing infested soil. In the latter case, each replication consisted of a cylinder where eight seeds were sown. Pots and cylinders were irrigated once a day. Five replicates of each treatment were placed in a randomised complete block design. Both experiments were repeated once.

Percentage of emerged plants was daily assessed. The plants were removed about 23 days after the sowing (DAS) and hypocotyls were evaluated to determine disease incidence and severity according to a 1-9 scale adapted from VAN SCHOONHOVEN and PASTOR-CORRALES (1987), where 1 - no visible symptoms, 3 - light discoloration without necrotic lesions, 5 - < 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm, 7 - 25-50% of the hypocotyl and root tissues covered with lesions combined with softening, rotting, and reduction of the root system, 9 - > 75% of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system or dead plants. Means of disease severity varying from 1 to 3 were classified as low, 3.1 to 7 as intermediate, and 7.1 to 9 as high. Plant height, plant dry weight and population density of *R. solani* were also evaluated.

To determine the population density of *R. solani* (KO and HORA, 1971), soil was mixed in each pot before samples were collected in the pot-experiment. In the cylinder-

experiment, samples were collected in each cylinder from the upper 10 cm of soil after plants were removed. At 60 DAI, population density of *R. solani* was also quantified, before the sowing, in samples collected from soil above the layer originally infested with the pathogen. Comparisons for emergence rate were done using the area under plant emergence curve (AUPEC). ANOVA was calculated and multiple range tests ($P = 0.05$) were used for mean separation with SPSS (SPSS Inc., Chicago).

2.3 Results

Due to the similarity in the results, data presented here are from the second run of the experiments. Plant height was positively correlated with plant dry weight at all evaluations ($r > 0.95$; $P < 0.01$) so that only data of the plant dry weight are used in the further analyses.

No infected plants were observed in the control (TC) and the pathogen could not be recovered from soil of this treatment in both experiments. Decreases in the emergence curves are related to post-emergence damping-off. Results of the treatment T1-4 are also presented in all Figures and Tables for comparisons.

2.3.1 Pot-experiment (beans sown in the top layer)

At the sowing done immediately after soil infestation (0 DAI), emergence of seedlings, final stand and plant weight were drastically reduced by *R. solani* in the two top layers (T1 and T2) (Fig. 1, Tab. 1). Post-emergence damping-off was also observed in T3 (Fig. 1) and plant dry weight in this treatment was significantly reduced by 36.7% compared to the control TC (Tab. 1). Disease incidence and severity were high in T1, T2, and T3 (Fig. 2), but only 22% of the plants were infected by the pathogen in bottom layer (T4) and disease severity was low, too (Fig. 2). The pathogen could hardly be recovered from soil in this treatment (Fig. 2).

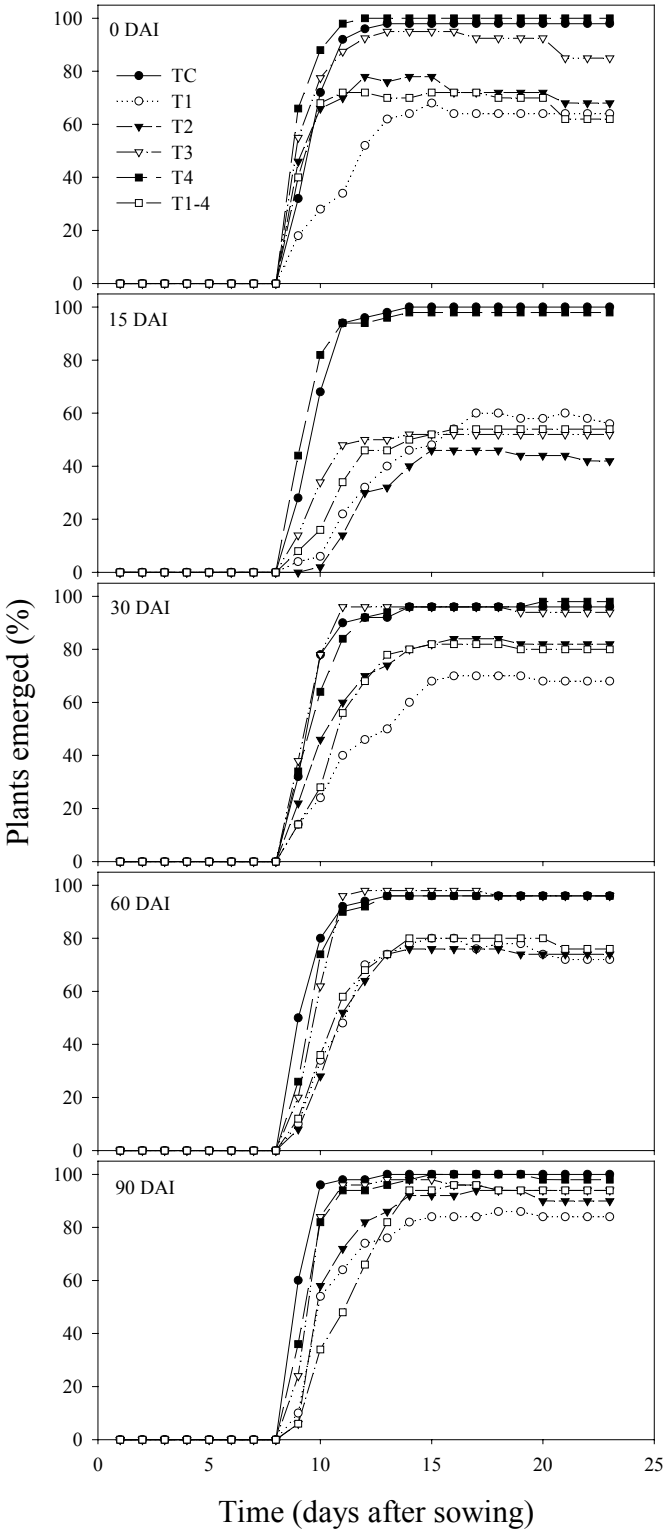

Figure 1. Effects of inoculum depth of *R. solani* (TC = non-infested control; T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm) on the emergence of bean seedlings when beans were sown in the top layer at **0**, **15**, **30**, **60** and **90 DAI**

Table 1. Effects of inoculum depth of *R. solani* on AUPEC (calculated up to 23 DAS) and plant dry weight (at 23 DAS) for 5 evaluation dates, characterised by the day (DAI) when beans were sown in the top layer

Treatments	AUPEC (%/Days)				
	0 DAI	15 DAI	30 DAI	60 DAI	90 DAI
TC*	140.3 a**	142.0 a	137.6 a	139.5 a	147.2 a
T1	86.1 c	68.8 b	87.1 c	102.7 b	115.7 b
T2	106.7 b	53.9 b	112.6 b	100.9 b	126.4 ab
T3	133.3 a	73.5 b	138.2 a	138.0 a	138.5 ab
T4	146.9 a	141.7 a	136.8 a	137.3 a	142.3 a
T1-4	*101.5*	*70.6*	*108.5*	*106.8*	*122.4*
	Dry weight (% in relation to TC)				
TC	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
T1	34.2 c	22.6 b	44.0 c	53.9 b	63.2 b
T2	34.8 c	12.0 b	66.0 b	46.2 b	80.1 ab
T3	63.3 b	48.7 b	104.6 a	81.5 a	95.6 a
T4	118.2 a	117.4 a	100.9 a	80.8 a	100.0 a
T1-4	*38.8*	*33.4*	*50.5*	*47.6*	*61.5*

* TC = non-infested control; T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm. **Values are means of 5 replicates. Means followed by the same letter in a column are not significantly different ($P = 0.05$)

In the second survival test, sown at 15 DAI, emergence, final stand and plant weight were more drastically reduced in T1 to T3 than immediately after infestation (Fig. 1, Tab. 1). Disease incidence and severity were again high in these treatments (Fig. 2). However, in T4 only 16% of the plants were infected and disease severity was rather low (Fig. 2). Population density of *R. solani* was significantly lower in T4 compared to T1 to T3 (Fig. 2).

In the following survival tests at 30 and 60 DAI, emergence and plant weight were reduced only in T1 and T2 (Fig. 1, Tab. 1). Disease severity was lower than in the previous evaluations, especially for T3 (Fig. 2). In contrast, disease severity in T4 was low at 30 DAI, but became intermediate at 60 DAI (Fig. 2). About 20% and 80% of plants were infected in T4 at 30 and 60 DAI, respectively (Fig. 2). The pathogen could more easily be recovered from soil in T1 (0.4 cfu/g of soil) compared to T4 (0.1 cfu/g of soil) at 30 DAI, but there were no differences among T1 to T4 at 60 DAI (Fig. 2).

When the bean seeds were sown at 90 DAI, emergence and plant weight were reduced only in T1 (Fig. 1, Tab. 1). Disease severity was intermediate for treatments T1 to T4 (Fig. 2). About 50% of plants were infected in T4 (Fig. 2). No differences were observed for population density of *R. solani* among T1 to T4 (Fig. 2).

The AUPEC values in T1 to T4 were related with inoculum depth and evaluation time (Tab. 1). AUPEC (and also the percentage of plants emerged and plant weight) were lowest in T1 and T2 and increased with deeper inoculum placement so that in T4 similar values as in TC, the disease free control, were reached. For disease incidence and severity the trend was

the other way round, i.e. the values decreased with deeper inoculum placement (Fig. 2). Nevertheless, also in T4 a disease level was reached, which cannot be neglected. A second trend was related to the evaluation over time: Initially there is a decrease in the AUPEC-values in T1 to T4, but after the second evaluation at 15 DAI the values increased. This effect was clearer if the inoculum was confined in the upper layers (T1 and T2).

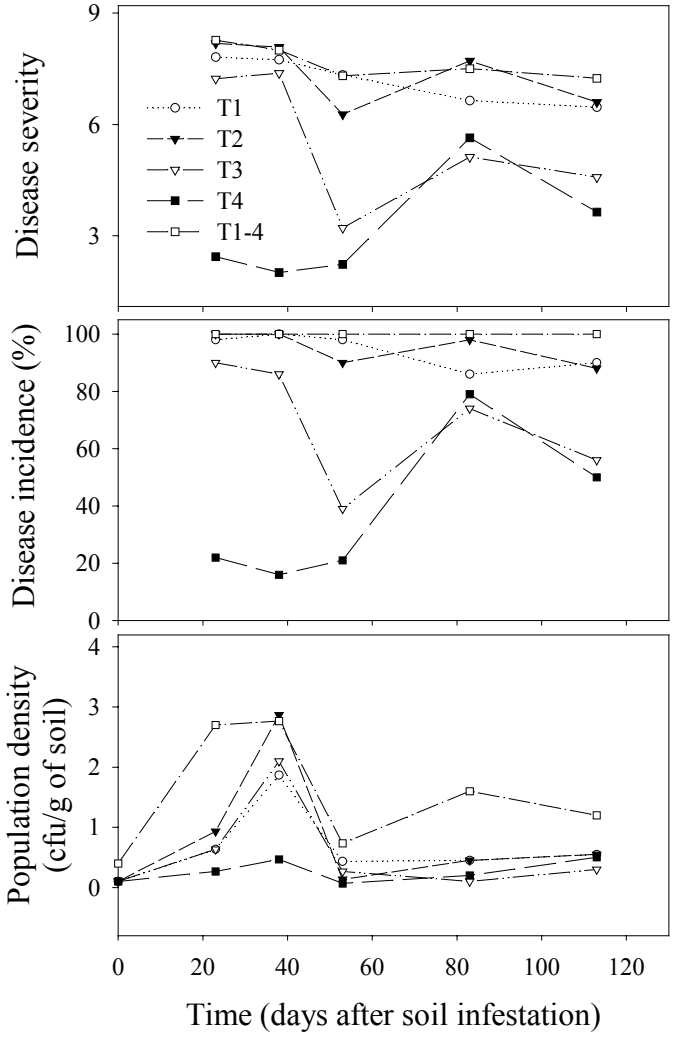

Figure 2. Effects of inoculum depth of *R. solani* (TC = non-infested control; T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm) over time on disease severity, disease incidence and population density at 23 DAS when beans were sown in the top layer

The trends described are reflected in the Figure 3 in which the function

$AUPEC(t,d) = 141.5\ [1 - (c_1 - c_2\ t)\ \exp(-c_3\ d)]$

was fitted to the data of treatments T1 to T4. The value 141.5 is the AUPEC mean value of the control (TC) at the four evaluation dates after the establishment of the pathogen in the soil

(between 15 and 90 DAI). The parameters *t* and *d* represent time (measured in days after soil infestation) and soil layer depth (1 to 4 for T1 to T4). The coefficient c_1 is a general constant that is related to AUPEC at $t = 0$ and $d = 0$. The coefficients c_2 and c_3 describe the increasing effect of time and soil layer, respectively. According to this equation, AUPEC increased monomolecularly with the deeper inoculum placement and linearly with time.

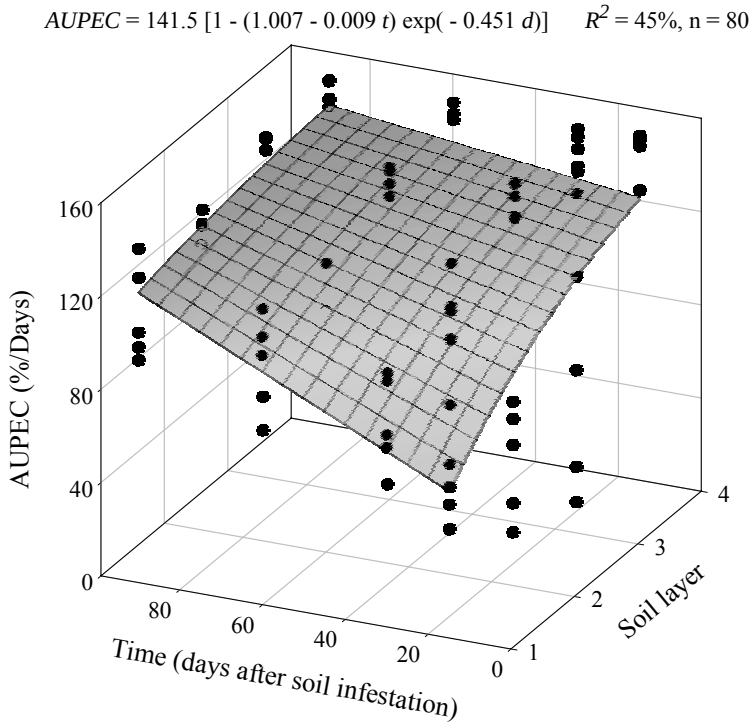

Figure 3. Effects of inoculum depth of *R. solani* over time on AUPEC (calculated up to 23 DAS) when beans were sown in the top layer. Soil layers 1 to 4 correspond to treatments T1 to T4

The population density of *R. solani* in the bottom layer (T4) was practically not altered over time. However, in the upper layers (T1, T2 and T3), the density increased in the beginning (at 0 and 15 DAI) and after a decrease (between 15 and 30 DAI) it remained constant (Fig. 2).

2.3.2 Cylinder-experiment (beans sown in the infested layer)

As an example for this experiment, the sowing performed at 30 DAI was chosen. The emergence curves of the six treatments at this evaluation date are presented in the Figure 4. In this survival test as well as in the tests carried out at other evaluation dates, the emergence of seedlings, final stand and plant weight were drastically reduced by *R. solani,* but the depth

where the inoculum was originally placed had no influence (Tab. 2). Using the AUPEC and dry weight data of individual cylinders for the four soil layers (T1 to T4), linear regression analyses revealed significant increases of both variables with time.

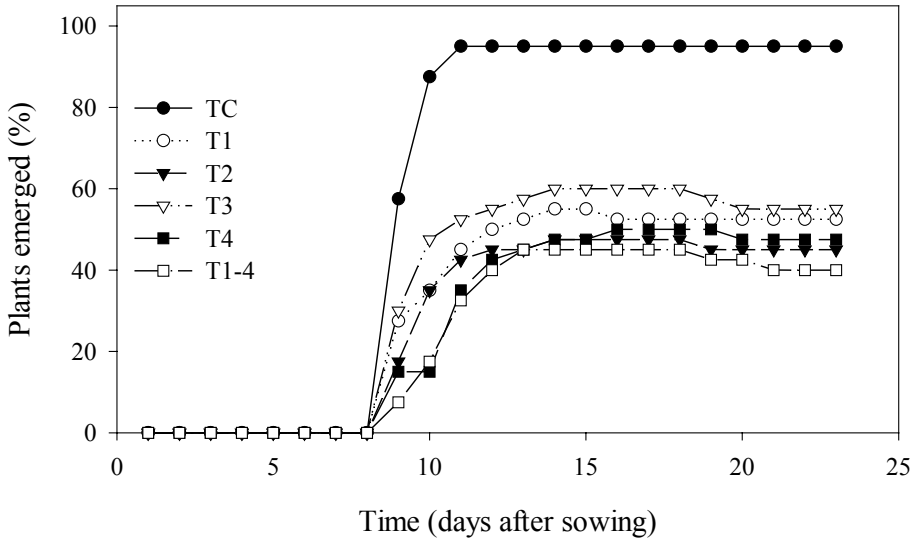

Figure 4. Effects of inoculum depth of *R. solani* (TC = non-infested control; T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm) on the emergence of bean seedlings when beans were sown in the infested layer at **30 DAI**

Disease incidence was 100% or close to it in the treatments that received *R. solani* inoculum and disease severity was consistently high at all evaluation dates (Fig. 5). No significant differences were observed among T1 to T4 for any factor evaluated. Density of *R. solani* increased up to 4.8 cfu/g of soil, but showed later a decreasing trend (Fig. 5).

At 60 DAI, population density of *R. solani* in samples collected from soil above the layer originally infested with the pathogen was 0.7, 0.7 and 0.05 cfu/g of soil in T2, T3 and T4, respectively.

In general, it can be stated for the cylinder-experiment that the inoculum survival was not influenced by the depth of the inoculum, because the percentage of plants emerged, plant weight and disease severity were similar in T1 to T4 at all evaluation times. The density of the pathogen in the soil samples, expressed as cfu/g of soil, was also similar in T1 to T4 (Fig. 5). Disease severity and population density decreased slightly over time (Fig. 5).

Table 2. Effects of inoculum depth of *R. solani* on AUPEC (calculated up to 23 DAS) and plant dry weight (at 23 DAS) for 5 evaluation dates, characterised by the day (DAI) when beans were sown in the infested layer

Treatments	0 DAI	15 DAI	30 DAI	60 DAI	90 DAI
			AUPEC (%/Days)		
TC*	123.9 a**	111.3 a	111.9 a	113.2 a	116.2 a
T1	45.5 b	36.3 b	60.2 b	74.1 b	99.9 ab
T2		72.9 ab	52.9 b	71.2 b	87.7 b
T3		64.3 b	66.6 b	60.3 b	85.7 b
T4		63.0 b	52.3 b	63.7 b	60.7 c
T1-4	*80.3*	*53.5*	*47.1*	*66.4*	*59.3*
			Dry weight (% in relation to TC)		
TC*	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
T1	13.8 b	19.1 b	29.6 b	45.2 b	54.2 b
T2		42.7 b	39.6 b	45.5 b	50.0 b
T3		35.8 b	56.0 b	33.1 b	46.7 b
T4		26.5 b	30.2 b	38.4 b	31.6 b
T1-4	*28.2*	*27.8*	*14.8*	*37.2*	*32.2*

* TC = non-infested control; T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm. **Values are means of 5 replicates. Means followed by the same letter in a column are not significantly different ($P = 0.05$)

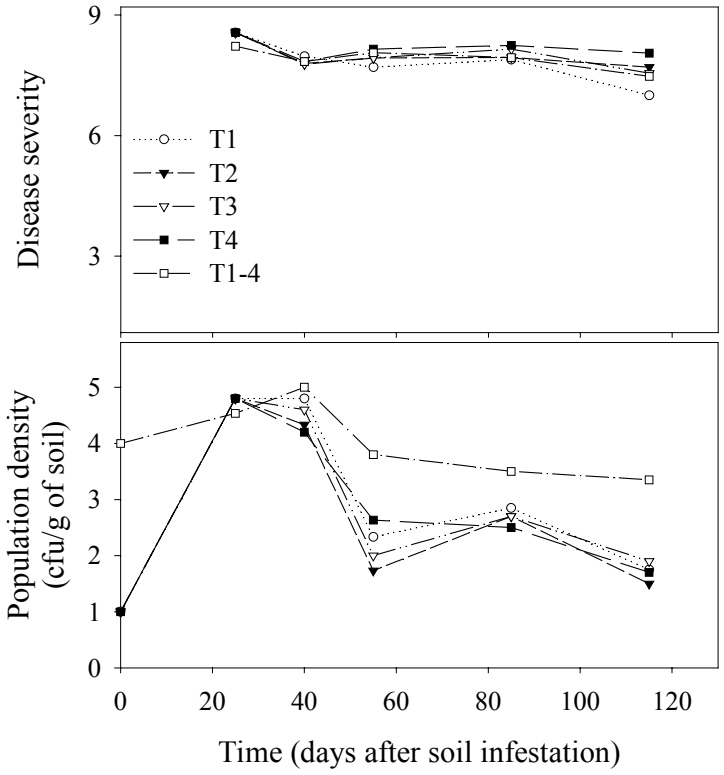

Figure 5. Effects of inoculum depth of *R. solani* (T1 = 0-5 cm; T2 = 5-10 cm; T3 = 10-15 cm; T4 = 15-20 cm; and T1-4 = 0-20 cm) over time on disease severity and population density at 23 DAS when beans were sown in the infested layer

2.4 Discussion

The burial of *R. solani* inoculum at different depths may affect the survival of the pathogen, as well as the disease severity and the damage on plants (SUMNER, 1996). In the pot-experiment, high disease incidences and severities were observed at all evaluation dates when the inoculum was confined to the upper 10 cm of soil (T2 and T3) (Fig. 2). In both treatments, the emergence of seedlings and the plant dry weight were consistently reduced by the pathogen (Fig.1, Tab. 1). Moreover, the Figure 3 indicated that the damaging effects of *R. solani* decreased with the deeper inoculum placement in the pot-experiment. These results are supported by several authors, which have shown that growth and aggressiveness of *R. solani* are increased when the pathogen is located in the upper soil layer (PAPAVIZAS et al., 1975; HIREMATH and PRASAD, 1985; RUPPEL, 1991; BAIRD et al., 1993; LEACH et al., 1993).

There is a general agreement stating that *R. solani* can rarely be isolated from subsoil in the field (PAPAVIZAS et al., 1975; SUMNER, 1996). PAPAVIZAS et al. (1975) found that the pathogen was confined almost entirely to the upper 5 cm of soil; very little activity was observed between 5 and 10 cm, and no activity below 10 cm. However, in the present study, when the soil layer 10-15 cm (T3) was infested with the pathogen, disease incidence and severity (Fig. 2), as well as emergence and weight of plants (Fig. 1, Tab. 1) varied greatly; disease severity was high in the survival tests at 0 and 15 DAI, but became intermediate later (30, 60 and 90 DAI) (Fig. 2). Although no effect on emergence was observed in T3 at 0 DAI (Fig. 1, Tab. 1), plant weight was reduced at this evaluation date as well as at 15 DAI (Tab. 1). This is in accordance to the results of HIREMATH and PRASAD (1985) that *R. solani* may survive even if it is buried deeper than 10 cm. They collected soil at 2.5 cm intervals to a 35 cm depth and incubated soil pellets on selective media. The pathogen grew from 80-98% of the pellets in the upper 15 cm of soil, and from 40-60% of the pellets in 15-25 cm depth, but not from pellets deeper than 25 cm. When fenugreek was cultivated continuously, the pathogen even survived to a depth of 30 cm. These findings also support the results presented in the pot-experiment. Although emergence of bean seedlings and plant weight were not significantly reduced at any evaluation date when the inoculum was placed deeper than 15 cm (T4) (Fig. 1, Tab. 1), infected plants were frequently observed in this treatment (Fig. 2). In addition, disease severity in T4 was low up to 30 DAI, but became intermediate later at 60 and 90 DAI (Fig. 2). It may have significant epidemiological implications, since diseased plants may be a source of inoculum for the next season.

In the pot experiment, disease severity and damage caused to plants were highest at 15 DAI. This confirms results of the Chapter 4 and is related to the time required by *R. solani* to establish itself in the soil. Disease severity was intermediate for T1 to T4 at 90 DAI (Fig. 2). The regression analyses for AUPEC and dry weight over time in both experiments (exemplified in the Figure 3 for AUPEC in the pot-experiment) could indicate a reduction of the survival ability of the pathogen. Moreover, in T3 disease severity was high (Fig. 2) and plant weight was significantly reduced at 0 DAI and 15 DAI (Tab. 1), but severity becomes intermediate (Fig. 2) and plant weight was not reduced at 30, 60 and 90 DAI (Tab. 1). Lower disease severities and damaging effects caused to plants at 60 and 90 DAI were clearer in another set of experiments carried out using a less aggressive *R. solani* inoculum, which was stored for 4 months at 4°C (data not shown).

Nevertheless, disease severity increased in T4 at 60 and 90 DAI in the pot-experiment (Fig. 2). In addition, inoculum confined even at 15-20 cm did not avoid high severity of disease (Fig. 5) and severe reduction of the plant growth (Tab. 2) at 90 DAI in the cylinder-experiment. Results of PAPAVIZAS et al. (1975) suggest that *R. solani* is highly dependent on plant tissue and almost disappears when the latter is exhausted. Moreover, the pathogen may not be able to colonize extensively plant residues when it is buried (PAPAVIZAS et al., 1975), because of its low tolerance to high concentrations of CO_2 (PAPAVIZAS et al., 1975; SUMNER, 1996). BAIRD et al. (1993) observed that the inoculum density of *R. solani* was greatly reduced after six months when peanut shells of pods containing inoculum were deep buried in the soil. However, our results indicate that, after 90 days in absence of host tissue, the survival of the pathogen was not consistently reduced when the inoculum was buried deeper than 15 cm (Figs. 2 and 5). At the evaluation time of the last survival test, sown at 90 DAI, the population density for T4 was similar to those observed for T1, T2 and T3 in both experiments (Figs. 2 and 5). The slow declines of *R. solani* activity may be due a gradual reduction of food bases in soil, because of decomposition and food exhaustion (PAPAVIZAS et al., 1975). This phenomenon can explain the variations observed in the curves of population density. In the beginning, the pathogen could grow rapidly from the suitable energy base provided by the rice grains, reaching the highest population density at the evaluation time of the second test, sown at 15 DAI (Figs. 2 and 5).

Several environmental factors may influence survival of propagules of *R. solani*. Edaphic factors include soil texture, compactation, and water potential. Survival of *R. solani* is also affected by tillage practices that influence vertical distribution in soil (SUMNER, 1996). Deep plowing such as obtained by mouldboard plows will result in a deep turning over

of infected organic debris and will thus encourage a more complete and faster decomposition than shallow plowing with disc harrows or subsoilers (SUMNER et al., 1986; ABAWI and PASTOR-CORRALES, 1990). Different soil preparation techniques and equipments can help in reducing the inoculum density of the pathogen as well as the incidence and severity of root rot in the field (HUSSEY and RONCADORI, 1977; LEWIS et al., 1983; SUMNER et al., 1986; LEACH et al., 1993). WIN and SUMNER (1988) suggested that a substantial reduction of bean yield could occur in conservation tillage, where infected plant residues normally are not deep buried.

Deep plowing of bean plant residues infected by *R. solani* to a depth of 20-25 cm has been recommended to reduce inoculum density and survival of the pathogen in the soil (PAPAVIZAS et al., 1975). This practice may also promote deeper and more extensive root formation that escapes root rot pathogens (ABAWI and PASTOR-CORRALES, 1990) and may be used as a component of an integrated management of root rot. Plowing in association with seed treatment increased bean yield more than when each component was used individually (LEWIS et al., 1983). A combination of plowing and biological control with *Trichoderma harzianum* reduced Rhizoctonia damage on cucumber (LEWIS and PAPAVIZAS, 1980). Our results showed that inoculum depth affected the vertical growth of *R. solani*. Although the pathogen survived in T4, it could hardly be recovered from soil samples collected above the layer originally infested in this treatment in the cylinder-experiment. This poor vertical growth and colonization demonstrates the benefit of plowing to reduce inoculum density. However, there is little information about how long the pathogen can survive in the soil when it is deep buried. In the Chapter 4, it was demonstrated that *R. solani* survived more than one year in the soil when the inoculum was homogenously distributed in pots. Deep turning (20-30 cm) of soil with a mouldboard plow reduced populations of *R. solani* but did not eliminate propagules of the pathogen (SUMNER et al., 1986). Considering the results presented here, plowing in short intervals of time or other soil movements could establish the contact between viable inoculum of the pathogen and bean seeds or seedlings in the next season. This may have important implications especially for tropical countries, where bean is frequently planted three times a year in the same field.

3. Effects of soil moisture and sowing depth on Rhizoctonia root rot of beans and its control by *Trichoderma harzianum*

Abstract

The effects of soil moisture and sowing depth on bean root rot caused by *Rhizoctonia solani* and its control by *Trichoderma harzianum* were studied under greenhouse conditions. The pathogen was grown on rice grains and the antagonist on wheat bran. Both fungi were inoculated before sowing. Disease severity, percentage of plants emerged, plant height and dry weight were evaluated three weeks after sowing. Emergence rate and growth of plants inoculated only with *R. solani* were not affected by soil moisture varying from 15 to 42% (v/v). Deep sowing significantly reduced the emergence rate and growth of plants inoculated only with *R. solani*. However, in the presence of the antagonist, the effect of sowing depth was not significant. At a sowing depth of 6.0 cm, the percentage of plants emerged was 50% in the presence of *T. harzianum*, but only 6.7% when the pathogen was inoculated alone. The antagonist protected bean seedlings from pre-emergence damping-off, reduced disease severity and increased plant growth in the presence of *R. solani*, especially in moist soil.

3.1 Introduction

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is a widely distributed disease of common bean (*Phaseolus vulgaris* L.) in the world (ABAWI, 1994). Bean production has substantially declined in the last years in Brazil due to soilborne pathogens such as *R. solani*, apparently because of the combination of soil moisture and cooler temperatures observed in areas under irrigation regimes (CARDOSO, 1994; VIEIRA and DE PAULA, 1998). Although HALL (1991) affirmed that soil moisture conditions have little effect on disease severity on beans, ABAWI and PASTOR-CORRALES (1990) asserted the disease may be more severe under moderate to high soil moisture conditions and moderate temperature. High disease severity on beans has frequently been correlated with high soil moisture (GALINDO et al., 1982; KOBRIGER and HAGEDORN, 1983). However, contradictory results have been found by other authors on beans (VAN BRUGGEN et al., 1986; FENILLE and SOUZA, 1999) and on crops other than beans (DAS and WESTERN, 1959; PITT, 1964; MAUGHAN and BARBETTI, 1983; HUISSMAN, 1988; TEO et al., 1988). Different results may partially be

related to problems with terminology that has been used to quantify the soil water status (PLOETZ and MITCHELL, 1985).

The disease may be reduced by various cultural practices such as sowing on raised beds during the wet rainy season, delayed sowing (until the soil has sufficiently been warmed up to reduce *R. solani* infection), crop rotation with non-host crops (wheat, oat, barley, and maize), deep plowing, use of more resistant cultivars, incorporation of organic residues as soil amendment and shallow sowing (ABAWI, 1994). Shallow sowing can be effective in reducing the damage caused by *R. solani*, since deep sowing extends the period of seedlings emergence and increase the severity of infection (MANNING et al., 1967; LEACH and GARBER, 1970).

Apart from cultural practices, biological control using *Trichoderma* species can reduce activities of *R. solani* (HARMAN et al., 1980; LEWIS and PAPAVIZAS, 1980, 1987, 1991; CHET and BARKER, 1981; BEAGLE-RISTAINO and PAPAVIZAS, 1985; LEWIS et al., 1995; KOK et al., 1996). Especially *T. harzianum* Rifai is one of the more intensively investigated biological control agent (HENIS et al., 1978; HADAR et al., 1979; ELAD et al., 1980a, 1981b, 1981c; CHET et al., 1982; MARSHALL, 1982; WU, 1982; DAL SOGLIO, 1998). *Trichoderma* species are particularly prevalent in humid environments and are relatively intolerant of low moisture levels; however they can be isolated from all climatic zones, including desert soils (KLEIN and EVELEIGH, 1998).

As *R. solani* is worldwide distributed, the exclusion and eradication of the pathogen are usually not effective field control measures. Generally, no single treatment provides a satisfactory control of *R. solani*, but it has been demonstrated that some practices used simultaneously may be effective to manage the disease (LEWIS and PAPAVIZAS, 1980; ABAWI, 1994). The integration of biological agents with additional strategies is increasingly recommended to enhance disease control (SWEETINGHAM, 1996). However, environmental and edaphic factors, including soil moisture, as well as cultural practices used to control root rot, like water management, may influence the antagonistic properties of *T. harzianum*. Similarly, little is known about the effects of some practices such as shallow sowing on the biological control of Rhizoctonia root rot using *T. harzianum*.

The objective of this study was to investigate the influence of soil moisture and sowing depth on the development of bean root rot caused by *R. solani* and its biological control by *T. harzianum*.

3.2 Material and methods

Two kinds of experiments were conducted under greenhouse conditions at Hanover, Germany: The moisture experiment and the sowing depth experiment. The fungi isolates used were taken from the collection of the Institute of Plant Diseases and Plant Protection, University of Hanover. They were maintained at 4°C. The isolate of *R. solani* (AG-4), originally isolated from bean, was grown on rice grains in 200 ml-Erlenmeyer flasks, while *T. harzianum* was grown on wheat bran. Two 5 mm diameter mycelial-agar disks were transferred from the margin of growing colonies to the flasks. After 6 days of incubation at 25°C in darkness, rice grains were totally colonized by *R. solani* and wheat bran by *T. harzianum*. The mass of rice grains was manually separated and the grains were dried on trays for 24 hours before soil infestation.

Soil-sand (2:1) was sterilized at 150-170°C for 24 hours. Immediately before sowing, the content of each pot (800 ml of soil-sand) was poured on a tray, carefully mixed with inoculum of both fungi at 3% (w/w), and put back in the pot. Ten seeds of the bean cultivar 'Dufrix' were sown per pot. Soil not infested with *R. solani* or *T. harzianum* received non-colonized rice grains and wheat bran, respectively.

Both experiments consisted of 16 treatments as a combination of presence and absence of *R. solani*/*T. harzianum*, and four soil moisture levels or four sowing depths. In the moisture experiment, soil moisture levels were determined with a soil moisture sensor (*ThetaProbe,* Delta-T Devices Ltd., Cambridge, U.K.) and kept at 42, 32, 23 and 15% (v/v). The pots were weighed to monitor water loss and irrigated once a day. In this experiment, bean seeds were sown 3 cm deep. In the sowing depth experiment, the seeds were sown at 1.5, 3.0, 4.5 and 6.0 cm and the soil moisture content was maintained approximately at 32%. The following combinations were tested: without both fungi (rt), without *R. solani*/with *T. harzianum* (rT), with *R. solani*/without *T. harzianum* (Rt) and with both fungi (RT). Three replicates of each treatment were placed in a randomised complete block design. Each replication consisted of a pot in which 10 seeds were sown. The pots were put at 23/18°C (day/night).

Daily observations were made on emergence. The plants were removed three weeks after sowing and hypocotyls were evaluated to determine the disease severity according to a 1-9 scale adapted from VAN SCHOONHOVEN and PASTOR-CORRALES (1987), with 1 - no visible symptoms, 3 - light discoloration without necrotic lesions, 5 - < 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm, 7 - 25-50% of the hypocotyl and root tissues covered with lesions combined with softening, rotting, and

reduction of the root system, 9 - > 75% of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system or dead plants. Plant height and dry weight were also determined.

The experiments were repeated once. Comparisons for emergence rate were done through the area under plant emergence curve (AUPEC). The non-parametric test of Kruskal-Wallis was used to compare root rot severities. Analysis of variance (ANOVA) was calculated followed by multiple range tests ($P = 0.05$) to separate the means for each fungi combination. In addition, the effects of the moisture level (ML) on some variables y, for instance AUPEC, plant height and dry weight, were investigated by means of regression analyses using the following model for the combined data of the four fungi combinations on the pot basis (altogether 48 pots):

$$y = (d_{rt}\, y_{rt} + d_{rT}\, y_{rT} + d_{Rt}\, y_{Rt} + d_{RT}\, y_{RT})\,(1 + b_1\, ML) \quad (1)$$

In this equation, the d_{ij} are dummy variables (0 or 1) to choose the fungi combination (rt, rT, Rt or RT) and the four parameters y_{ij} represent the estimated level of the dependent variable of the fungi combination for moisture level 0. The coefficient b_1 describes the increasing effect of the moisture level (measured in percentage of moisture content).

As in most cases the estimated values y_{rt} and y_{rT} were not significantly different, meaning that *T. harzianum* had no effect in the absence of *R. solani*, also the following model was fitted to the combined data to assess the effects of the pathogen and the antagonist on AUPEC, plant height and dry weight:

$$y = y_a\,[1 - d_R\, b_R\,(1 - d_T\, b_T)]\,(1 + b_2\, ML) \quad (2)$$

Here again d_R *and* d_T are dummy variables (0 or 1) describing if *R. solani* and *T. harzianum* has been added to the soil. The parameter y_a is an estimate of the dependent variable at moisture level 0 (ML = 0) when *R. solani* is not applied ($d_R = 0$). The coefficient b_R reflects the decrease due to the application of the pathogen, while b_T quantifies how *T. harzianum* reduces the negative effect of *R. solani*. The coefficient b_2 characterises again the increasing effect of the moisture level. From this equation, the antagonistic effect of *T. harzianum* in the presence of *R. solani* can be deduced as an increase in y by the factor $[b_R\, b_T / (1 - b_R)]$.

Similar models were used to investigate the effects of *R. solani* and *T. harzianum* in combination with different sowing depths (SD, measured in cm) on some dependent variables y like AUPEC, plant height and dry weight:

$$y = (d_{rt}\, y_{rt} + d_{rT}\, y_{rT} + d_{Rt}\, y_{Rt} + d_{RT}\, y_{RT})\,(1 - b_1\, SD) \quad (3)$$

$$y = y_a\,[1 - d_R\, b_R\,(1 - d_T\, b_T)]\,(1 - b_2\, SD) \quad (4)$$

The only difference between the models for soil moisture level (ML) and sowing depth (SD) is the negative sign of the coefficients b_1 and b_2, which reflects the fact that the independent variables, for instance plant dry weight, decrease with the sowing depth.

The analysis of variance and the regression analyses were carried out with the Statistical Analysis System (SAS Institute, Carey, NC) and Sigma Plot (SPSS Inc., Chicago).

3.3 Results

As the tendencies in each experiment and the repetition were similar, only the data from the second run of experiments are presented. Plant height and dry weight were highly positively correlated with each other ($r > 0.92$; $P < 0.01$), but negatively correlated with severity ($r > - 0.909$; $P < 0.01$). Root rot and hypocotyl symptoms were observed in all plants inoculated with *R. solani* (treatments Rt and RT).

3.3.1 Effect of soil moisture levels

The final emergence rate was similar for the treatments rt and rT (Fig. 1), although plants inoculated with *T. harzianum* emerged earlier, resulting in higher AUPEC values (Tab. 1). The pathogen reduced consistently the emergence so that plants in the treatment Rt emerged three days later and the final emergence rate was only between 23 and 43% (Fig. 1). Differences in emergence rate between treatments Rt and RT were dramatic, indicating that the antagonist protected the bean seedlings from pre-emergence damping-off in the presence of *R. solani* (Fig. 1). The percentage of emerged plants in the treatment RT varied from 77 to 93%. In spite of the biological control provided by *T. harzianum*, the severity indexes were always higher than 7 for plants inoculated with both fungi (Tab. 1). Disease severity ratings were on average 8.45 and 7.43 on plants in the treatments Rt and RT, respectively, but in both treatments the severity did not differ significantly among the moisture levels.

Characterising the emergence of seedlings by AUPEC, the mean values for the different fungi combinations are 108, 117, 27 and 87%/Days for treatments rt, rT, Rt and RT, respectively (Tab. 1). The effect of the biological control by *T. harzianum* results from the comparison of the treatments Rt and RT (Tab. 1 and Fig. 2). Due to the antagonist, AUPEC increased by 192, 215, 336 and 195%, plant height by 504, 827, 613 and 175%, and plant dry weight by 221, 539, 270 and 80%, respectively for soil moisture contents of 42, 32, 23 and 15%.

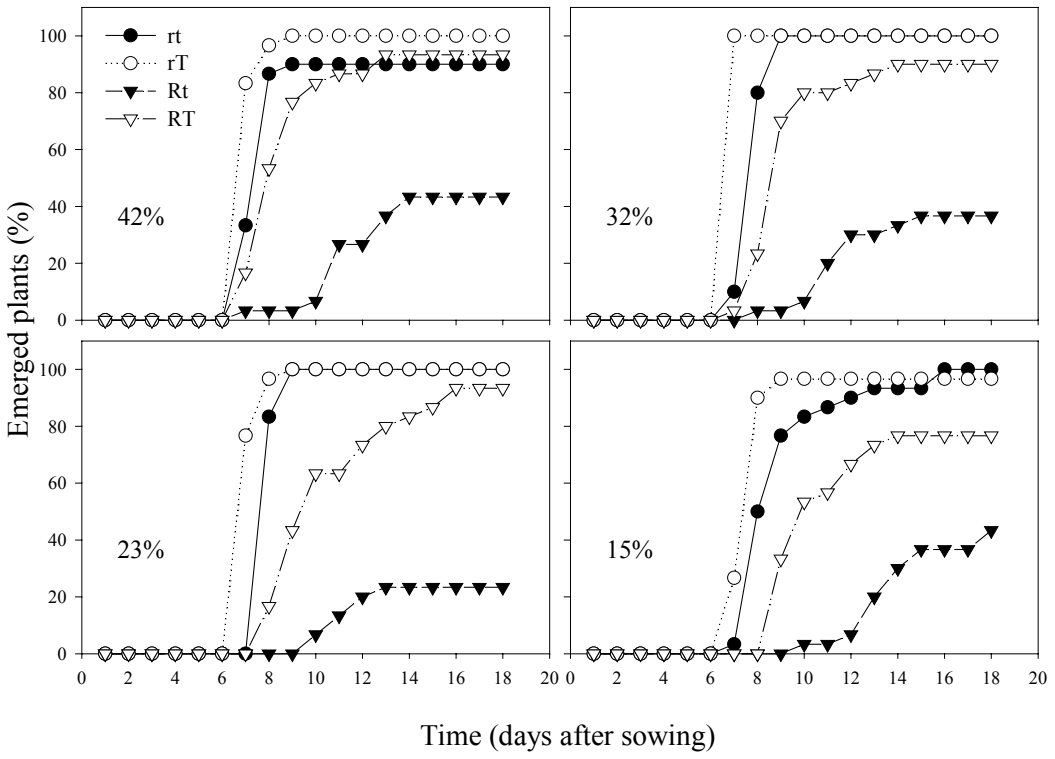

Figure 1. Effect of four soil moisture levels (%, v/v) on emergence of bean seedlings inoculated with *R. solani* and on the biological control with *T. harzianum*. (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

Table 1. AUPEC values (%/Days) and severity (1-9 scale) for each fungi combination and **soil moisture level**

Soil moisture	Treatments							
	rt		rT		Rt		RT	
	AUPEC	Severity	AUPEC	Severity	AUPEC	Severity	AUPEC	Severity
42%	104.8 a*	1.0	118.8 a	1.0	34.3 a	8.40	100.2 a	7.47
32%	113.5 a	1.0	120.0 a	1.0	29.2 a	8.67	92.0 ab	7.67
23%	113.3 a	1.0	118.5 a	1.0	19.2 a	8.63	83.7 ab	7.20
15%	101.8 a	1.0	111.8 a	1.0	23.8 a	8.10	70.5 b	7.40

* Values are means for three replicates. For each fungi combination, means followed by the same letter are not significantly different ($P = 0.05$). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

A general trend can be observed for the effect of soil moisture levels on emergence and plant growth: At decreasing moisture levels, AUPEC (Tab. 1) and plant growth (Fig. 2) were reduced, although not always significantly. Results of ANOVA indicated that the percentage of plants emerged (Fig. 1) and the plant growth expressed in plant height and dry weight (Fig. 2) were not significantly affected by soil moisture in the treatment Rt. Values observed for

disease severity were also similar in this treatment (Tab. 1). On the other hand, in the presence of *T. harzianum*, AUPEC (Tab. 1), plant height and dry weight (Fig. 2) were more reduced by *R. solani* at drier soils. At decreasing soil moisture levels, AUPEC (Tab. 1) and plant growth (Fig. 2) were stronger reduced in the treatment RT (compared to rT): reductions of 16, 23, 29 and 37% for AUPEC, 45, 53, 60 and 67% for plant height, and 37, 33, 59 and 54% for plant weight were observed respectively for soil moisture contents of 42, 32, 23 and 15%.

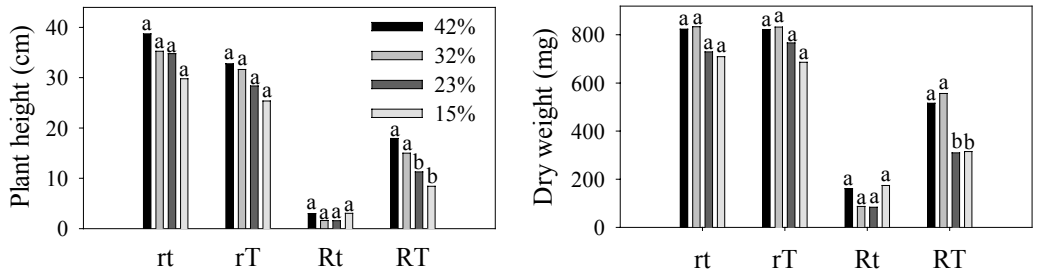

Figure 2. Effect of four soil moisture levels (%, v/v) on plant height and dry weight. Values of plant height and dry weight are means of three replicates. For each fungi combination, means followed by the same letter are not significantly different ($P = 0.05$). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

The application of model 1 resulted in the equations 5-7, which permitted to estimate AUPEC, plant height (PH) and dry weight (DW) for each treatment and the effect of the soil moisture levels. All estimated parameter values were significantly different from 0 ($P < 0.05$) and the coefficients of determination R^2 varied from 91.5 (eq. 5) to 94.6 (eq. 6). For each increasing percent of moisture content, AUPEC, plant height and dry weight increased by 0.48, 1.47 and 1.1%, respectively.

$$AUPEC = (d_{rt}\ 95.43 + d_{rT}\ 103.34 + d_{Rt}\ 23.61 + d_{RT}\ 76.62)\ (1 + 0.0048\ ML) \quad (5)$$

$$PH = (d_{rt}\ 24.5 + d_{rT}\ 20.9 + d_{Rt}\ 1.62 + d_{RT}\ 9.48)\ (1 + 0.0147\ ML) \quad (6)$$

$$DW = (d_{rt}\ 590.34 + d_{rT}\ 592.61 + d_{Rt}\ 95.63 + d_{RT}\ 327.86)\ (1 + 0.011\ ML) \quad (7)$$

Model 2 was also applied for the variables AUPEC, plant height and dry weight, resulting in equations 8 to 10. The estimated parameter values were significantly different from 0 ($P < 0.05$) and the coefficients of determination R^2 varied from 90.8 (eq. 8) to 92.9 (eq. 10). The equations show that *R. solani* reduced AUPEC, plant height and dry weight by 76, 93 and 84%, respectively. However, in the presence of *T. harzianum*, the reduction of AUPEC, plant height and dry weight by the pathogen was only 22.8, 58.6 and 44.5%,

respectively. Looking at the increase of the variables, the antagonistic effects are given by the factors 2.21, 4.51 and 2.47 for AUPEC, PH and DW, respectively.

$$AUPEC = 99.44\ [1 - d_R\ 0.76\ (1 - d_T\ 0.70)]\ (1 + 0.0048\ ML) \quad (8)$$

$$PH = 22.67\ [1 - d_R\ 0.93\ (1 - d_T\ 0.37)]\ (1 + 0.0148\ ML) \quad (9)$$

$$DW = 591.51\ [1 - d_R\ 0.84\ (1 - d_T\ 0.47)]\ (1 + 0.011\ ML) \quad (10)$$

3.3.2 Effect of sowing depth

The final emergence rate was similar for the treatments rt and rT (Fig. 3). Emergence took longer and pre-emergence damping-off was more severe the deeper the seeding was performed in the treatment Rt (Fig. 3). However, deep sowing did not reduce AUPEC (Tab. 2) and plant growth (Fig. 4) in the treatment RT. At 1.5 cm, plants in the treatment Rt emerged only one day later than plants in the treatment RT, and the final number of plants emerged was similar. At 6.0 cm, plants in the treatment Rt emerged three days later than plants in the treatment RT. The corresponding disease severity ratings for treatments RT and Rt were on average 8.15 and 8.7, respectively.

The mean values for AUPEC in four treatments rt, rT, Rt and RT were 119.7, 123.9, 26.4 and 66.2%/Days, respectively (Tab. 2). The variation of AUPEC values was highest at 6.0 cm: 112.7, 122.3, 6.0 and 52.7 %/Days, for treatments rt, rT, Rt and RT, respectively (Tab. 2).

Emergence and plant height were in general reduced at deep sowing, but not in all treatments. In the treatment Rt, plant height (Fig. 4) and dry weight of aerial parts of plants (data not shown) were higher and disease severity (Tab. 2) lower at 1.5 cm compared to other sowing depths. Pre-emergence damping-off was most severe at 6.0 cm (Fig. 3). At this sowing depth, 50% of the plants inoculated with *R. solani* emerged in the presence of *T. harzianum*, but only 6.7% in the absence of the antagonist. In both treatments inoculated with *R. solani* (Rt and RT), the disease severity showed an increasing tendency with sowing depth (Tab. 2) although the differences among the four sowing depths were not significant.

The model 3 applied to AUPEC, plant height (PH) and dry weight (DW) resulted in the equations 11-13, which permitted to estimate the general effects of the treatments and the effect of sowing depth. The estimated parameter values for AUPEC and plant height were significantly different from 0 ($P < 0.05$) and the coefficients of determination R^2 varied from 91.6 (eq. 11) to 94.5 (eq. 12). Sowing depth had a negative effect on AUPEC and plant height so that they were reduced by 2.2 and 5.07%, respectively, for each cm depth. On the other hand, the effect of sowing depth on plant dry weight was not significant.

$$AUPEC = (d_{rt}\ 130.40 + d_{rT}\ 134.91 + d_{Rt}\ 29.51 + d_{RT}\ 72.24)\ (1 - 0.022\ SD) \quad (11)$$

$$PH = (d_{rt}\ 55.49 + d_{rT}\ 51.66 + d_{Rt}\ 3.63 + d_{RT}\ 13.02)\ (1 - 0.0507\ SD) \quad (12)$$

$$DW = (d_{rt}\ 1118.58 + d_{rT}\ 1180.55 + d_{Rt}\ 103.16 + d_{RT}\ 386.14)\ (1 + 0.0047\ SD) \quad (13)$$

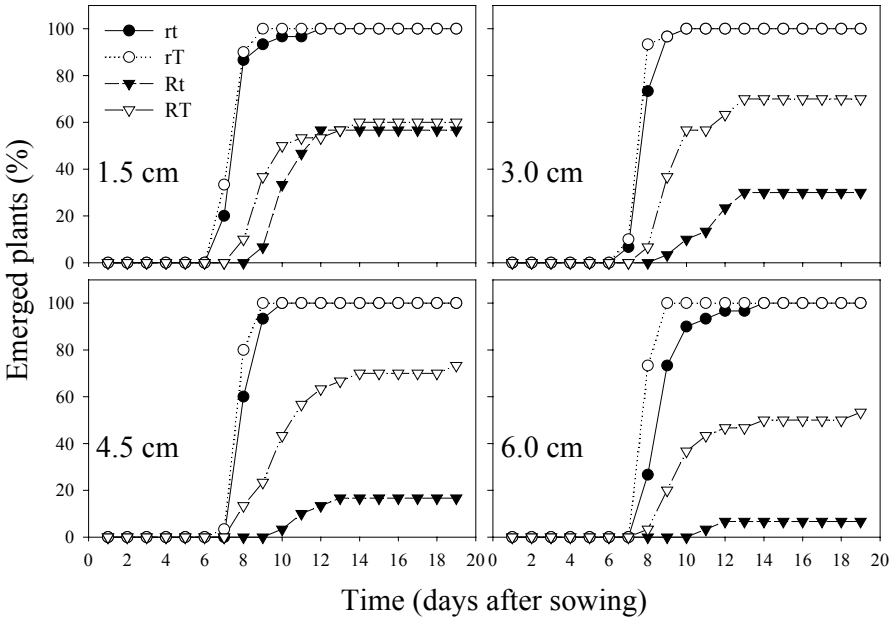

Figure 3. Effect of sowing depth (cm) on emergence of bean inoculated with *R. solani* and on the biological control with *T. harzianum*. (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

Table 2. AUPEC values (%/Days) and severity (1-9 scale) for each fungi combination and **sowing depth**

Sowing depth	Treatments							
	rt		rT		Rt		RT	
	AUPEC	Severity	AUPEC	Severity	AUPEC	Severity	AUPEC	Severity
1.5 cm	123.3 a*	1.0	125.7 a	1.0	56.8 a	8.33	65.0 a	7.97
3.0 cm	122.3 a	1.0	124.5 a	1.0	27.5 b	8.77	74.5 a	8.07
4.5 cm	120.3 a	1.0	123.2 a	1.0	15.2 bc	8.80	72.7 a	8.10
6.0 cm	112.7 a	1.0	122.3 a	1.0	6.0 c	8.93	52.7 a	8.47

* Values are means for three replicates. For each fungi combination, means followed by the same letter are not significantly different ($P = 0.05$). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

In the same way, the equations 14 to 16 resulted from the application of the model 4 to AUPEC, plant height (PH) and dry weight (DW). The estimated parameter values for AUPEC and plant height were significantly different from 0 ($P < 0.05$) and the coefficients of determination R^2 varied from 91.4 (eq. 14) to 94.2 (eq. 15). The equations indicated that the pathogen reduced AUPEC, plant height and dry weight by 78, 93 and 91%, respectively.

However, in the presence of the antagonist, the reduction of AUPEC, plant height and dry weight by *R. solani* was only 46.0, 75.3 and 66.4%, respectively. Regarding again the increase of the variables, the antagonistic effects are given by the factors 1.45, 2.52 and 2.73 for AUPEC, PH and DW, respectively.

$$AUPEC = 132.67\ [1 - d_R\ 0.78\ (1 - d_T\ 0.41)]\ (1 - 0.0221\ SD) \quad (14)$$

$$PH = 53.63\ [1 - d_R\ 0.93\ (1 - d_T\ 0.19)]\ (1 - 0.0509\ SD) \quad (15)$$

$$DW = 1150.44\ [1 - d_R\ 0.91\ (1 - d_T\ 0.27)]\ (1 + 0.0045\ SD) \quad (16)$$

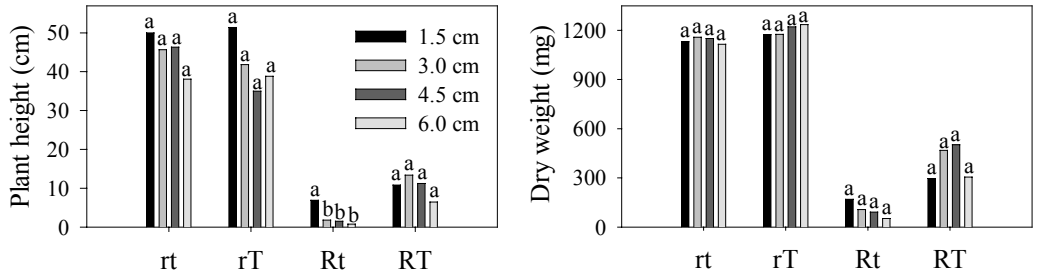

Figure 4. Effect of sowing depth (cm) on plant height and dry weight. Values of plant height and dry weight are means of three replicates. For each fungi combination, means followed by the same letter are not significantly different ($P = 0.05$). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

3.4 Discussion

The influence of soil moisture on incidence and severity of diseases caused by *R. solani* on different crops has been studied by several authors (DAS and WESTERN, 1959; PITT, 1964; LEWIS and PAPAVIZAS, 1977; SUMNER and BELL, 1982; MAUGHAN and BARBETTI, 1983; SHEHATA et al., 1984; TEO et al., 1988; HUISSMAN, 1988), but there is no general agreement whether high or low soil moisture favours the fungus.

Also for Rhizoctonia root rot on beans, contradictory results about the effect of soil moisture have been published. Several authors affirm that moderate to high soil moisture favours bean root rot (ABAWI, 1994; ABAWI and PASTOR-CORRALES, 1990). GALINDO et al. (1982) recorded that disease severity increased, as soil moisture and relative humidity were higher during incubation. According to KOBRIGER and HAGEDORN (1983), disease severity commonly decreases in the field, when early irrigation is reduced. ABAWI (1994) recommended to plant beans on raised beds during the wet rainy season to reduce the severity of the disease. On the other hand, CANADAY (1998) found that soil moisture levels had negligible effects on Rhizoctonia root rot in field experiments. FENILLE and SOUZA

(1999) observed the same results in experiments with low and high soil moisture levels (20 and more than 80% of field capacity, respectively). VAN BRUGGEN et al. (1986) reported that disease incidence did not depend on soil moisture; however, the largest lesions developed at lower moisture levels (-9.5 bar), but the time period available for lesion expansion was longer. They found also that *R. solani* delayed emergence and reduced the plant growth rate, particularly at low soil moisture levels. Our results, especially based on the equations 5 to 10, indicated that increasing soil moisture in general enhanced AUPEC and plant growth. However, the statistical analyses done for each fungi combination separately showed that different soil moisture levels did not influence the emergence rate and plant growth in the treatment Rt (Tab. 1, Fig. 2). In this treatment (and also in RT) disease severity did not differ among the soil moisture levels. These results support the findings of FENILLE and SOUZA (1999), who presented evidence that the incidence of Rhizoctonia root rot on beans was not dependent on soil moisture. It has even been demonstrated that incidence and severity of root diseases caused by *R. solani* may decrease at very high soil moisture levels (ROTH and RIKER, 1943; WRIGHT, 1957; BATEMAN, 1961b; KUMAR et al., 1999), apparently because the lack of aeration is a limiting factor for *R. solani* (BATEMAN, 1961b; PLOETZ and MITCHELL, 1985). A deleterious effect of wet soil on *R. solani* survival was observed in the Chapter 4.

One reason for the contradictory results in literature could be the use of ambiguous terms to quantify the soil water status (PLOETZ and MITCHELL, 1985). According to SHEHATA et al. (1984), this confusion related to effects of soil moisture on *R. solani* in different crops may also be due to failure to distinguish between the effects of moisture tension and aeration. Moreover, the results may differ depending on the *R. solani* anastomosis group (TEO et al., 1988). Chemical, physical and biological characteristics of the soil may affect drastically results of experiments involving soil moisture and activities of soilborne pathogens. Furthermore, different methods of experimentation have been used so that comparisons among results are very difficult.

Apart from the influence of soil moisture, the increasing effect of high relative humidity on bean root rot should not be neglected (GALINDO et al., 1982; Chapter 5). It may have important implications for disease management, because high relative humidity caused by excessive irrigation should be avoided in areas with high inoculum potential of *R. solani*. Moreover, soil moisture may influence indirectly soil temperature and consequently disease development (BENSON and BAKER, 1974a; VAN BRUGGEN et al., 1986; TEO et al., 1988), since bean plants emerge more rapidly at high temperatures and thus could escape

infection (ZAUMEYER and THOMAS, 1957). According to TEO et al. (1988), the disease on canola could be controlled to a certain extend by later seeding to avoid low soil temperatures, when *Rhizoctonia* isolates in an area are predominantly AG 2-1 and soil moisture is high. These authors demonstrated that high soil moisture might lower soil temperature and favour AG 2-1.

There is a general agreement in literature that bean root rot is enhanced when bean seeds are deeper sown. CROSSAN (1965), WESTER and GOTH (1965) and MANNING et al. (1967), for instance, showed that deep sowing increases root and hypocotyl rot on beans. Lower incidence and severity of disease were obtained by sowing bean seeds 2.5 cm deep compared to 7.5 cm (MANNING et al., 1967). In California, sowing depth of 1.5-2.5 cm reduced the disease incidence on beans to a level avoiding the application of fungicides (LEACH and GARBER, 1970). Severe outbreaks of lupin hypocotyl rot in Western Australia, caused by *R. solani* AG 11, were usually associated with sowing depths greater than 6 cm (SWEETINGHAM, 1996). The results presented here also support the promoting effect of deeper sowing on bean root rot. Equations 11 and 14 indicated that AUPEC was in general reduced by 2.2% for each cm depth, although the percentage of plants emerged was about 100% in the treatments rt and rT 14 days after sowing. ROSA (1990) found no effects of different depths on percentage of bean plants emerged, but observed that deep sowing diminished the velocity of emergence. Our results showed that sowing depth dramatically affected pre-emergence damping-off at 3.0, 4.5 and particularly at 6.0 cm (Fig. 3). The effect of sowing depth on plant dry weight was not significant because the root system of plants emerged from deeply sown seeds was longer. Plant height (Fig. 4) and dry weight of aerial parts of plants (data not shown) in the treatment Rt were highest at 1.5 cm, and disease severity lowest (Tab. 2). Severity of root rot increased with higher sowing depth (Tab. 2). Deep sowing extends the period of seedlings emergence which favours the seedlings contact with *R. solani* and increases the probability of damping-off and root rot. It is known that colonization of *R. solani* is limited or slow in older hypocotyls (MANNING et al., 1967). Thus, deep sowing increases the exposition of growing hypocotyl to the pathogen. According to ARAUJO (1998), deep sowing favours soilborne pathogens less severely in sandy soils.

The antagonistic effect of *T. harzianum* on *R. solani* root rot of beans is known (CHET et al., 1979; HADAR et al., 1979; ELAD et al., 1980a; MARSHALL, 1982). ELAD et al. (1980a) reported that *T. harzianum* significantly reduced disease severity, delayed the progress and incidence of *R. solani* damping-off, increased the yield of beans under field conditions and reduced disease incidence in greenhouse experiments. In our experiments, *T.*

harzianum increased emergence of seedlings in the presence of *R. solani* (Figs. 1 and 3), although severity indexes were high for plants inoculated with both fungi (Tabs. 1 and 2). In addition, plant growth was consistently higher for plants in the treatment RT than in the treatment Rt (Fig. 2 and 4). According to the regression analyses with the models 2 and 4, in the presence of the pathogen, *T. harzianum* was more effective in increasing emergence rate than plant growth.

A significant effect of soil moisture was observed in the treatment RT, indicating that AUPEC and plant growth were increased at high soil moisture (Tab. 1). The increases in height and weight of plants inoculated with *R. solani* due to *T. harzianum* were lower at 15% soil moisture compared to the higher moisture levels. In addition, the antagonistic effects were enhanced at increasing soil moisture levels, comparing treatments rT and RT. These results support the findings of LIU and BAKER (1980) who observed more persistent suppressive effects of *Trichoderma* spp. in moist soil than in drier soil. *Trichoderma* species seem to be more prevalent under high moisture conditions (KLEIN and EVELEIGH, 1998), although HUISSMAN (1988) reported that they were apparently not greatly affected by soil moisture. In the Chapter 4, we discuss the effects of soil moisture on the survival of *R. solani* and *T. harzianum* and suggested that the antagonistic ability and survival of *T. harzianum* was affected by soil moisture, but it apparently was dependent on temperature and inoculum potential of both fungi in the soil.

LIU and BAKER (1980) suggested that the induction of suppressiveness to *R. solani* might be enhanced by manipulating the frequency of irrigation in order to maintain the soil moist and favour *T. harzianum*. However, at very high soil moisture conditions the establishment of *T. harzianum* seems to be reduced (Chapter 4). The combined use of *T. harzianum* and moderate soil moisture to reduce *R. solani* infection on beans under field conditions is feasible, but recommendations of soil moisture levels may be done for each situation in particular. Integration of water management with other cultural practices is recommended. Water management combined with sub-soiling increased bean yield in soil infested by *R. solani* and other soilborne pathogens (SILBERNAGEL, 1981).

Deep sowing did not reduce AUPEC (Tab. 2) and plant growth (Fig. 4) in the treatment RT. At 1.5 cm, percentage of emerged plants and plant height were similar in the treatments Rt and RT. However, differences between both treatments were dramatic at 3, 4.5 and 6 cm (Figs. 3 and 4). Studying the effects of sowing depths on Rhizoctonia root rot on beans, MANNING et al. (1967) suggested that cultural practices that hasten tissue maturity, such as shallow sowing, might reduce the disease. We observed that *T. harzianum* increased the

emergence of seedlings and plant growth in the presence of *R. solani* even if seeds were placed at 6 cm deep. These results show that integration of *T. harzianum* with shallow sowing may improve the root rot control in the field.

The antagonist raised the emergence rate of plants not inoculated with *R. solani* in the moisture experiment. This effect was also observed in the Chapter 4 and could suggest a direct effect of *T. harzianum* on the seedlings, which improves plant emergence. Plant growth promotion by *Trichoderma* spp. has been reported (CHANG et al., 1986; HARMAN et al., 1989; LYNCH et al., 1991), which is undoubtedly influenced by variations in the rhizosphere environment (BAILEY and LUMSDEN, 1998). ELAD et al. (1980a) observed that a wheat bran preparation of *T. harzianum* significantly increased weight of bean plants when applied to non-infested soil, but non-inoculated wheat bran had no significant effect on plants. Promotion of plant development by *T. harzianum* may be more relevant under stress situations that delay emergence, like deep sowing. Plants not infected with *R. solani* emerged more rapidly in the presence of *T. harzianum*, when seeds were sown at 6 cm (Fig. 3).

The results presented here demonstrate the potential of *T. harzianum* for use as a component of an integrated disease management program to control *Rhizoctonia* root rot on beans. It has been shown that the effectiveness of introduced antagonists can be enhanced by cultural practices (SWEETINGHAM, 1996). The improved antagonism of *T. harzianum* under conditions adverse for soilborne pathogens emphasizes the potential of integrating various means of control (ELAD et al., 1980b). The reduction of Rhizoctonia fruit rot of cucumber was greater with a combination *Trichoderma*-moldboard plowing (LEWIS and PAPAVIZAS, 1980). In the same way, the integration of *T. harzianum* and solarization (ELAD et al., 1980b, 1981b; CHET et al., 1982), sublethal doses of pentachloronitrobenzene (PCNB) (CHET et al., 1979; HADAR et al., 1979) or methyl bromide (STRASHNOW et al., 1985) had best results.

The commercial use of antagonists to control *Rhizoctonia* root rot on beans in Brazil, where large areas are planted with this crop, could be feasible, for example, for fields with high inoculum potential. However, the erratic and inconsistent results in fields due to climatic and edaphic variables have limited the introduction and acceptance of commercial biocontrol agents (LEWIS and KULIK, 1996). Moreover, *Trichoderma* species do not appear to be very competitive in non-sterilized soil (ADAMS, 1990). For a successful application of these fungi, it is essential to use highly efficient isolates and an inoculum carrier that permits the antagonist to become established in the soil.

The information presented here reinforces the concept of biocontrol as an alternative strategy against Rhizoctonia root rot. In soils where *R. solani* has been detected, sowing deeper than 3 cm is not recommended. Management practices may also include sowing of high quality seeds and the adjustment of soil water content to permit a rapid emergence of seedlings and to favour *T. harzianum*.

4. Effect of soil moisture on the survival of *Rhizoctonia solani* and *Trichoderma harzianum*

Abstract

The effect of soil moisture on the survival of the soilborne pathogen *Rhizoctonia solani* and of its antagonist *Trichoderma harzianum*, as well as on the dynamics of both microorganisms in the soil were studied under greenhouse conditions. Inoculum of *R. solani* was grown on rice grains, that of *T. harzianum* on wheat bran. Both fungi were inoculated at the same time, and the sowing of bean seeds to test the survival was carried out immediately after soil infestation and 20, 60, 180 and 360 days after soil infestation (DAI), and 3, 6, 12 and 18 DAI in a complementary experiment. Soil moisture was periodically monitored and kept at levels varying from 15 to 57% (v/v). The pathogen survived in the soil and caused disease at all tested dates. However, in the first survival tests (0, 20 and 60 DAI), severity of root rot initially increased, but decreased later (180 and 360 DAI). On the other hand, dry weight of *R. solani*-infected plants was reduced in the initial tests, but increased later so that at 360 DAI values as in the treatment control were reached. Soil moisture did not affect the severity of root rot. The pathogen could easily be recovered even from dryer soil, but in the presence of *T. harzianum* this was hardly possible. The antagonist improved the emergence of seedlings and led to higher weights of plants grown in *R. solani*-infested soil. However, when the pathogen was well established in the soil, antagonistic protection was lower. Consistent antagonistic effects were observed until 180 DAI, but at 360 DAI they were hardly detectable. The antagonist improved plant growth until 60 DAI even on plants not infected by *R. solani*. The antagonistic ability and the survival of *T. harzianum* were greater in soils held at intermediate soil moisture levels than in wet or dry soils, but were also influenced by the inoculum potential of both fungi in the soil.

4.1 Introduction

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is a very important disease occurring throughout the bean-production areas of many countries of Latin America and Africa (ABAWI and PASTOR-CORRALES, 1990; HALL, 1991). The pathogen survives in the soil as sclerotia and melanized mycelium, free or embedded in soil organic debris (BOOSALIS

and SCHAREN, 1959), originating from parasitized crops and weeds host and/or from saprophytically colonized crop residues (HERR, 1976; SUMNER, 1996).

Environmental and edaphic factors may influence *R. solani* survival (SUMNER, 1996). Incidence and severity of root rot and the resultant damage vary considerably among plantings from one growing season to another, as a result from direct and indirect effects of prevailing environmental and soil conditions on the pathogen (ABAWI and PASTOR-CORRALES, 1990; SUMNER, 1996). Therefore, some cultural practices can be implemented in order to reduce inoculum levels in the soil, but no single treatment provides satisfactory results (SWEETINGHAM, 1996).

Manipulation of the frequency of irrigation to maintain a moderate soil moisture level has been suggested as a component of an integrated management program to control Rhizoctonia root rot (LIU and BAKER, 1980). High soil moisture levels and relative humidity are known to increase disease severity on beans (GALINDO et al., 1982; ABAWI and PASTOR-CORRALES, 1990), although contradictory results have been found depending on soil chemical, physical and biological properties, moisture levels as well as on experimental methodologies (VAN BRUGGEN et al., 1986; FENILLE and SOUZA, 1999). Nevertheless, there is a general agreement in the literature stating that the survival of *R. solani* in the soil is favoured by low soil moisture (PAPAVIZAS and DAVEY, 1962; BENSON and BAKER, 1974b; PLOETZ and MITCHELL, 1985).

Integration of water management with other cultural practices or with biological control measures has been recommended to reduce inoculum levels in the field (SILBERNAGEL, 1981; ABAWI and PASTOR-CORRALES, 1990; DE PAULA and ZAMBOLIM, 1998). The effectiveness of the biological control of Rhizoctonia root rot with *Trichoderma harzianum* seems to increase with soil moisture (Chapter 3). LIU and BAKER (1980) observed that survival of *Trichoderma* spp. and induction of suppressiveness to *R. solani* generally were enhanced by manipulating of frequency of irrigation to favour *T. harzianum*. *Trichoderma* spp. are particularly prevalent in humid environments and relatively intolerant to low moisture levels; however they can be isolated from all climatic zones, including desert soils (KLEIN and EVELEIGH, 1998).

The purpose of this study was to investigate the influence of soil moisture on the survival of both *R. solani* and *T. harzianum* and on the dynamic of both microorganisms in the soil. In addition, the development of Rhizoctonia root rot on beans and the biological control with *T. harzianum* were evaluated for a period of one year under different water regimes.

4.2 Material and methods

Two experiments were conducted under greenhouse conditions at Hanover, Germany, using similar methods. A long-term experiment on survival was carried out for a period of one year. A complementary short-term experiment was conducted only for 18 days focussing on the establishment of the fungi in the soil. The isolates of *R. solani* and *T. harzianum* were taken from the fungi collection of the Institute of Plant Diseases and Plant Protection, University of Hanover. They were maintained at 4°C. The isolate of *R. solani* (AG-4) was originally isolated from bean. *R. solani* was grown on rice grains and *T. harzianum* on wheat bran in 200 ml-Erlenmeyer flasks. Two 5 mm diameter mycelial-agar disks were transferred from the margin of growing colonies to the flasks. After 6 days of incubation at 25°C in darkness, rice grains were totally colonized by *R. solani* and wheat bran by *T. harzianum*. The mass of rice grains was manually separated and the grains were dried on trays for 24 hours.

Soil (Fruhstorfer Erde Typ 1, Industrie-Erdenwerk Archut, Lauterbach) was sieved and mixed with sand (2:1). The mixture was sterilized twice at 100°C for 24 hours. The content of each pot (800 ml of soil-sand) was poured on a tray and carefully mixed with inoculum of both fungi at 3% (w/w) in the long-term experiment and 1% (w/w) in the short-term experiment, and put back in the pot. Treatments not infested with *R. solani* or *T. harzianum* received non-colonized rice grains and wheat bran, respectively.

The long-term experiment began on November 2000. During the winter, the temperature in the greenhouse was maintained at 18/23°C (night/day). The short-term experiment was carried out on May 2001 and repeated on August 2001 under temperatures varying from 18 to 32°C.

During the experiments, the pots were weighted to monitor water loss and irrigated once a day. Soil moisture content was periodically monitored with a soil moisture sensor (*ThetaProbe,* Delta-T Devices Ltd., Cambridge, U.K.) and kept at four levels in the long-term experiment: 57, 42, 30 and 20% (v/v), and at three levels in the short-term experiment: 42, 27 and 15%.

The following combinations were tested for both experiments: without both fungi (rt), without *R. solani*/with *T. harzianum* (rT), with *R. solani*/without *T. harzianum* (Rt) and with both fungi (RT). To test the survival, ten seeds of bean cultivar ‘Dufrix’ were sown using a sowing depth of 3 cm. In the long-term experiment, sowing was performed immediately after the soil infestation and 20, 60, 180 and 360 days after the soil infestation (DAI), and in the short-term experiment, immediately after the soil infestation, 3, 6, 12 and 18 DAI. Five

replicates of each treatment were placed in a randomised complete block design. Each replication consisted of a pot in which 10 seeds were sown.

After sowing, daily observations were made on plant emergence. Disease severity, plant height and dry weight were evaluated about 22 days after sowing (DAS). Plants were removed and hypocotyls were evaluated to determine the disease severity according to a 1 to 9 scale adapted from VAN SCHOONHOVEN and PASTOR-CORRALES (1987), with 1 - no visible symptoms, 3 - light discoloration without necrotic lesions, 5 - < 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm, 7 - 25-50% of the hypocotyl and root tissues covered with lesions combined with softening, rotting, and reduction of the root system, 9 - > 75% of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system or dead plants. Determinations of population density in number of colony-forming units (cfu)/g of soil were performed according to KO and HORA (1971) for *R. solani* and according to ELAD et al. (1981a) for *T. harzianum*.

Comparisons of emergence were done through area under plant emergence curve (AUPEC). The non-parametric test of Kruskal-Wallis was used to compare root rot severities. Analysis of variance (ANOVA) was calculated and multiple range tests ($P = 0.05$) were used for mean separation with the Statistical Analysis System (SAS Institute, Cary, NC).

In the long-term experiment, the data of the survival test at different dates were combined and non-linear functions of time (t) were fitted to the data of the treatments Rt and RT for root rot severity (RRS), given as disease scores between 1 and 9, and relative dry weight (RDW), expressed as percentage in relation to the treatments rt and rT, respectively. As RRS increased initially, reached a maximum and decreased later, the following function was used to describe the time course:

$$RRS(t) = 1 + c_1 \, (t/c_2)^{(c_2 \, c_3)} \, e^{c_3 \, (c_2 - t)} \qquad (1)$$

In this function, $(c_1 + 1)$ reflects the maximum value possible (< 9). The parameter c_2 is the time when this maximum is reached and c_3 is related to the decline of the disease later in time. The relative dry weight declined in the time shortly after the soil infestation, reached a minimum value (≥ 0) and increased then with time to approach 100% again. The following function was therefore used for its description:

$$RDW(t) = 100 - (100 - c_1 \, (t/c_2)^{(c_2 \, c_3)} \, e^{c_3 \, (c_2 - t)} \qquad (2)$$

The parameter c_1 is the minimum value of this function, which was restricted to values above 0. The second parameter c_2 is the time when this minimum is reached, and c_3 is related

to the increase later in time. Both functions were fitted to the data of the individual soil moisture levels as well as to combined data, using Sigma-Plot (SPSS Inc., Chicago).

4.3 Results

4.3.1 Long-term experiment on survival

Plant dry weight was positively correlated with plant height at all evaluations ($r > 0.95$; $P < 0.05$) so that only data of plant dry weight are used in the further analyses. The pathogen survived in the soil and caused disease at all tested dates. Emergence of seedlings and plant growth was consistently reduced at soil moisture content of 57% in all treatments.

At the sowing done immediately after the soil infestation (0 DAI), *T. harzianum* improved emergence and plant dry weight of plants not inoculated with *R. solani* at all soil moisture levels (Fig. 1, Tab. 1). The emergence of seedlings and plant weight, except for the highest moisture level, were reduced by *R. solani*, comparing treatments rt and Rt (Fig. 1, Tab. 1). For a soil moisture content of 30 and 20%, the emergence and the weight of plants of treatment RT were higher than of Rt, reflecting the antagonistic effect of *T. harzianum* on *R. solani*; however, at 42% soil moisture, the antagonist had no effect on the emergence curve and on the dry weight, while for 57% the emergence was even reduced in the presence of the antagonist (Fig. 1, Tab. 1). Effects of soil moisture levels on AUPEC and plant weight for each fungi combination are presented in the Table 1, indicating that, in general, plants developed better at intermediate moisture levels.

The percentage of plants emerged as well as the dry weights were more drastically reduced in the survival tests at 20 and 60 DAI (data not shown) than in the other tests. At 20 and 60 DAI, almost 100% damping-off was observed when plants were inoculated with *R. solani* in the presence or absence of *T. harzianum*. The antagonist again improved emergence of seedlings and raised dry weight of plants not inoculated with *R. solani* at both sowing dates (data not shown), although not consistently as in the first test at 0 DAI.

In the following survival test at 180 DAI, no effect of *T. harzianum* on emergence and weight was observed on plants not inoculated with *R. solani* (Fig. 2, Tab. 2). The pathogen consistently delayed emergence of seedlings, reduced final stand and plant weight at all soil moisture levels (Fig. 2, Tab. 2). Emergence and weight of plants inoculated with *R. solani* were increased in the presence of *T. harzianum* at soil moisture content of 42 and 30%, comparing treatments RT and Rt (Fig. 2, Tab. 2). Effects of moisture levels on AUPEC and

plant weight for each fungi combination (Tab. 2) had in general the same trends as observed at 0 DAI.

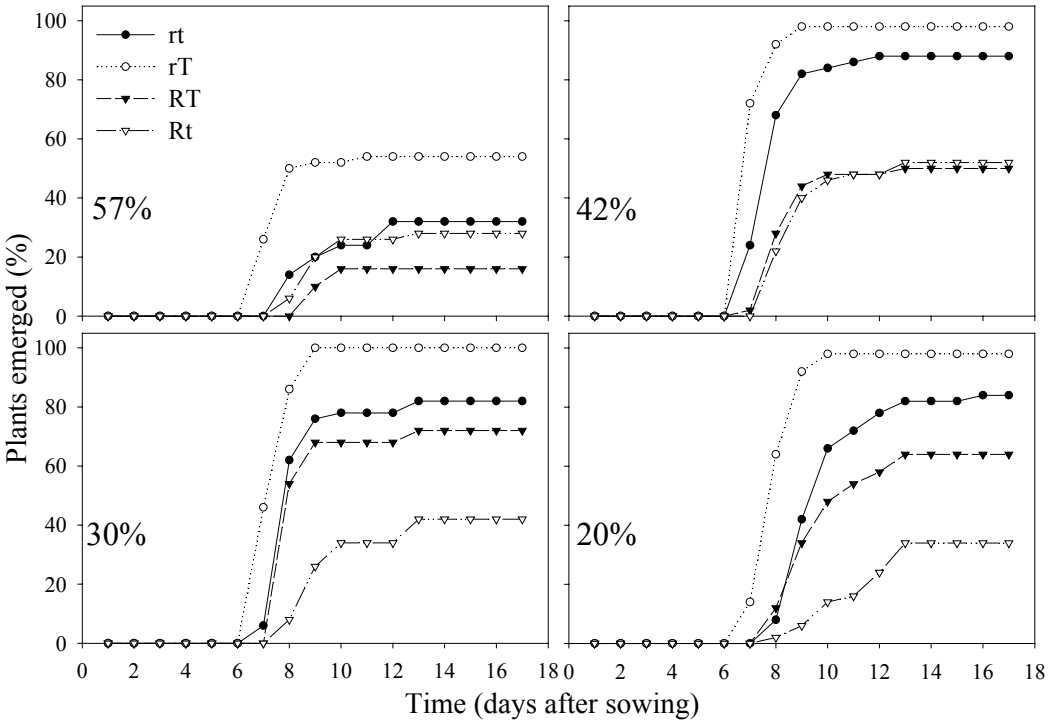

Figure 1. Emergence of bean seedlings at four soil moisture levels and at different combinations of presence and absence of *R. solani* and *T. harzianum* at **0 DAI** (long-term experiment)

Table 1. Effect of soil moisture levels on AUPEC and plant dry weight at different combinations of presence and absence of *R. solani* and *T. harzianum* at **0 DAI** (long-term experiment)

Soil moisture	AUPEC (%/Days)			
Levels	rt	rT	Rt	RT
57%	29.0 b	57.2 b	25.8 b	14.6 b
42%	90.4 a	105.9 a	49.0 a	49.2 a
30%	82.6 a	105.9 a	36.7 ab	72.2 a
20%	72.2 a	99.6 a	24.9 b	55.8 a
	Dry weight (mg)			
57%	539.2 b	1473.8 b	544.4 ab	324.6 b
42%	1746.8 a	2396.8 a	908.2 a	825.6 ab
30%	1565.2 a	2080.2 ab	807.0 ab	1222.2 a
20%	1232.2 a	2070.0 ab	391.8 b	954.4 a

* Values are means for five replicates. For each fungi combination, means followed by the same letter in a column are not significantly different (P = 0.05). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

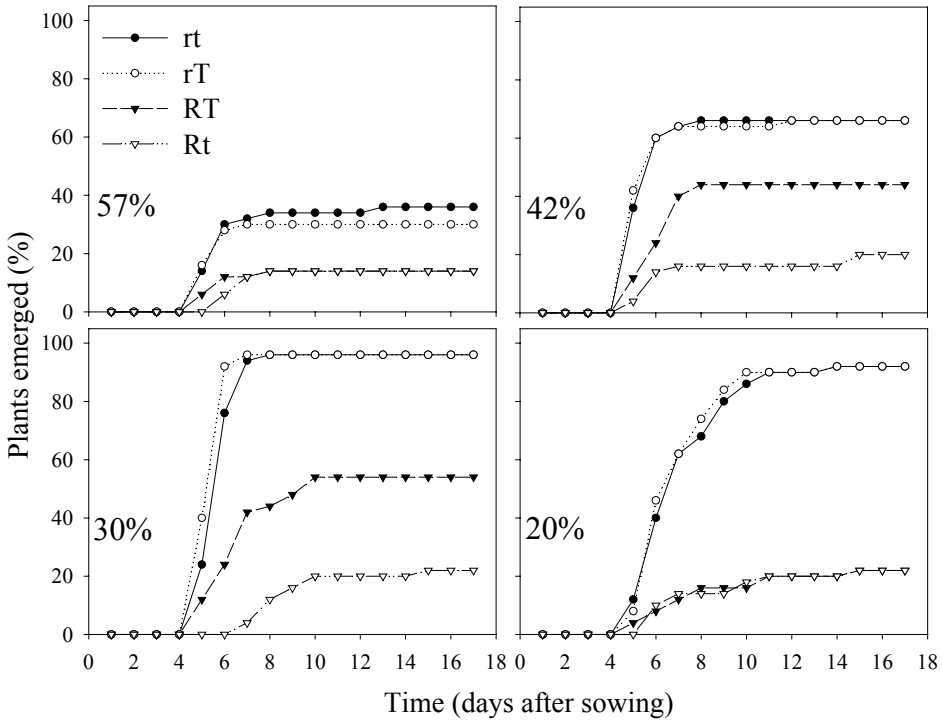

Figure 2. Emergence of bean seedlings at four soil moisture levels and at different combinations of presence and absence of *R. solani* and *T. harzianum* at **180 DAI** (long-term experiment)

Table 2. Effect of soil moisture levels on AUPEC and plant dry weight at different combinations of presence and absence of *R. solani* and *T. harzianum* at **180 DAI** (long-term experiment)

Soil moisture levels	AUPEC (%/Days)			
	rt	rT	Rt	RT
57%	36.5 c	32.1 c	13.7 a	14.6 b
42%	70.3 b	69.8 b	17.4 a	44.4 a
30%	99.8 a	102.4 a	16.5 a	51.5 a
20%	84.2 ab	86.0 ab	18.3 a	18.3 b
	Dry weight (mg)			
57%	1450.4 b	1248.8 c	313.0 a	288.6 b
42%	2257.0 ab	2175.2 b	398.4 a	937.8 a
30%	2900.2 a	2947.2 a	207.6 a	1111.2 a
20%	2159.0 ab	2364.6 ab	407.6 a	418.6 b

* Values are means for five replicates. For each fungi combination, means followed by the same letter in a column are not significantly different (P = 0.05). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

In the final survival test at 360 DAI, the emergence curves of all treatments in the moisture levels 30% and 20% were very similar (Fig. 3). At 42% soil moisture, *R. solani*

reduced the percentage of emerged plants and the plant weight, but *T. harzianum* could compensate part of this reduction (Fig. 3, Tab. 3). Dry weight (Tab. 3) was very similar in all treatments showing the same trend with respect to soil moisture: The values for the highest and the lowest moisture levels were nearly identical and always reduced compared to the two intermediate moisture levels.

A general trend was observed: In the highest moisture level, emergence and plant weight in all treatments were lower compared to the other levels; here *T. harzianum* could not compensate for the negative effect of *R. solani*, but even intensified the reducing effect (Figs. 1-3, Tabs. 1-3).

The data of the five survival tests were combined to show the dynamics over the whole period of the long-term experiment. The severity of root rot (Fig. 4) increased initially, was quite high at the second and third tests, but decreased thereafter. Generally the course of the curves was rather similar in the treatments RT and Rt as well as in the four moisture levels. Neglecting the moisture levels, function (1) was fitted to the combined data resulting in reasonable fits (R^2 = 55.4% in RT and 63.1% in Rt). Based on the estimated parameter values it can be concluded that under the influence of *T. harzianum* (treatment RT) the maximum was reached slightly earlier (at day 88 compared to day 91 in Rt), and the decrease was a little bit quicker (c_3 = 0.0023 compared to 0.0020). Looking at the fitted curves in the different moisture levels separately (Fig. 4), it is evident that in the highest and lowest level *T. harzianum* did not influence the severity curves. In the intermediate levels, the antagonistic effect is reflected by the lower maximum severity, which is also reached earlier in the treatment RT compared to Rt. In both moisture levels, root rot severities were significantly lower at 180 DAI and 360 DAI when *T. harzianum* was also inoculated.

The data for the relative dry weight (RDW) of the five observation dates were also combined to conclude about the dynamics. In the treatments that received *R. solani* inoculum, dry weight declined after the soil infestation to a minimum value (close to 0) and increased then (Fig. 5). Equation (2) was fitted to the combined data (not considering the four moisture levels) and resulted in coefficients of determination of 64.4% in treatment Rt and 76.8% in RT. In the treatment including *T. harzianum*, the minimum was reached at day 80, in the other treatment at day 95. Also the parameter value of c_3 was slightly higher in RT than in Rt, so that the dry weight increased quicker later in the experiment. Again, the differences in the curves at both intermediate moisture levels (Fig. 5) reflect the better conditions for the biological control by *T. harzianum.* In treatment RT, the minimum value is reached earlier

and the increase in dry weight is stronger than in treatment Rt. In the lowest moisture level, no effect of the antagonist is detectable, while in the highest level the effect is not clear.

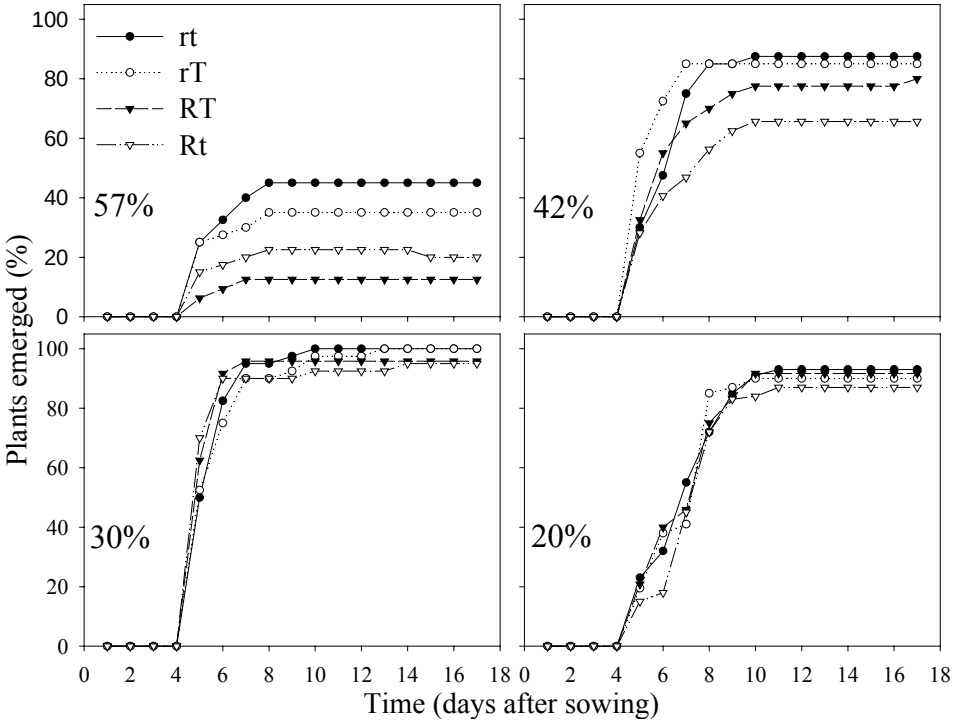

Figure 3. Emergence of bean seedlings at four soil moisture levels and at different combinations of presence and absence of *R. solani* and *T. harzianum* at **360 DAI** (long-term experiment)

Table 3. Effect of soil moisture levels on AUPEC and plant dry weight at different combinations of presence and absence of *R. solani* and *T. harzianum* at **360 DAI** (long-term experiment)

Soil moisture levels	AUPEC (%/Days)			
	rt	rT	Rt	RT
57%	37.4 c	29.4 c	18.6 c	10.5 b
42%	70.1 b	72.6 ab	51.7 b	62.8 a
30%	83.6 a	81.3 a	80.2 a	82.7 a
20%	68.4 b	67.5 b	63.0 b	68.2 a
	Dry weight (mg)			
57%	1061.9 b	1009.7 b	940.0 b	960.0 b
42%	1740.8 a	2028.0 a	1489.0 a	1758.5 a
30%	1761.6 a	1699.0 a	1563.3 a	1572.5 a
20%	1084.1 b	1117.7 b	1003.7 b	1006.9 b

* Values are means for five replicates. For each fungi combination, means followed by the same letter in a column are not significantly different (P = 0.05). (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

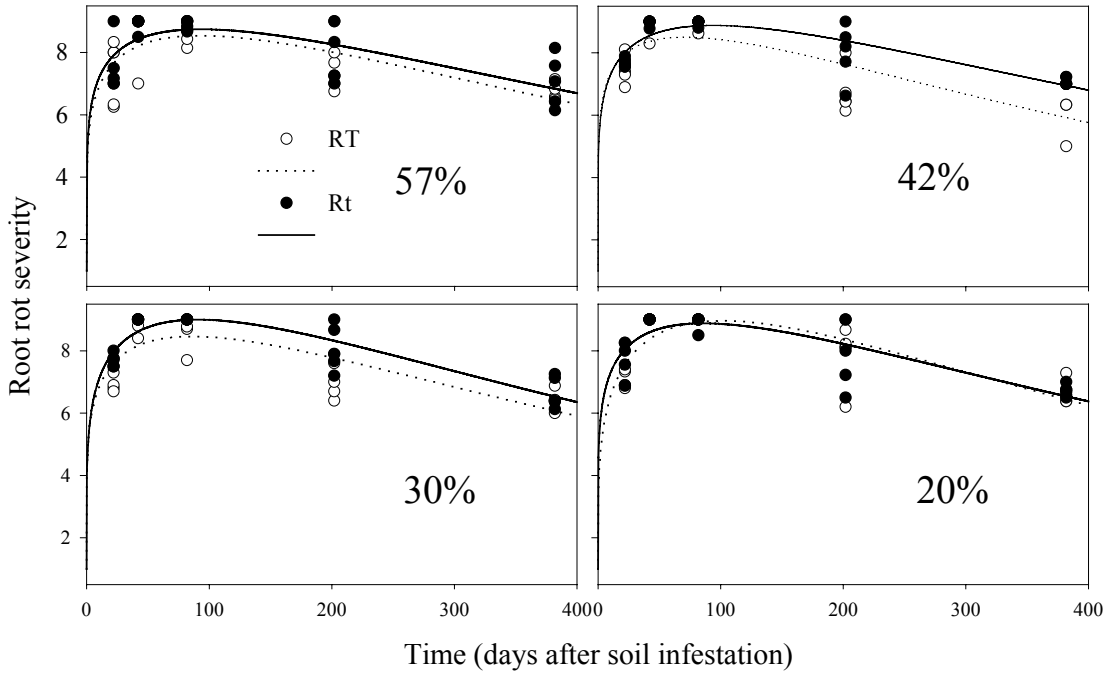

Figure 4. Root rot severity in five survival tests in the treatments RT and Rt at four soil moisture levels. The disease severities are assigned to the evaluation day, which was always done at 22 DAS

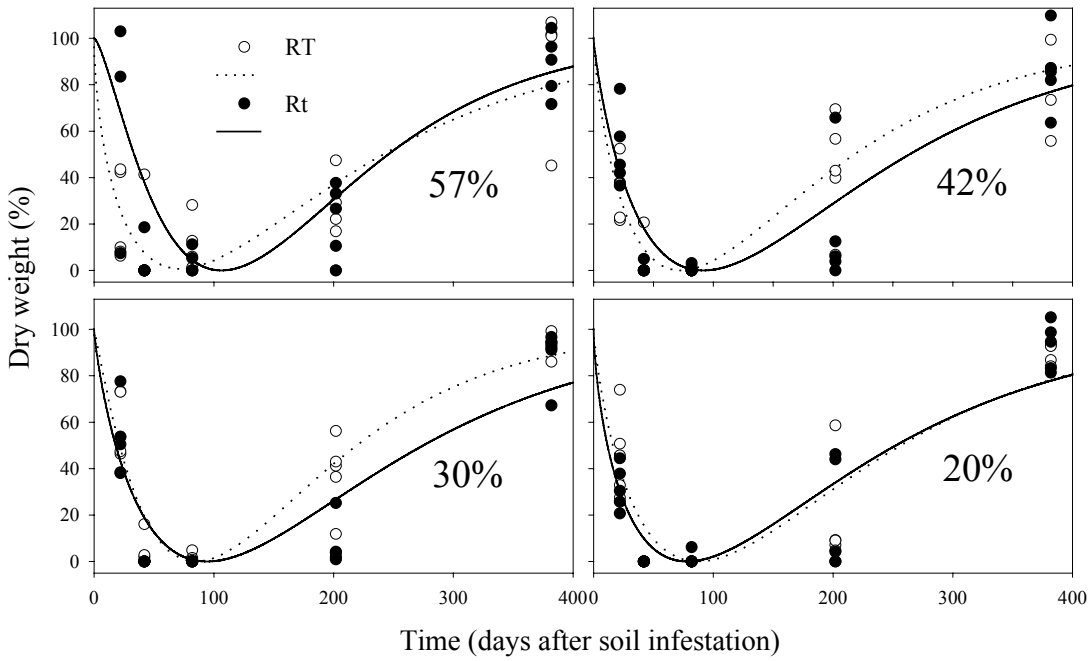

Figure 5. Relative plant dry weight (expressed as percentage in relation to the treatments rt and rT, respectively) in five survival tests in the treatments RT and Rt at four soil moisture levels. The values are assigned to the evaluation day, which was always done at 22 DAS

At the end of the last survival test, population density of both fungi was assessed (Tab. 4). Density of *R. solani* was significantly higher in the driest soil. In addition, it was consistently lower in treatment RT than in treatment Rt, except for the soil moisture content of 20% with identical values. The population density of *T. harzianum* was higher in the soil containing *R. solani* than without the pathogen. In the absence of *R. solani*, the density of the antagonist was significantly lower in the driest soil.

Table 4. Population density of *R. solani* and *T. harzianum*, expressed as cfu/g of soil, determined at the end of the long-term experiment

Treatments	Soil moisture content (%, v/v)	Population density of *R. solani* (cfu/g of soil)	Population density of *T. harzianum* (cfu/g of soil)
RT	57	-	3.60×10^5 a
	42	-	4.46×10^5 a
	30	-	3.82×10^5 a
	20	-	1.28×10^5 b
RT	57	1.50 c*	6.00×10^5 a
	42	2.37 b	10.40×10^5 a
	30	2.13 bc	9.57×10^5 a
	20	3.88 a	8.80×10^5 a
Rt	57	1.70 c	-
	42	3.26 b	-
	30	3.22 b	-
	20	3.88 a	-

*Values are means of 10 replicates for *R. solani* and 5 replicates for *T. harzianum*. For each fungi combination, means followed by the same letter are not significantly different ($P = 0.05$)

4.3.2 Short-term experiment on the establishment phase

As the tendencies in the experiments of May and August 2001 were similar, only the data from the first run of the experiment are presented. The antagonist improved emergence of seedlings and plant growth only in the presence of *R. solani* (data not shown). Disease severity was lower in the treatment RT compared to the treatment Rt at all sowing dates (Fig. 6). No significant effects of soil moisture levels were observed on disease severity in both treatments (Fig. 6). Density of *R. solani* was always lower in the treatment RT compared to treatment Rt (Fig. 7). In both treatments it increased initially and decreased later at soil moisture content of 42 and 27%, but remained relatively constant at 15% (Fig. 7). In the treatment Rt, the density of *R. solani* was significantly higher at soil moisture content of 15% at the end of the short-term experiment (Fig. 7).

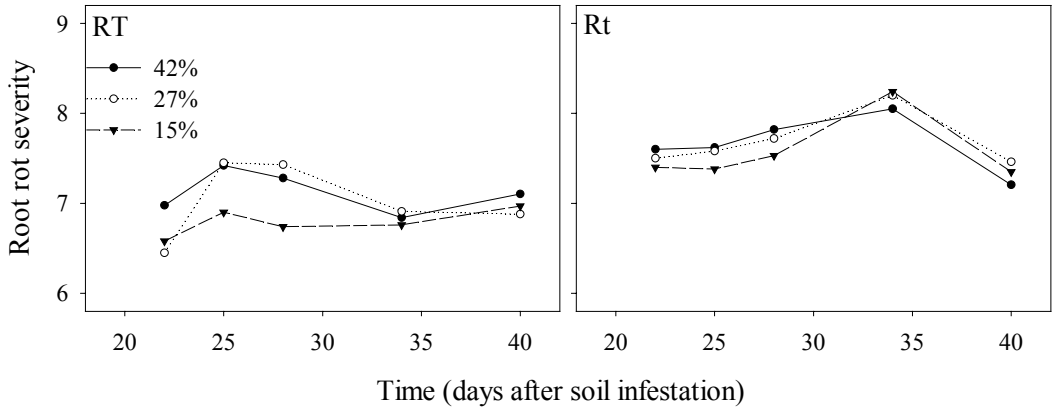

Figure 6. Root rot severity over time in treatments RT and Rt at three soil moisture levels. The disease severities are assigned to the evaluation days of the tests, taking place always at 22 DAS (short-term experiment)

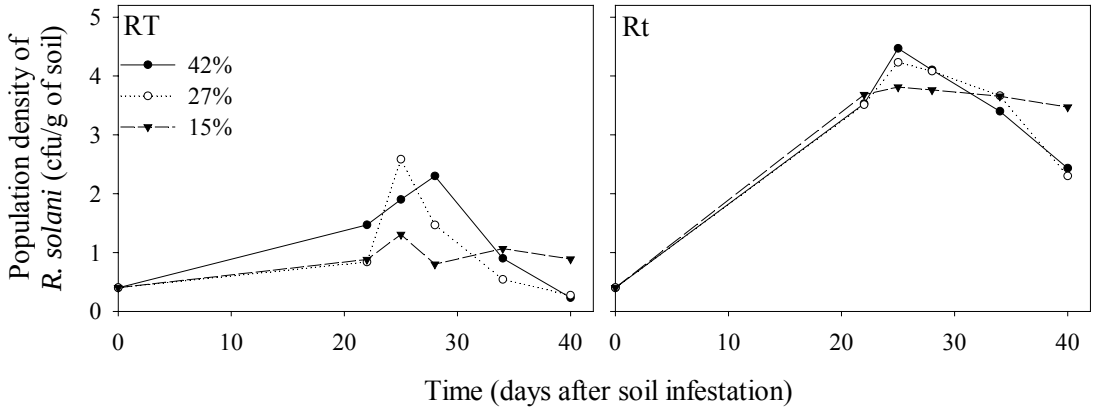

Figure 7. Population density of *R. solani*, expressed as cfu/g of soil, over time at three soil moisture levels in treatments RT and Rt. The first data were collected immediately after the soil infestation, the other are assigned to the evaluation days of the tests, taking place always at 22 DAS (short-term experiment)

Population density of *T. harzianum* was increased at 42% soil moisture (Fig. 8). In the presence of *R. solani*, the density of *T. harzianum* continuously increased and the antagonist could more easily be isolated from the soil in the treatment RT compared to rT; on the other hand, in absence of *R. solani*, density of *T. harzianum* increased initially and decreased later (Fig. 8).

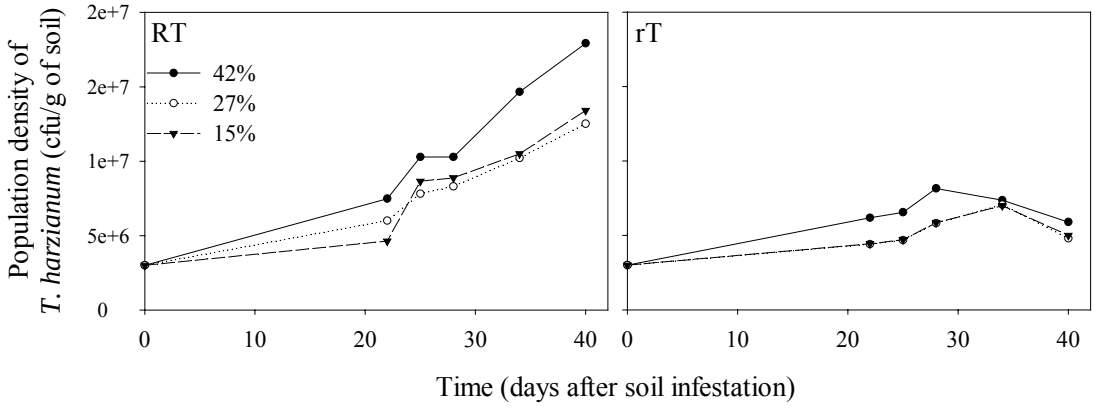

Figure 8. Population density of *T. harzianum*, expressed as cfu/g of soil, over time at three soil moisture levels in treatments RT and rT. The first data were collected immediately after the soil infestation, the other are assigned to the evaluation days of the tests, taking place always at 22 DAS (short-term experiment)

4.4 Discussion

Our results indicated that *R. solani* effectively survived in the soil in absence of host tissue at least one year after the soil infestation. However, after an initial increase, the severity of root rot decreased over time (Fig. 4). On the other hand, the relative dry weight of *R. solani*-infected plants was initially reduced, but increased later so that at 360 DAI values close to 100% were reached (Fig. 5), although the population density of the pathogen at the end of the long-term experiment was relatively high (Tab. 4). It is known that *R. solani* can survive in the soil for a long period (ABAWI and PASTOR-CORRALES, 1990). In the experiments of the Chapter 2, the survival of the pathogen was only slightly reduced after 90 days in absence of host tissue even when the inoculum was buried deeper than 15 cm. BELL and SUMNER (1987) observed that several *R. solani* isolates of AG-4 survived more than 9.4 months in a fallow soil.

We observed that *R. solani* became better established in the soil some time after the soil infestation. Disease severity and damaging effects of *R. solani* on plant development were highest in the tests at 20 and 60 DAI in the long-term experiment (Figs. 4 and 5). Based on the analysis of all survival tests, we predicted that the maximum values for the disease severity would be reached around day 90 in the long-term experiment, while in the short-term experiment the highest disease severity was already noticed at day 34 in treatment Rt. This discrepancy is probably caused by the differences in the temperature regimes (cooler in the long-term experiment) and in the inoculum levels (higher in the long-term experiment).

Although the disease severity was consistently high on plants infected with *R. solani* also in the presence of *T. harzianum* (Figs. 4 and 6), the antagonist frequently promoted emergence of seedlings and growth of plants in the treatment RT. In the short-term experiment, the antagonist changed the timing of *R. solani* establishment so that the highest severity values were earlier observed in treatment RT than in treatment Rt (Fig. 6). Antagonistic effects of *T. harzianum* on bean plants infected with *R. solani* have frequently been described (CHET et al., 1979; ELAD et al., 1980a; HADAR et al., 1979; MARSHALL, 1982). ELAD et al. (1980a) reported that the antagonist significantly reduced disease severity, delayed the progress and incidence of *R. solani* damping-off, and increased the yield of beans.

In both experiments, the population density of the pathogen recovered from soil was lower in the presence of the antagonist (Tab. 4, Fig. 7), while the final population density of *T. harzianum* was higher in the presence of *R. solani* (Tab. 4, Fig. 8). A comparison of the densities of both fungi over time in the short-term experiment (Figs. 7 and 8) shows that after the establishment phase an increase in density of *T. harzianum* propagules was accompanied by a decrease in density of *R. solani* (Figs. 7 and 8), which confirms results obtained by LIU and BAKER (1980).

In the survival tests at 20 and 60 DAI, no antagonistic effects of *T. harzianum* were detected. ELAD et al. (1980a) observed that the efficiency of the biological control by *T. harzianum* on *R. solani* was positively correlated with the inoculum level of the antagonist prepared on wheat bran and negatively with the level of infestation by the pathogen. The high *R. solani* inoculum level used in the long-term experiment apparently increased the establishment of the pathogen in the soil and then the antagonistic protection failed, although the inoculum level of the antagonist was also high. After 6 months (180 DAI), antagonistic effects could again be observed, apparently because the ability of *R. solani* to survive declined. These results are supported by HADAR et al. (1979), who affirmed that nutrient-rich food base, like wheat bran, allows *T. harzianum* to continue its growth in the soil and to attack soilborne pathogens. LO et al. (1996) found that an isolate of *T. harzianum* persisted at elevated levels in creeping bentgrass for over 8 months. Although *T. harzianum* was isolated from soil and disease severity was lower in the presence of the antagonist at the end of the long-term experiment, the promotion of emergence and of the growth of *R. solani*-infected plants was very slight in the last test at 360 DAI. Exhaustion of the suitable energy base provided by wheat bran may account for this observation. PAPAVIZAS (1982) found that without a food base, *T. harzianum* did not establish in the rhizosphere of beans and, in

addition, the survival of the antagonist was considerably reduced after 12 days, although some isolates could be recovered from soil even after 130 days.

In both experiments, different soil moisture levels did not affect substantially the disease severity in the treatment Rt (Figs. 4 and 6). However, the pathogen could more easily be recovered from drier soil at the end of the experiments (Tab. 4, Fig. 7). Soil moisture affecting *R. solani* survival has been studied at different conditions. According to PLOETZ and MITCHELL (1985), the pathogen is an active saprophyte within a wide range of water potentials. However, they observed that the survival of *R. solani* AG-4 was greater in soils held at intermediate water potentials of -2 to -15 bars than in moister or drier soils, and suggested that the lethal loss of water probably occurred in soils held at -100 and 1500 bars (air-dried soil). In the present study, population density of the pathogen was more reduced in wet soil at the end of the long-term experiment (Tab. 4). A reduced availability of oxygen or increased levels of CO_2, volatiles, alcohols, or other compounds found in very moist or flooded soil are normally indicated as causes of the reduction of the *R. solani* survival in wet soils (PLOETZ and MITCHELL, 1985). Several authors have found that *R. solani* survival is lower in moist than in drier soils (BAKER and MARTINSON, 1970; BENSON and BAKER, 1974b; PLOETZ and MITCHELL, 1895), which supports the results presented here. BENSON and BAKER (1974b) investigated the survival of *R. solani* (AG-4) for 24 days at water potentials of -0.7, -18 and -540 bars (air-died soil). At -0.7 and -18 bars, propagule densities increased 2 to 8 days after infestation and then quickly decreased. However, propagule densities remained relatively constant at -540 bars for the entire experiment. In the short-term experiment, we also observed that *R. solani* population density remained relatively constant at the soil moisture content of 15% (Fig. 7). PAPAVIZAS and DAVEY (1962) reported that the saprophytic activity of *R. solani* was significantly higher at lower soil moisture levels (20 to 50% water holding capacity) than at high soil moisture (70 to 90% WHC). Mycelium of *R. solani* grew best and produced most disease on lettuce, when the soil moisture level was lower, about 40% of saturation (DAS and WESTERN, 1959). On the other hand, LIU and BAKER (1980) found that inoculum density of *R. solani* remained relatively constant during a 140-day period at water potentials varying from -1.35 to -7000 bars.

Although *R. solani* apparently survives better in drier soils, the growth rate of the pathogen may decrease at very low moisture levels. BELL and SUMNER (1987) reported that *R. solani* isolates of AG 2-1 and AG 2-2 may become quiescent during prolonged moisture deficits. BENSON and BAKER (1974a) observed that at a given temperature, the growth rate

of *R. solani* in the soil was not significantly different at matric potentials between -0.02 and -5.4 bars, but decreased from -5.4 to -50 bars.

Different results have frequently been found when effects of soil moisture on *R. solani* activities in the soil or on root rot development are investigated. The terminology used to quantify soil water status (PLOETZ and MITCHELL, 1985) or to distinguish between effects of moisture tension and aeration (SHEHATA et al., 1984) is often confusing. Different experimental purposes and methodologies do not allow direct comparisons. Many other factors may influence the response of *R. solani* to different soil moisture levels, for instance the size of the propagule (PLOETZ and MITCHELL, 1985) or the anastomosis group (TEO et al., 1988). In addition, a significant interaction between temperature and soil moisture has been shown (BENSON and BAKER, 1974a; VAN BRUGGEN et al., 1986). Also chemical, physical and biological characteristics of the soil may drastically influence the results of experiments. In the Chapter 3, a soil moisture content varying from 15 to 42% did not affect the severity of Rhizoctonia root rot on beans when plants were inoculated only with *R. solani*. However, in the presence of the antagonist *T. harzianum*, the damage caused on plants was higher at drier soils. The results presented here for the short-term experiment also indicated that the presence of *T. harzianum* changed the shape of the curves of root rot severity over time at different soil moisture levels, comparing treatments RT and Rt (Fig. 6).

The antagonistic ability and survival of *T. harzianum* were affected by soil moisture. At 57% soil moisture, emergence and plant dry weight were frequently lower in the treatment RT than in Rt (Figs. 1-3, Tabs. 1-3), indicating that the excess of water can disturb the establishment of the antagonist in the soil. KNUDSEN and BIN (1990) affirmed that reduced availability of oxygen in wet soils might adversely affect hyphal proliferation of *T. harzianum*. Moreover, the availability of nutrients may also be affected (HJELJORD and TRONSMO, 1998). Our results presented evidence that *T. harzianum* better survived in soils held at intermediate moisture levels than in wet or drier soils. Root rot was consistently less severe at the soil moisture levels 42 and 30% in the treatment RT (Fig. 4). In the same way, the curves of the relative dry weight over time indicated that the antagonistic effects were more pronounced at the intermediate moisture levels (Fig. 5). We also observed that *T. harzianum* population density was highest at 42% soil moisture at the end of both experiments (Tab. 4, Fig. 8). According to KLEIN and EVELEIGH (1998), *Trichoderma* species are particularly prevalent in humid environments and relatively intolerant of low moisture levels. Several authors have studied survival and activities of *Trichoderma* spp. in the presence of *R. solani*. LIU and BAKER (1980) found that survival of *Trichoderma* spp. generally was

enhanced and suppressive effects were more persistent in moist soil than in drier soils. NELSON and HOITINK (1983) reported that survival of *R. solani* AG-4 was reduced at high water potentials (soil incubated at -0.022 bar) as a result of the activity of *T. harzianum*. PLOETZ and MITCHELL (1985) suggested that the lower ability of *R. solani* to survive in moist soils was due the enhancement of *T. harzianum* activities. According to HJELJORD and TRONSMO (1998), reduction of activities of *Trichoderma* spp. under dry conditions is apparently related to increased energy costs of osmoregulation.

Although the survival of *T. harzianum* seems to be increased at moist conditions, the activities and growth of the antagonist were not negligible at the low soil moisture content of 20 or 15%. KNUDSEN and BIN (1990) observed that soil moisture between -0.3 and -5 bars did not significantly affect radial growth rates of *T. harzianum* in the soil and hyphal density was significantly higher at -5 bars.

As mentioned earlier for *R. solani*, several factors may also influence *T. harzianum* activities at different soil moisture levels and explain different results in the literature. Different temperatures and inoculum potentials of both fungi used in both experiments may account for some dissimilarity in the observed effects of soil moisture on the disease development and on the biological control in these experiments.

The antagonist improved emergence of seedlings and weight of plants non-inoculated with *R. solani* (Fig.1, Tab. 1), but this effect did not persist more than two months after the soil infestation. Plant growth promotion by *Trichoderma* species has been frequently described in the literature (LINDSEY and BAKER, 1967; CHANG et al., 1986; WINDHAM et al., 1986; INBAR et al., 1994; KLEIN and EVELEIGH, 1998) and can even result in increasing yield (HARMAN et al., 1989; LYNCH et al., 1991). ELAD et al. (1980a) reported that wheat bran preparation of *T. harzianum* increased bean plant development in the absence of pathogens, while not inoculated wheat bran had no significant effect on the plants. Plant growth promotion by *T. harzianum* has been explained as a result of the control of minor pathogens (HARMAN et al., 1989; BAILEY and LUMSDEN, 1998) or of induced resistance (ZIMAND et al., 1996). However, direct effects of *Trichoderma* on seeds and seedling vigour have also been demonstrated (BJÖRKMAN et al., 1998). In the short-term experiment, improvement of emergence and plant weight by *T. harzianum* in treatment rT (data not shown) was not as expressive as in the long-term experiment. As the short-term experiment was carried out at higher temperatures than the long-term experiment, seedlings in treatment rt emerged so fast as seedlings in treatment rT in that experiment. According to BAILEY and LUMSDEN (1998), plant growth promotion by *Trichoderma* spp. may be influenced by

variations in the rhizosphere environment as well as by the many interactions that take place in the soil between *Trichoderma* spp., other microorganisms, changes in the soil environment, and the plant root.

The mechanisms of *T. harzianum* biocontrol on *R. solani* have not always been elucidated, but competition for nutrients, mycoparasitism, and production of inhibitory compounds and hydrolytic enzymes have frequently been described (ELAD, 1996; HJELJORD and TRONSMO, 1998). In culture, *T. harzianum* is capable of directly attacking and lysing *R. solani* (ELAD et al., 1980a). Chitinolytic enzymes and glucanases are produced by *T. harzianum* in vitro (CHET and BAKER, 1981; LORITO et al., 1994) and in the rhizosphere in the presence of both fungi (DAL SOGLIO et al., 1998).

The potential use of *Trichoderma* species as a biological control agent has been reviewed (PAPAVIZAS, 1985; CHET, 1990; ADAMS, 1990; HJELJORD and TRONSMO, 1998). Although there is considerable information on the influence of antagonists of bean soilborne pathogens, the availability and success of commercial preparations of biocontrol agents are very limited. Available preparations are normally expensive and exhibit variable results when used in naturally infested fields (ABAWI and PASTOR-CORRALES, 1990). It is known that under sterile conditions, the propagation of *T. harzianum* in soil is much greater and the biological control more efficient than under non-sterile conditions (ADAMS, 1990; KOK et al., 1996), although control in naturally infested soil has been demonstrated (HADAR et al., 1979). Many biocontrol agents including *Trichoderma* spp. are currently available in commercial products for the control of Rhizoctonia-caused diseases (LEWIS and KULIK, 1996). The reliability of biological control systems can be improved by the use of formulations that provide conducive environments for the antagonists and systems that economically produce propagules of high quality (HARMAN, 1991).

Strategies to control bean rot pathogens include fallow fields (ABAWI and PASTOR-CORRALES, 1990). In fact, BELL and SUMNER (1987) indicated that fallowing fields for one summer would reduce *R. solani* inoculum density but would not eliminate the pathogen in undisturbed soil. Cropping systems that favour the maintenance of *T. harzianum* may help reduce the survival of *R. solani* and the crop losses due to root rot. Considering that *R. solani* persists for the longest periods in soils with low matric potential, these observations suggest that induction of suppressiveness to *R. solani* may be enhanced by manipulation of the frequency of irrigation in order to maintain the soil moist (LIU and BAKER, 1980). Conversely, keeping the soil continuously wet by irrigation may help to hasten propagule decline (HADAR et al., 1982). According to ABAWI and PASTOR-CORRALES (1990),

flooding the soil for 4-6 weeks is highly effective in controlling all root rot pathogens of beans.

The results presented here are of importance in the elaboration of practical biological control strategies of Rhizoctonia root rot on beans, involving water management. However, *R. solani*, *T. harzianum* and bean plants may react differently to alternating soil moisture levels compared to constant levels. Therefore, further research is needed to extrapolate our findings to the field under the wide range of moisture conditions.

5. Interactions between Rhizoctonia root rot and aerial diseases of common beans

Abstract

The effects of the co-inoculation of the soilborne pathogen *Rhizoctonia solani* and the aerial pathogens *Colletotrichum lindemuthianum* or *Uromyces appendiculatus* at different inoculum levels were studied on the disease dynamics and on the growth of bean plants under greenhouse conditions. Bean seeds were sown in soil infested with *R. solani* inoculum produced on rice grains. Additional experiments in which bean seedlings were transplanted to infested soil were also carried out. Conidial suspensions of *C. lindemuthianum* and uredospores of *U. appendiculatus* were inoculated onto leaves at plant developmental stages V2 and V3, respectively. Interactions between root rot and the aerial diseases were observed depending on the inoculum levels and on the timing of *R. solani* inoculation. Anthracnose severity tended to be higher on plants infected by *R. solani*. On the other hand, *R. solani* infection significantly reduced diameter of pustules and rust severity. At *R. solani* low levels, root rot severity and population density of the soilborne pathogen in the soil were magnified at high levels of *C. lindemuthianum* or *U. appendiculatus* when seedlings were transplanted to *R. solani*-infested soil. In these experiments, a synergistic interaction between root rot and anthracnose was observed to affect the plant dry weight. Antagonistic effects on the plant dry weight could be detected for the combination root rot/rust only when bean seeds were sown in infested soil.

5.1 Introduction

Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is a widely distributed disease of common bean (*Phaseolus vulgaris* L.) occurring in the bean-production areas around the world (ABAWI, 1994). The pathogen can singly attack and cause damage to beans, but also in combination with other soilborne pathogens. These pathogens may interact with each other, resulting in root-disease complexes (ABAWI and PASTOR-CORRALES, 1990). Interactions involving *R. solani* and other bean soilborne pathogens, such as *Fusarium oxysporum* f. sp. *solani* (HALL, 1991) and *Pythium ultimum* (ABAWI et al., 1985), or nematodes like *Meloidogyne incognita* (REDDY et al., 1979) are frequently observed. Occasionally these interactions are synergistic (ABAWI and PASTOR-CORRALES, 1990).

Interactions among bean aerial diseases have also been studied (BASSANEZZI et al., 1998; CARNEIRO et al., 2000; JESUS JUNIOR et al., 2001; LOPES, 2001), but their importance has been demonstrated in only a few cases. Some of these interactions involving anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. and/or rust, caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*, could alter disease development (YARWOOD, 1977; DALLA PRIA et al., 1994; BASSANEZZI et al., 1998) and cause reduction on yield (JESUS JUNIOR et al., 2001).

Although interacting diseases usually affect the same plant organ (WALLER and BRIDGE, 1984), aerial and soilborne pathogens can simultaneously attack different parts of the bean plant (BIRD, 1969; YARWOOD, 1977; BOOKBINDER and BLOOM, 1980; GBAJA and CHANT, 1985). Interactions between diseases caused by aerial and soilborne pathogens may have significant implications for assessing crop losses and selecting appropriate management strategies. In addition, infection rates, maximum disease levels and the shapes of the progress curves may be changed by interacting diseases (HAU, 2001). Damage due *R. solani* on bean is commonly magnified if biological stresses such as other pathogens are present (ABAWI and PASTOR-CORRALES, 1990). On other crops than beans, for instance on cucumber and pepper, it has been reported that Rhizoctonia root rot increases when plants are infected by virus (BATEMAN, 1961a; PIECZARKA and ZITTER, 1981). On the other hand, Alternaria leaf blight on sunflower was more severe if plants were infected by *R. solani* (BHOWMIK and SINGH, 1977).

Although interactions involving Rhizoctonia root rot and diseases caused by aerial pathogens may frequently occur on bean plants, their importance for disease assessment and management is little investigated. The purpose of this study was to determine possible effects of interactions between Rhizoctonia root rot and the aerial diseases anthracnose or rust on the dynamics of the diseases occurring simultaneously and on the growth of bean plants.

5.2 Material and methods

The experiments with both disease combinations (root rot/anthracnose and root rot/rust) were conducted under greenhouse conditions in the Institute of Plant Diseases and Plant Protection (IPP), University of Hanover, Germany. Two kinds of experiments were carried out differing in the time of the attack of the soilborne pathogen: For an early attack, bean seeds were sown in *R. solani*-infested soil, while for a late attack, bean seedlings were transplanted to infested soil.

An isolate of *R. solani* (AG-4) maintained at 4°C was taken from the fungi collection of the IPP and grown on rice grains. The rice grains (without hull) were washed and soaked overnight, washed again and autoclaved in 200 ml-flasks. Mycelia were produced on PDA. After 3 days of incubation at 25°C, three 5 mm diameter mycelial-agar disks were transferred from the margin of growing colonies to flasks with rice grains. After 6 days of incubation at 25°C in darkness, the grains were totally colonized. The mass of grains was manually separated and the grains were dried on trays for 24 hours.

In the experiments in which bean seeds were sown in infested soil, rice grains colonized by *R. solani* were carefully mixed with soil previously sterilized (150-170°C for 24 hours) on a tray. After putting back the soil in 800 ml pots, 5 seeds of cultivar 'Dufrix' were sown per pot. Similarly, 14 and 25 days old-seedlings, respectively for the combinations root rot/anthracnose and root rot/rust, were transplanted to pots containing soil, which was 5 days previously infested with *R. solani* inoculum. Concentrations of *R. solani* of 0.2 (RS1), 0.6 (RS2) and 1.8% (w/w) (RS3) were used, corresponding to population densities of 0.5, 1.2 and 3.8 cfu/g of soil, respectively. Control treatments without *R. solani* inoculum (RS0) were used in all experiments.

Conidia of *C. lindemuthianum* were grown on sterile bean pods. Mycelia were initially produced on PDA. After 6 days of incubation at 25°C, 5 mm diameter mycelial-agar disks were transferred from the margin of growing colonies to flasks with bean pods. After 12 days of incubation at 25°C, inoculum was obtained by addition of distilled water and scraping the surface of the pods with a spatula. The conidial concentration was adjusted to 1.2×10^4 (CL1), 1.2×10^5 (CL2) and 1.2×10^6 conidia/ml (CL3). Primary leaves of plants at developmental stage V2 (about 20 days old) were inoculated with suspensions of conidia. Plants not inoculated with *C. lindemuthianum* received only water (CL0). Plants were incubated for 72 hours in a mist chamber (20-22°C, high relative humidity) and disease evaluation was done seven days after inoculation (DAI).

In the experiments with bean rust, bean plants at developmental stage V3 (about 30 days old) were inoculated with uredospores suspensions of *U. appendiculatus* at concentrations of 4×10^2 (UA1), 4×10^3 (UA2) and 4×10^4 uredospores/ml (UA3). Plants not inoculated with *U. appendiculatus* received only water (UA0). Plants were incubated for 24 hours in a mist chamber (20-22°C, high relative humidity) and disease evaluation was done 14 DAI.

Each experiment consisted of 16 treatments and 4-6 replicates whereby a pot with 5 plants represented each replicate. The pots of each treatment were placed in a randomised complete block design in the greenhouse and irrigated once a day to maintain the soil

moisture approximately at 100% field capacity. Percentage of emerged plants was evaluated daily. Plant height, plant dry weight and severity of diseases were evaluated at 27 days after the sowing (DAS) for the combination root rot/anthracnose and at 40 DAS for the combination root rot/rust. Evaluations of root rot severity were done on a 1-9 disease scale adapted from VAN SCHOONHOVEN and PASTOR-CORRALES (1987), where 1 - no visible symptoms, 3 - light discoloration without necrotic lesions, 5 - < 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm, 7 - 25-50% of the hypocotyl and root tissues covered with lesions combined with softening, rotting, and reduction of the root system, and 9 - 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system or dead plants. Another 1-9 disease scale of VAN SCHOONHOVEN and PASTOR-CORRALES (1987) was used for evaluations of anthracnose, where 1 = no visible symptoms; 3 = presence of very few and small lesions on the primary vein of the lower leaf side, that cover approximately 1% of the leaf surface area; 5 = presence of several small lesions on the primary and secondary veins; 7 = presence of numerous enlarged lesions on the lower side of the leaf, associated with necrotic lesions observed on the upper leaf surface; 9 = severe necrosis on 25% or more of the plant tissues which results in death of the infected tissues. Rust severity was assessed using a diagrammatic scale (GODOY et al., 1997) to estimate the average severity in percentage. Rust pustule diameter was additionally measured in the experiment in which seedlings were transplanted to infested soil. Population density of *R. solani*, expressed as number of colony-forming units (cfu)/g of soil, was determined according to KO and HORA (1971).

The disease and host parameters were determined as mean values per plant in each pot. In case a plant died due to pre-emergence damping-off, a disease score of 9 for root rot was assigned to this plant, while for the leaf diseases the mean value of all other plants in the same pot was taken.

The effect of the inoculation with *R. solani* and *C. lindemuthianum* or *U. appendiculatus* on the dry weight (DW) of plants (mean values per pot) was estimated using the following multiple regression equations including a mixed term for the simultaneous occurrence of both diseases:

$$DW = DW_H - b_{RS} (y_{RS} - 1) - b_{CL} (y_{CL} - 1) - b_{RS/CL} (y_{RS} - 1) (y_{CL} - 1) \quad (1)$$

$$DW = DW_H - b_{RS} (y_{RS} - 1) - b_{UA}\, y_{UA} - b_{RS/UA} (y_{RS} - 1)\, y_{UA} \quad (2)$$

In these equations, DW_H is an estimate of the dry weight of a healthy plant. The regression coefficients b_{RS}, b_{CL} and b_{UA} represent the reduction in dry weight due to root rot (severity score y_{RS}), anthracnose (severity score y_{CL}), and rust (disease severity in percentage y_{UA}), respectively. The parameters $b_{RS/CL}$ and $b_{RS/UA}$ describe the combined effect of root rot/anthracnose and root rot/rust, respectively.

The experiments were once repeated. Comparisons for anthracnose and root rot severities were done with the non-parametric test of Kruskal-Wallis. Multiple regression analyses and ANOVA were performed and multiple range tests ($P = 0.05$) used for mean separation with Sigma Plot and SPSS (SPSS Inc., Chicago).

5.3 Results

The tendencies in each experiment and its repetition are similar, so that only the data from the second run of experiments are presented. It should be noted that the experiments were carried out at different times of the year so that plant growth parameters cannot be compared. Generally, root rot increased by use of a mist chamber after the inoculation of the aerial pathogens. Plant height was positively correlated with plant dry weight in all experiments ($r > 0.95$; $P < 0.01$) so that only data of the plant dry weight are used in the further analyses.

5.3.1 Interaction root rot/anthracnose

5.3.1.1 Early attack of *R. solani* (bean seeds sown in infested soil)

Higher levels of *C. lindemuthianum* resulted in higher anthracnose severities for all *R. solani* levels (Fig. 1). Anthracnose severity was always lowest on plants growing in the soil not infested with *R. solani* (treatment RS0), but significantly only for the two highest *C. lindemuthianum* levels (Fig. 1). Root rot severity was high for all inoculum levels of *R. solani*, but no effect of inoculum level of *C. lindemuthianum* on root rot severity was observed (Fig. 2).

The mean plant dry weight per pot showed a high variation, but was clearly negative affected by root rot (Fig. 3). Accordingly, the stepwise fitting of equation (1) to this data revealed a significant negative effect of root rot, while the effects of anthracnose and of the interaction of both diseases were insignificant.

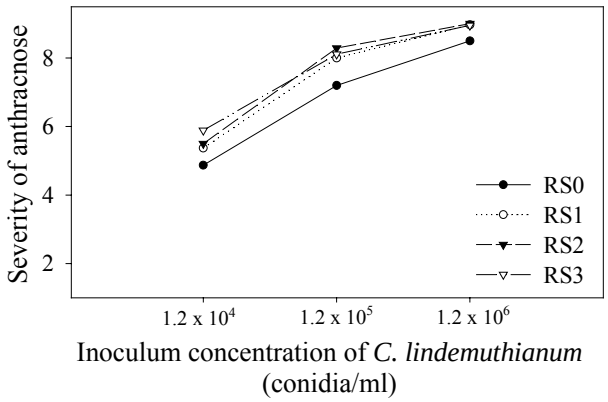

Figure 1. Effects of different inoculum levels of *C. lindemuthianum* and *R. solani* (RS0 = non-infested control, RS1 = 0.2% (w/w), RS2 = 0.6%, and RS3 = 1.8%) on anthracnose severity at 27 DAS when bean seeds were sown in *R. solani*-infested soil

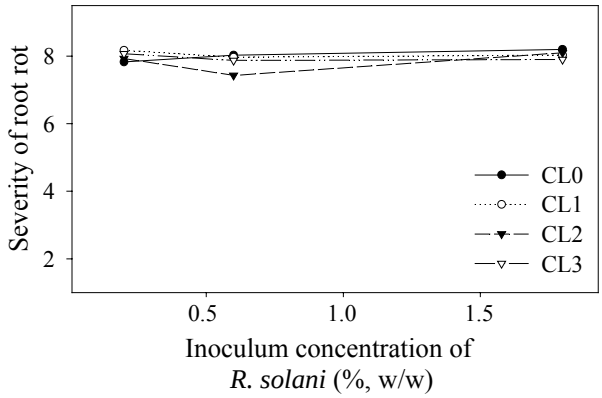

Figure 2. Effects of different inoculum levels of *R. solani* and *C. lindemuthianum* (CL0 = only water, CL1 = 1.2 x 10^4, CL2 = 1.2 x 10^5, and CL3 = 1.2 x 10^6 conidia/ml) on root rot severity at 27 DAS when bean seeds were sown in *R. solani*-infested soil

5.3.1.2 Late attack of *R. solani* (bean seedlings transplanted in infested soil)

For anthracnose, the increasing relationship between inoculum concentration of *C. lindemuthianum* and the resulting disease score was similar like in the experiment with the early attack (Fig. 4). Again, the disease level of anthracnose tended to be lower in the treatments without soil infestation with *R. solani*. At a *R. solani* inoculum level of 0.2%, root rot severity was significantly higher at CL3 compared to CL0 (Fig. 5); similarly, *R. solani* population density was significantly increased at the highest *C. lindemuthianum* inoculum level (Fig. 5).

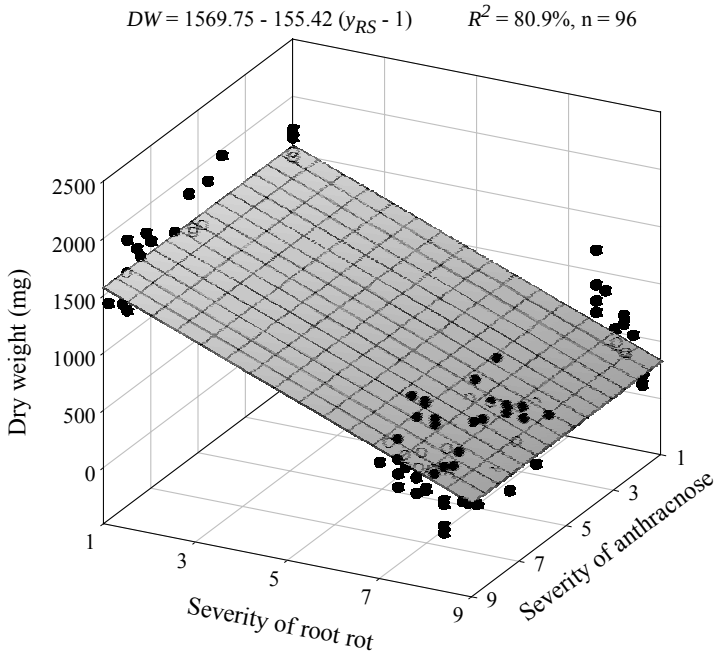

Figure 3. Effects of co-infection of *R. solani* and *C. lindemuthianum* on dry weight of bean plants at 27 DAS when bean seeds were sown in *R. solani*-infested soil

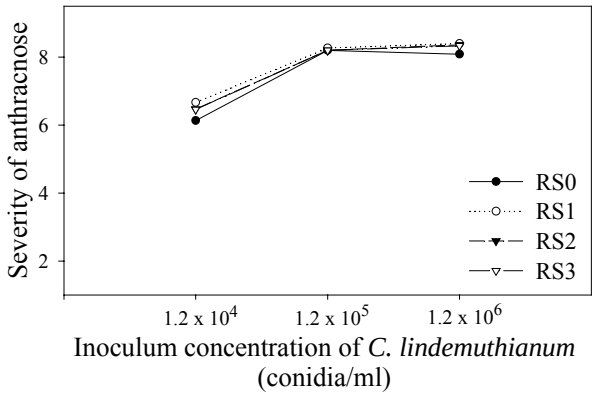

Figure 4. Effects of different inoculum levels of *C. lindemuthianum* and *R. solani* (RS0 = non-infested control, RS1 = 0.2% (w/w), RS2 = 0.6%, and RS3 = 1.8%) on anthracnose severity at 27 DAS when 14 days old-seedlings were transplanted to *R. solani*-infested soil

In this experiment, both diseases reduced plant dry weight (Fig. 6). In the stepwise regression procedure using equation (1), a significant effect of root rot and of the disease interaction was determined, while the effect of anthracnose alone could be neglected.

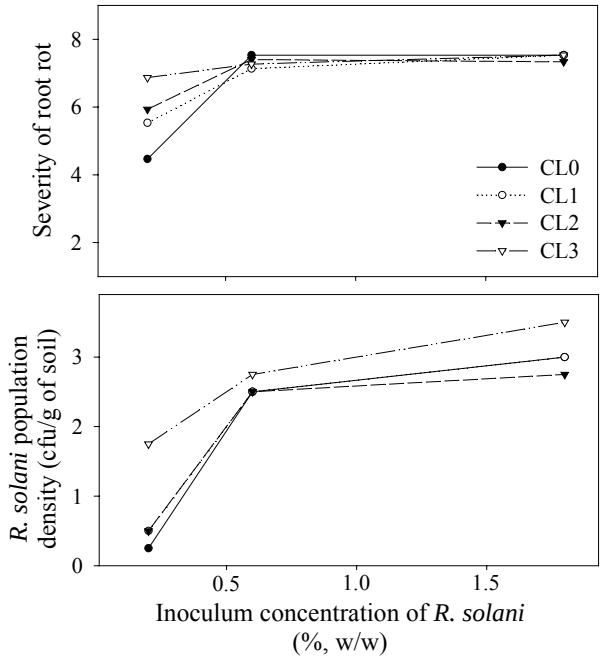

Figure 5. Effects of different inoculum levels of *R. solani* and *C. lindemuthianum* (CL0 = only water, CL1 = 1.2 x 10^4, CL2 = 1.2 x 10^5, and CL3 = 1.2 x 10^6 conidia/ml) on root rot severity at 27 DAS when 14 days old-seedlings were transplanted to *R. solani*-infested soil

5.3.2 Interaction root rot/rust

5.3.2.1 Early attack of *R. solani* (bean seeds sown in infested soil)

As expected, higher concentrations of uredospores resulted in higher rust severities, which tended to be lower in the presence of *R. solani* (Fig. 7). Root rot severity was high and similar for all levels of *R. solani* and no effect of bean rust on root rot severity could be detected (Fig. 8).

Plant weight was obviously reduced by both diseases (Fig. 9). All three regression coefficients of the regression equation (2) were significantly different from 0. The positive sign of the interaction term indicated an antagonistic effect of the two diseases on plant dry weight.

5.3.2.2 Late attack of *R. solani* (bean seedlings transplanted in infested soil)

Severity of bean rust and root rot increased at higher inoculum levels of *U. appendiculatus* and *R. solani*, respectively (Figs. 10 and 11). Pre-infection with *R. solani* significantly reduced rust severity at the highest levels of *U. appendiculatus* and *R. solani*; similarly,

diameter of rust pustules was consistently reduced at the highest *R. solani* levels (Fig. 10). When the soilborne pathogen was inoculated at 0.2%, root rot severity was significantly higher at UA3 compared to UA0 (Fig. 11); in the same way, *R. solani* population density was highest at UA3 (Fig. 11).

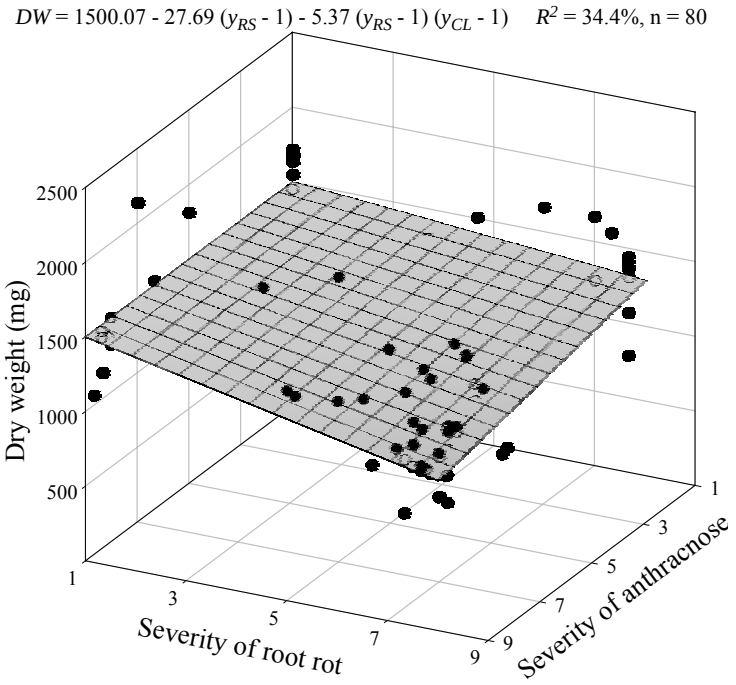

Figure 6. Effects of co-infection of *R. solani* and *C. lindemuthianum* on dry weight of bean plants at 27 DAS when 14 days old-seedlings were transplanted to *R. solani*-infested soil

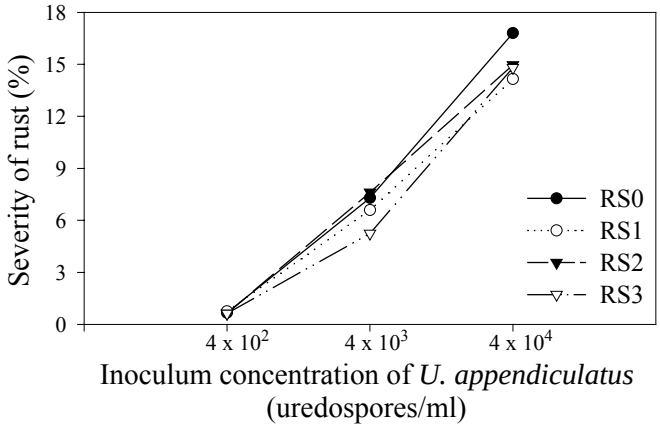

Figure 7. Effects of different inoculum levels of *U. appendiculatus* and *R. solani* (RS0 = non-infested control, RS1 = 0.2% (w/w), RS2 = 0.6%, and RS3 = 1.8%) on rust severity at 40 DAS when bean seeds were sown in *R. solani*-infested soil

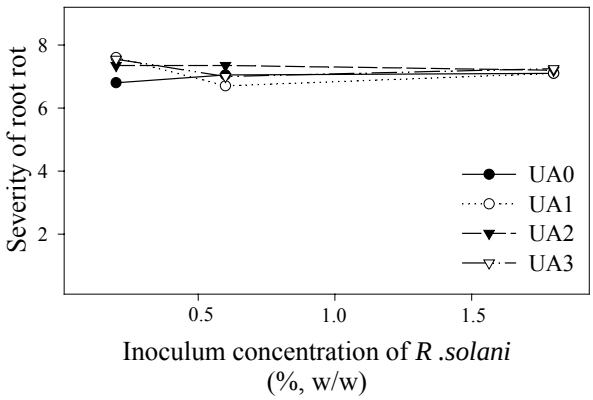

Figure 8. Effects of different inoculum levels of *R. solani* and *U. appendiculatus* (UA0 = only water, UA1 = 4 x 10^2, UA2 = 4 x 10^3, and UA3 = 1.2 x 10^4 uredospores/ml) on root rot severity at 40 DAS when bean seeds were sown in *R. solani*-infested soil

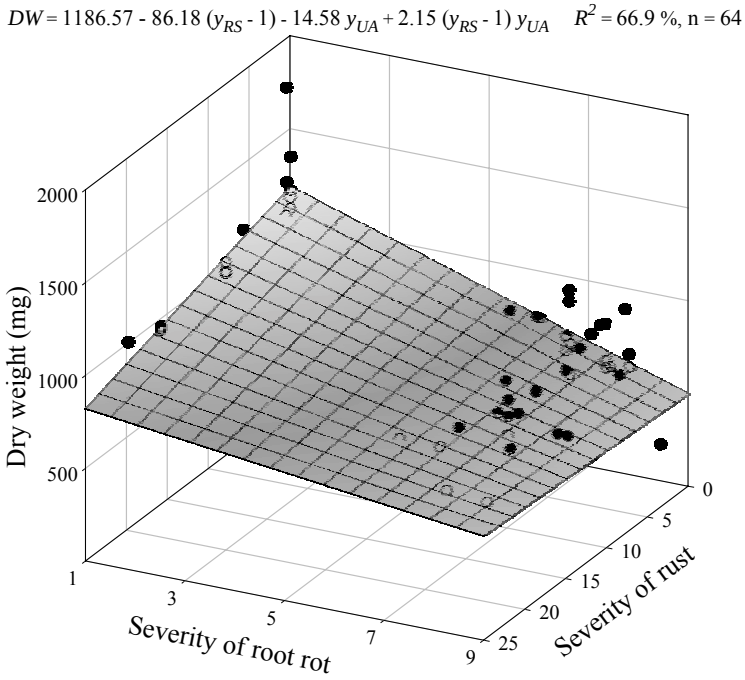

Figure 9. Effects of co-infection of *R. solani* and *U. appendiculatus* on dry weight of bean plants at 40 DAS when bean seeds were sown in *R. solani*-infested soil

Both root rot and rust reduced plant dry weight (Fig. 12). These effects are reflected in the significant regression coefficients of equation (2) for both diseases, but no significant interaction of root rot and rust was detected, which is in contrast to the situation when bean seeds were sown in infested soil.

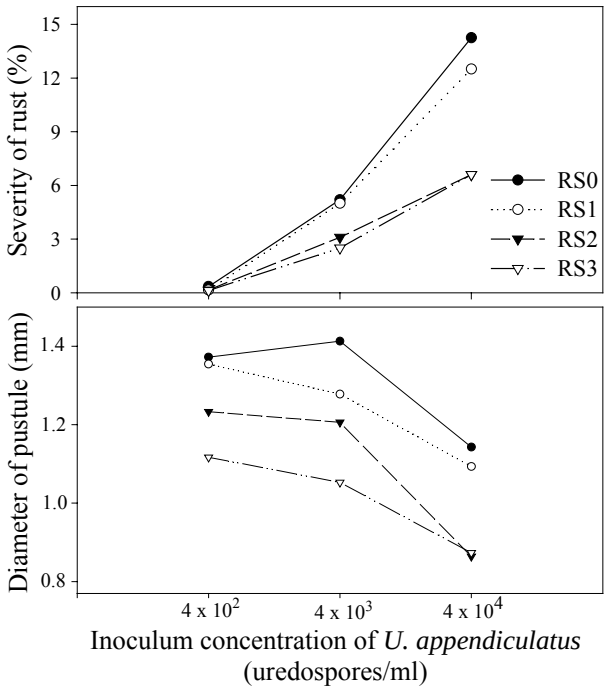

Figure 10. Effects of different inoculum levels of *U. appendiculatus* and *R. solani* (RS0 = non-infested control, RS1 = 0.2% (w/w), RS2 = 0.6%, and RS3 = 1.8%) on rust severity at 40 DAS when 25 days old-seedlings were transplanted to *R. solani*-infested soil

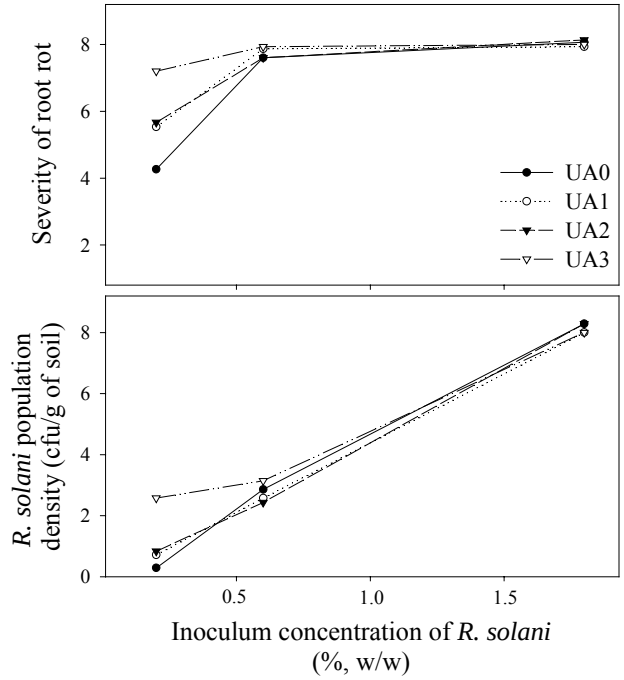

Figure 11. Effects of different inoculum levels of *R. solani* and *U. appendiculatus* (UA0 = only water, UA1 = 4 x 10^2, UA2 = 4 x 10^3, and UA3 = 1.2 x 10^4 uredospores/ml) on root rot severity at 40 DAS when 25 days old-seedlings were transplanted to *R. solani*-infested soil

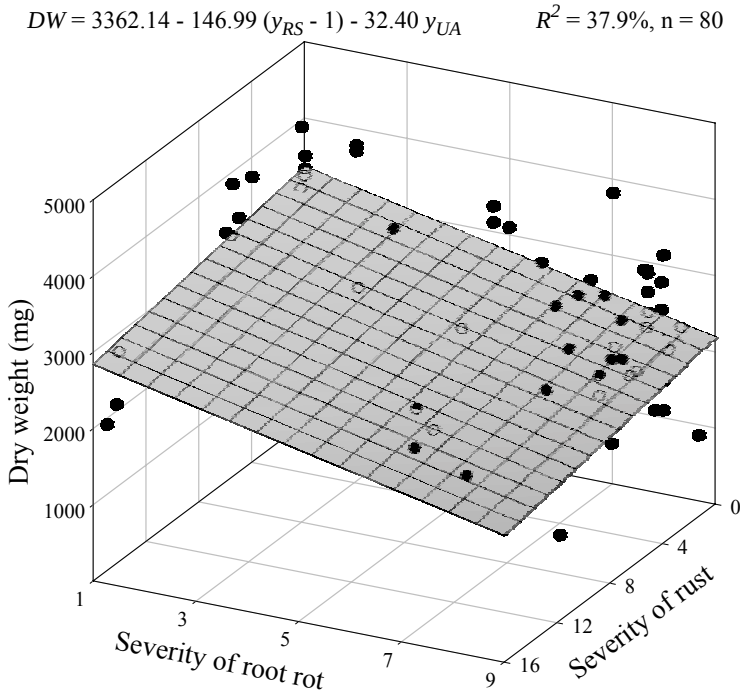

Figure 12. Effects of co-infection of *R. solani* and *U. appendiculatus* on dry weight of bean plants at 40 DAS when 25 days old-seedlings were transplanted to *R. solani*-infested soil

5.4 Discussion

In our experiments, severities of anthracnose and rust were altered when bean plants were pre-inoculated with *R. solani*, but in different ways. In the presence of the soilborne pathogen, anthracnose severity tended to be higher (Figs. 1 and 4). This is in accordance with the rather general statement of WALLER and BRIDGE (1984) that many leaf spot diseases are more severe under stress caused by root diseases. Several mechanisms have been proposed to explain the increase in host susceptibility and enhancement of the activity of interacting soilborne and aerial pathogens. These mechanisms include transmission of signals by the host (that causes metabolic or systemic changes) and changes of the nutritional status (POWELL, 1971; EVANS and HAYDOCK, 1993). Some authors have found that diseases caused by non-obligate pathogens like *C. lindemuthianum* increase when the host is infected with a destructive pathogen like *R. solani*: NICHOLSON et al. (1985) found that corn plants infected by *Pratylenchus hexincisus* developed significantly more leaf blight, caused by *Colletotrichum graminicola*, and proposed that leaf senescence hastened by the nematode infection favoured anthracnose leaf blight. BHOWMIK and SINGH (1977) also observed that Alternaria leaf blight on sunflower was more severe on plants infected with *R. solani*. Similarly, Verticilium wilt reduced plant vigour and caused premature senescence on potato

plants, which increased early blight severity, caused by *Alternaria solani* (HARRISON, 1974). Our results suggested that during the process of colonization of plant tissues by *C. lindemuthianum*, the phase of slow senescence and of eventual death of infected cells (BAILEY et al., 1992) was apparently accelerated in *R. solani*-infected plants. Thus root rot should be recognized as a potential stress factor that can increase anthracnose severity on beans.

On the other hand, rust severity and diameter of pustules tended to be lower on *R. solani*-infected plants (Figs. 7 and 10). Antagonistic effects involving *U. appendiculatus* and the nematode *M. incognita* were reported by BOOKBINDER and BLOOM (1980). These authors observed that rust severity was lower on bean plants attacked by the nematode. Moreover, nematode infection significantly reduced uredial diameters, which was reflected in a lower sporulation capacity of uredia on leaves of nematode-infected plants. The most important mechanisms that explain antagonism among pathogens infecting simultaneously different parts of the plant are competition (HARRISON, 1974; JOHNSON et al., 1986), antibiosis (BOOKBINDER and BLOOM, 1980) and induced host plant resistance (McINTYRE and DODDS, 1979; GESSLER and KUC, 1982). We assume that physiological functions were modified in *R. solani*-infected plants, consequently altering the host response to the subsequent *U. appendiculatus* infection. Suppressed host photosynthesis reduced *U. appendiculatus* sporulation on beans (COHEN and ROTEM, 1970) and could be involved in the observed response to the *R. solani* infection. In the absence of *R. solani*, the diameter of pustules was also reduced on plants more severely infected by *U. appendiculatus* (Fig. 10), possibly as a result of competition and reduction of the photosynthetic surface. Moreover, senescence caused by *R. solani* may also affect the rust infection, since appressoria formation, infection frequency and diameter of uredia decrease considerably on adult leaves (VON ALTEN, 1983; STAVELY and PASTOR-CORRALES, 1984).

Root rot severity was affected neither by *C. lindemuthianum* nor by *U. appendiculatus* infection in the experiments in which seeds were sown in *R. solani*-infested soil (Figs. 2 and 8). However, when bean seedlings were transplanted to infested soil, the highest level of *C. lindemuthianum* (Fig. 5) or *U. appendiculatus* (Fig. 11) enhanced root rot severity and the population density of the soilborne pathogen at *R. solani* inoculum level of 0.2%. According to ABAWI and PASTOR-CORRALES (1990), damage due *R. solani* may be magnified if biological stresses, such as other pathogens, are present. Although effects of interactions involving root and aerial diseases cannot be anticipated, it has been demonstrated that stem, stalk, and root rots caused by less specialized fungi are commonly more severe on plants

infected by virus (BEUTE and LOCKWOOD, 1968; BIRD, 1969; DIAZ-POLANCO et al., 1969; TU and FORD, 1971; NITZANY et al., 1973; PRATT et al., 1982; CHANT and GBAJA, 1986; EVANS and STEPHENS, 1989). The susceptibility of cucumber seedlings to Rhizoctonia damping-off increased on CMV infected plants, maybe due to the increased transport of materials from the roots to the cotyledons (BATEMAN, 1961a). PIECZARKA and ZITTER (1981) observed similar effects for the combination *R. solani* and tobacco mosaic virus (TMV-P) or pepper mottle virus (PeMV) on pepper. Conversely, McCARTER and HALPIN (1961) observed no significant increase in pathogenicity when white clover plants were infected by *R. solani* and bean yellow mosaic virus (BYMV). We assume that the increased root rot severity in the presence of the aerial pathogens operated probably through changes in the host physiology involving mechanisms such as reduction of photosynthesis, increased plant respiration and reduced translocation of photosynthates. Several researchers have demonstrated that the photosynthetic competence of plants is reduced in the presence of *C. lindemuthianum* (BASSANEZI et al., 1997; LOPES and BERGER, 2001) or *U. appendiculatus* (LIVNE and DALY, 1966; RAGGI, 1980; LOPES and BERGER, 2001). An increase of plant respiration has been reported for both pathogens (LIVNE and DALY, 1966; WONG and THROWER, 1978). Moreover, reduced translocation of photosynthates (ZAKI and DURBIN, 1965) and lower growth of the root system (SO and THROWER, 1976) have been described for rust. In the experiments in which seedlings were transplanted to infested soil, the period of possible contact between host tissue and *R. solani* was shorter compared to the experiments in which seeds were sown in infested soil. In these experiments, soil was infested with *R. solani* inoculum immediately before planting. The long time gap between the inoculation of *R. solani* and of the aerial pathogens and the drastic effect of *R. solani* in causing seedling pre- and postemergence damping-off and reducing plant growth could explain why root rot severity was not affected by the aerial diseases. On the other hand, when seedlings were transplanted to infested soil, the weakness in the root system caused by anthracnose or rust apparently favoured the root rot development and the increase of the *R. solani* population in the soil, at least at low *R. solani* inoculum levels. Some mechanisms have been proposed to explain how aerial pathogens contribute to increase severity of diseases caused by soilborne pathogens. EVANS and STEPHENS (1989) observed a reduced ability of virus-infected asparagus plants to wall-off and lignify infection courts of *Fusarium* spp. BIRD (1969) suggested that the reduction of the amount of nitrogen available caused by tobacco mosaic virus indirectly increases the growth rate of *M. javanica*. It is known that increased root exudations of virus-infected plants may change nutrients utilizable by fungi and alter the

soil microflora (BEUTE and LOCKWOOD, 1968; DIAZ-POLANCO et al., 1969; TU and FORD, 1971; PIECZARKA and ZITTER, 1981; PRATT et al., 1982; EVANS and STEPHENS, 1989).

Effects of rust on other bean diseases have been described (YARWOOD, 1969; YARWOOD, 1977; STAVELY and PASTOR-CORRALES, 1984). It is known that rust predisposes bean plants to infection of the soil inhabitants pathogens *Thielaviopsis basicola* (YARWOOD, 1977) and *Pseudomonas syringae* pv. *phaseolicola* (YARWOOD, 1969), but the mechanisms were not determined. BOOKBINDER and BLOOM (1980) observed that the mean number of root galls of *M. incognita* per gram of root was reduced by *U. appendiculatus* infection on bean when both pathogens were applied simultaneously and when the fungus was applied first. According to them, this response may be related to a reduced root growth, which caused less egg production.

Our results were observed depending on the inoculum levels. Reduction of rust severity and diameter of pustules was significant only when *R. solani* was inoculated at 0.6 and 1.8%, when the seedlings were transplanted to infested soil (Fig. 10). On the other hand, enhancement of root rot severity and population density of the soilborne pathogen at the highest level of *C. lindemuthianum* or *U. appendiculatus* was not observed at all *R. solani* inoculum levels (Fig. 11). YARWOOD (1969) also found that the interaction between rust and halo blight, caused by *P. syringae* pv. *phaseolicola*, was dependent on disease levels; halo blight was increased in the presence of *U. appendiculatus*, but heavy rust infection suppressed the occurrence of halo blight lesions. The results presented here were also dependent on timing of *R. solani* infection, so that the effects of the aerial diseases on root rot could be observed only when the seedlings were transplanted to infested soil (Figs. 5 and 11). Methods of experimentation and assessment affecting results of interactions studies have been discussed by HYDE (1981) and SIKORA and CARTER (1987).

The combined effect of root rot and anthracnose on plant dry weight was not significant when seeds were sown in *R. solani*-infested soil (Fig. 3). However, a significant synergistic effect was observed when the seedlings were transplanted to infested soil (Fig. 6), indicating that the damage caused by both diseases was more than the sum of the damage caused by each disease alone. On the other hand, a significant antagonistic effect between root rot and rust was observed on plant dry weight only when beans seeds were sown in infested soil (Fig. 9), suggesting that the damage caused by the diseases in combination was less than the sum of the damage caused by each disease individually. Although anthracnose severity tended to increase and rust to decrease in the presence of *R. solani*, the effects of combined diseases on

plant dry weight were not always synergistic for the combination root rot/anthracnose or antagonistic for the combination root rot/rust. For the same disease combinations and conditions, different effects can be observed on dynamic of diseases and crop loss. BOOKBINDER and BLOOM (1980) observed an additive effect on plant weight when bean plants were simultaneously infected with *U. appendiculatus* and *M. incognita*, but fungal uredia were reduced in diameter and sporulation capacity and *M. incognita* produced fewer root galls, and fewer eggs per egg mass.

Interactions involving Rhizoctonia root rot and aerial diseases may frequently occur in many production systems. It is evident that the results presented here may not be promptly extrapolated to field situations. However, it seems reasonable to suppose that strategies of disease assessment and/or management may be altered, since these interactions can occur in the field. The assessment of plant susceptibility to aerial pathogens can be limited by interactions with soilborne pathogens, particularly in interactions involving low levels of root infection (JENKINS and JONES, 1980). Moreover, root diseases reduce the capacity of plants for compensatory growth that they normally show when infected by aerial pathogens (WALLER and BRIDGE, 1984). Interactions between root rot and aerial diseases are very complex phenomena. This work emphasizes the necessity of more studies to investigate the real importance of these interactions at field conditions and to develop adequate management strategies for combined diseases.

6. Final conclusion

The control of Rhizoctonia root rot caused by *Rhizoctonia solani* on beans was investigated in different greenhouse experiments. Strategies like burial of the inoculum, shallow sowing and biological control using the antagonist *Trichoderma harzianum* were effective in controlling the disease.

The effects of inoculum depth on root rot progression and on survival of *R. solani* were investigated in Chapter 2. The sowing was carried out immediately after soil infestation and 15, 30, 60 and 90 days after soil infestation (DAI). Disease incidence and severity were high, but emergence of seedlings and plant weight were consistently reduced at all evaluation dates when *R. solani* inoculum was confined in the upper 10 cm of soil. Damaging effects of *R. solani* on plant development were less severe with deeper placement of inoculum. Emergence of seedlings and plant weight were not reduced when the inoculum was buried deeper than 15 cm, but infected plants were frequently observed. This has a significant epidemiological implication as diseased plants can be a source of inoculum for the next season. In PVC cylinders with removable rings, where the sowing was performed directly on infested soil, high disease severities were observed at all evaluation dates. Emergence and plant weight were reduced by *R. solani* independently of the depth in which the inoculum was originally buried. Pathogen survival was not consistently reduced after 90 days in the absence of host tissue in both experiments. Our results showed that inoculum depth affected the vertical growth of *R. solani*. Although the pathogen survived when the inoculum was placed deeper than 15 cm, it could hardly be recovered from soil samples collected above the layer originally infested in this treatment in the cylinder-experiment at 60 DAI. This poor ability for vertical growth and colonisation demonstrates the benefit of plowing to reduce inoculum density. Considering the results presented here, plowing in short time intervals or other soil movements may enable a contact between viable inoculum of the pathogen and bean seeds or seedlings in the next season. This has to be considered especially in tropical countries, where beans are frequently planted three times a year in the same field.

In Chapter 3, we observed that deep sowing significantly reduced the emergence rate and growth of plants inoculated only with *R. solani*. Effects on preemergence damping-off were dramatic at 3.0, 4.5 and particularly at 6.0 cm. However, we observed that the effectiveness of *T. harzianum* was enhanced in association with shallow sowing, since the effect of sowing depth was not significant in the presence of the antagonist. Therefore, the associated use of both strategies of disease control is recommended.

We observed in the Chapter 3 that *T. harzianum* protected bean seedlings from preemergence damping-off, reduced disease severity and increased plant growth in the presence of *R. solani*, especially in moist soil. Soil moisture levels varying from 15 to 42% (v/v) did not affect emergence rate and growth of plants infected only with *R. solani*. We concluded that the control of the disease could be enhanced by manipulating the frequency of irrigation to maintain a moist soil and to favour *T. harzianum*. The combined use of *T. harzianum* and moderate soil moisture to reduce *R. solani* infection on beans under field conditions is feasible, but recommendations of soil moisture levels must be done for each situation individually. Observations from the Chapter 5 revealed that the disease was severe when plants were in the mist chamber under high relative humidity. This may also be important for disease management, because high relative humidity caused by excessive irrigation should be avoided in areas with high inoculum potential of *R. solani*.

The survival of *R. solani* and *T. harzianum* and the dynamics of the microorganisms in the soil were affected by soil moisture levels varying from 15 to 57% (v/v) (Chapter 4). In experiments where both fungi were inoculated at the same time, and the sowing was carried out immediately after soil infestation and 20, 60, 180 and 360 DAI, the pathogen survived in the soil at least one year and caused root rot at all tested dates. However, in the first survival tests (0, 20 and 60 DAI), severity of root rot initially increased, but decreased later (180 and 360 DAI). On the other hand, dry weight of *R. solani*-infected plants was reduced in the initial tests, but increased later so that at 360 DAI values as in the treatment control were reached. Although soil moisture did not affect severity of root rot, the pathogen could easily be recovered from dryer soil. It could hardly be recovered from soil in the presence of *T. harzianum*. Consistent antagonistic effects were observed until 180 DAI. However, when the pathogen was well established in the soil, antagonistic protection was lower. Although *T. harzianum* was isolated from the soil, and disease severity was lower in the presence of the antagonist at 360 DAI, the promoting effects on emergence and growth of *R. solani*-infected plants were very slight. Exhaustion of a suitable energy base provided by wheat bran may account for this observation. The antagonist improved plant growth even on plants not infected with *R. solani*, but this effect was observed only until 60 DAI. In an additional experiment carried out at higher temperatures, this effect was less pronounced because higher temperatures promoted the emergence of seedlings. The antagonistic ability and survival of *T. harzianum* were greater in soils with an intermediate moisture level than in wet or dry soils, but depended on the inoculum potential of both fungi in the soil. Although the survival of *T. harzianum* seems to be increased in moist conditions, we observed that the activities and

growth of the antagonist were not negligible even at soil moistures of 20 or 15%. A deleterious effect of wet soil on the survival of *R. solani* and on the establishment of *T. harzianum* was observed. We concluded that an elaboration of practical biological control strategies of Rhizoctonia root rot on beans involving water management is feasible. Nevertheless, bean plants and both fungi may react differently to alternating soil moisture levels compared to constant levels. Therefore, additional research should be done before our findings are extrapolated to the field under the wide range of moisture conditions.

Additionally, we investigated the effects of the co-inoculation of *R. solani* and *C. lindemuthianum* or *U. appendiculatus* on the disease dynamics and on the growth of bean plants. Interactions between root rot and the aerial diseases were observed depending on the inoculum levels. Anthracnose severity tended to be higher on plants infected by *R. solani*. We concluded that root rot should be recognized as a potential stress factor that can increase anthracnose severity on beans. Our results suggested that during the process of colonisation of plant tissues, the phase of slow senescence and of eventual death of infected cells was apparently accelerated in *R. solani* infected plants. On the other hand, *R. solani* infections significantly reduced the diameter of rust pustules and subsequently the rust severity. We assume that physiological functions were modified in *R. solani* infected plants, altering consequently the host response to the subsequent *U. appendiculatus* infection. Suppressed host photosynthesis reducing *U. appendiculatus* sporulation on beans could be involved in the observed response to *R. solani* infection. Moreover, we observed that, in the absence of *R. solani*, the diameter of pustules was also reduced on plants more severely infected by *U. appendiculatus*, possibly as a result of competition and reduction of the photosynthetic surface. Senescence caused by *R. solani* may also affect the rust infection. At low levels of *R. solani*, the root rot severity and population density of the soilborne pathogen in the soil were magnified at high levels of *C. lindemuthianum* or *U. appendiculatus* when seedlings were transplanted to *R. solani* infested soil. We suggest that the increased root rot severity in the presence of the aerial pathogens operated probably through changes in the host physiology, involving mechanisms like reduction of photosynthetic competence of plants, increased plant respiration, reduced translocation of photosynthates, and lower growth of the root system. We concluded that the weakness in the root system development caused by anthracnose or rust apparently favoured the root rot development and increased *R. solani* population in the soil, at least at low *R. solani* inoculum levels. In these experiments, a synergistic interaction between root rot and anthracnose was observed with respect to the plant dry weight. Antagonistic effects on the plant dry weight were seen for the combination root rot - rust but only when

bean seeds were sown in infested soil. Our results were dependent on the timing of *R. solani* infection, so that the effects of the aerial diseases on root rot could be observed only when the seedlings were transplanted into infested soil. Interactions involving Rhizoctonia root rot and aerial diseases may frequently occur in many production systems, which may alter strategies of disease assessment and/or management. We emphasise the need for more studies on the actual importance of these interactions at field conditions and for the development of adequate management strategies to control simultaneously occurring diseases.

7. References

Abawi, G. S. (1994). Pudriciones radicales. In: Pastor-Corrales, M. A. and Schwartz, H. F. (eds.) Problemas de Producción del Frijol en los Trópicos. Cali: CIAT, pp. 121-184.

Abawi, G. S. and Pastor-Corrales, M. A. (1990). Root rots of beans in Latin America and Africa: Diagnosis, Research Methodologies, and Management Strategies. Cali: CIAT. 114p.

Abawi, G. S., Crosier, D. C. and Cobb, A. C. (1985). Root rot of snap beans in New York. N.Y. Food Life Sci. Bull. 110. 7p.

Adams, B. P. (1990). The potential of mycoparasites for biological control of plant diseases. Ann. Rev. Phytopathol. 28, 59-72.

Araújo, G. A. A. (1998). Preparo do solo e plantio. In: Vieira, C., de Paula, T. J. and Borém, A. (eds.) Feijão: Aspectos Gerais e Cultura no Estado de Minas. Viçosa (Brazil): Ed. UFV, pp. 99-122.

Bailey, B. A, O'Connell, R. J., Pring, R. J. and Nash, C. (1992). Infection strategies of *Colletotrichum* species. In: Bailey, B. A. and Jeger, M. J. (eds.) *Colletotrichum*: Biology, Pathology and Control. Wallingford: CAB International, pp. 88-120.

Bailey, B. A. and Lumsden, R. D. (1998). Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In: Harman, G. E. and Kubicek, C. P. (eds.) *Trichoderma & Gliocladium*, Vol. 2, Enzymes, Biological Control and Commercial Applications. London: Taylor & Francis Ltd., pp. 185-204.

Baird, R. E., Bell, D. K., Sumner, D. R., Mullinix, B. G. and Culbreath, A. K. (1993). Survival of *Rhizoctonia solani* AG 4 in residual peanut shells in soil. Plant Dis. 77, 973-975.

Baker, R. and Martinson, C. A. (1970). Epidemiology of diseases caused by *Rhizoctonia solani*. In: Parmeter, J. R. Jr. (ed.) *Rhizoctonia solani*: Biology and Pathology. Berkeley: Univ. Calif. Press, pp. 172-188.

Bassanezi, R. B., Amorim, L., Bergamin Filho, A. and Hau, B. (1998). Effects of bean line pattern mosaic virus on the monocyclic components of rust and angular leaf spot of *Phaseolus* bean at different temperatures. Plant Pathol. 47, 289-298.

Bassanezi, R. B., Martins, C. C., Godoy, C. V., Amorim, L. and Bergamin Filho, A. (1997). Efeito da antracnose na eficiência fotossintética do feijoeiro. Fitopatol. Bras. 22, 520-524.

Bateman, D. F. (1961a). Synergism between cucumber mosaic virus and Rhizoctonia damping-off of cucumber. Phytopathology 51, 574-575. (Abstr.)

Bateman, D. F. (1961b). The effect of soil moisture upon development of poinsettia root rots. Phytopathology 51, 445-451.

Beagle-Ristaino, J. E. and Papavizas, G. C. (1985). Biological control of *Rhizoctonia* stem canker and black scurf in potato. Phytopathology 75, 560-564.

Bell, D. K. and Sumner, D. R. (1987). Survival of *Rhizoctonia solani* and other soilborne basidiomycetes in fallow soil. Plant Dis. 71, 911-915.

Benson, D. M. and Baker, R. (1974a). Epidemiology of *Rhizoctonia solani* preemergence damping-off of radish: Inoculum potential and disease potential interaction. Phytopathology 64, 957-962.

Benson, D. M. and Baker, R. (1974b). Epidemiology of *Rhizoctonia solani* preemergence damping-off of radish: survival. Phytopathology 64, 1163-1168.

Beute, M. K. and Lockwood, J. L. (1968). Mechanism of increased root rot in virus infected peas. Phytopathology 58, 1643-1651.

Bhowmik, T. P. and Singh, A. (1977). Combined effect of *Rhizoctonia* root rot and *Alternaria* leaf blight on sunflower. Indian Phytopathol. 30, 195-197.

Bird, A. F. (1969). The influence of tobacco ring spot virus and tobacco mosaic virus on the growth of *Meloidogyne javanica*. Nematologica 15, 201-209.

Björkman, T., Blanchard, L. M. and Harman, G. E. (1998). Growth enhancement of shrunken-2 sweet corn by *Trichoderma harzianum* 1295-22: effect of environmental stress. J. Am. Soc. Hort. 123, 35-40.

Bookbinder, M. G. and Bloom, J. R. (1980). Interaction of *Uromyces phaseoli* and *Meloidogyne incognita* on bean. J. Nematol. 12, 177-182.

Boosalis, M. and Scharen, A. L. (1959). Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* associated with plant debris. Phytopathology 49, 192-198.

Canaday, C. H. (1998). Differences in the incidence of Rhizoctonia root rot of snap bean associated with different potash fertilizers. Phytopathology 88, S124. (Abstr.)

Cardoso, J. E. (1994). Podridões radiculares. In: Sartorato, A. and Rava, C. A. (eds.) Principais Doenças do Feijoeiro Comum e seu Controle. Brasília: EMBRAPA-SPI, pp. 151-164.

Carneiro, S. M. T. P. G., Amorim, L., Bergamin Filho, A., Hau, B. and Bianchini, A. (2000). Dinâmica de área foliar, desfolha e variáveis de área foliar sadia em feijoeiros com infecções isoladas e conjuntas de *Phaeoisariopsis griseola* e *Colletotrichum lindemuthianum*. Summa Phytopathol. 26, 406-412.

Chang, Y. C., Chang, Y. C. and Baker, R. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70, 145-148.

Chant, S. R. and Gbaja, I. S. (1986). Effect of co-infection by *Fusarium oxysporum* and cowpea mosaic virus on the growth and colonization of cowpea seedlings (*Vigna unguiculata* (L.) Walp.). J. Phytopathol. 116, 81-87.

Chet, I. (1990). Biological control of soil-borne plant pathogens with fungal antagonists in combination with soil treatments. In: Hornby, D. (ed.) Biological Control of Soil-borne Plant Pathogens. Wallingford: CAB International, pp. 15-26.

Chet, I. and Barker, R. (1981). Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. Phytopathology 71, 286-290.

Chet, I., Elad, Y., Kalfon, A., Hadar, Y. and Katan, J. (1982). Integrated control of soil-borne and bulb-borne pathogens in iris. Phytoparasitica 10, 229-236.

Chet, I., Hadar, Y., Elad, Y., Katan, J. and Henis, Y. (1979). Biological control of soilborne plant pathogens by *Trichoderma harzianum*. In: Schippers, B. and Gams, W. (eds.) Soil Borne Plant Pathogens. London: Academic Press, pp. 585-591.

Cohen, Y. and Rotem, J. (1970). The relationship of sporulation to photosynthesis in some obligatory and facultative parasites. Phytopathology 60, 1600-1604.

Crossan, D. F. (1965). Field and greenhouse experiments for control of Rhizoctonia root rot of snapbean. Phytopathology 55, 503. (Abstr.)

Dal Soglio, F. K., Bertagnolli, B. L., Sinclair, J. B., Yu, G. Y. and Eastburn, D. M. (1998). Production of chitinolytic enzymes and endoglucanase in the soybean rhizosphere in the presence of *Trichoderma harzianum* and *Rhizoctonia solani*. Biol. Control 12, 111-117.

Dalla Pria, M., Bianchini, A. and Souza, E. A. (1994). Avaliação da resistência de dez cultivares de feijoeiro ao vírus do mosaico-em-desenho. In: Anais do 5º Seminário sobre Pragas, Doenças e Plantas Daninhas do Feijoeiro. Piracicaba (Brazil): FEALQ, 22.

Danielson, R. M. and Davey, C. B. (1973). The abundance of *Trichoderma* propagules and the distribution of species in forest soils. Soil Biol. Biochem. 5, 485-494.

Das, A. C. and Western, J. H. (1959). The effect of inorganic manures, moisture and inoculum on the incidence of root disease caused by *Rhizoctonia solani* Kühn in cultivated soil. Ann. Appl. Biol. 47, 37-48.

De Paula, T. J. and Zambolim, L. (1998). Doenças. In: Vieira, C., de Paula, T. J. and Borém, A. (eds.) Feijão: Aspectos Gerais e Cultura no Estado de Minas. Viçosa (Brazil): Ed. UFV, pp. 375-433.

Diaz-Polanco, C., Smith, S. H. and Hancock, J. G. (1969). Effect of virus infection on stem rot of squash caused by *Fusarium solani* f. sp. *cucurbitae*. Phytopathology 59, 18-22.

Elad, Y. (1996). Bacterial and fungal cell-wall hydrolytic enzymes in relation to biological control of *Rhizoctonia solani*. In: Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (eds.) *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht: Kluwer Academic Publishers, pp. 455-462.

Elad, Y., Chet, I. and Henis, Y. (1981a). A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9, 59-67.

Elad, Y., Chet, I. and Henis, Y. (1981b). Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. Plant and Soil 60, 245-254.

Elad, Y., Chet, I. and Katan, J. (1980a). *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70, 119-121.

Elad, Y., Hadar, Y., Hadar, E., Chet, I. and Henis, Y. (1981c). Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation. Plant Dis. 65, 675-677.

Elad, Y., Katan, J. and Chet, I. (1980b). Physical, biological and chemical control integrated for soilborne diseases in potato. Phytopathology 70, 418-422.

Evans, K. and Haydock, P. P. J. (1993). Interactions of nematodes with root-rot fungi. In: Khan, M. W. (ed.) Nematode Interactions. London: Chapman & Hall, pp. 104-133.

Evans, T. A. and Stephens, C. T. (1989). Increased susceptibility to Fusarium crown and root rot in virus-infected asparagus. Phytopathology 79, 253-258.

Fenille, R. C. and Sousa, N. L. (1999). Efeitos de materiais orgânicos e da umidade do solo na patogenicidade de *Rhizoctonia solani* Kühn GA-4 HGI ao feijoeiro. Pesq. Agropec. Bras. 34, 1959-1967.

Galindo, J. J., Abawi, G. S. and Thurston, H. D. (1982). Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. Plant Dis. 66, 390-394.

Gams, W. and Bissett, J. (1998). Morphology and identification of *Trichoderma*. In: Kubicek, C. P. and Harman, G. E. (eds.) *Trichoderma & Gliocladium*, Vol. 1, Enzymes, Biological Control and Commercial Applications. London: Taylor & Francis Ltd., pp. 3-34.

Gbaja, I. S. and Chant, S. R. (1985). The effects of co-infection by Sunn-Hemp mosaic virus (SHMV) and *Fusarium oxysporum* on the growth of French bean. Phytopathol. Z. 113, 252-259.

Gessler, C. and Kúc, J. (1982). Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens. Phytopathology 72, 1439-1441.

Godoy, C. V., Carneiro, S. M. T. P. G., Iamauti, M. T., Dalla Pria, M., Amorim, L. Berger, R. D. and Bergamin Filho, A. (1997). Diagrammatic scales for bean diseases: Development and validation. Z. Pflanzenkr. Pflanzenshutz 104, 336-345.

Hadar, E., Elad, Y., Hadar, Y. and Chet, I. (1982). Build-up and decline of *Rhizoctonia solani* inoculum under field conditions. Plant Soil 65, 303-307.

Hadar, Y., Chet, I. and Henis, Y. (1979). Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69, 64-68.

Hall, R. (1991). Compendium of Bean Diseases. St. Paul: APS Press, 73p.

Harman, G. E. (1991). Seed treatments for biological control of plant disease. Crop Prot. 10, 166-171.

Harman, G. E., Chet, I. and Baker, R. (1980). *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. Phytopathology 70, 1167-1172.

Harman, G. E., Taylor, A. G. and Stasz, T. E. (1989). Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatment. Plant Dis. 73, 631-637.

Harrison, M. D. (1974). Interactions between foliar sprays and soil fumigation in the yield response of potatoes. Phytopathology 64, 860-864.

Hau, B. (2001). Some remarks on interactions among diseases with respect to their dynamics and crop losses. 8th International Workshop on Plant Disease Epidemiology. Ouro Preto (Brazil): International Society of Plant Pathology, pp. 110-119.

Henis, Y., Ghaffer, A. and Baker, R. (1978). Integrated control of *Rhizoctonia solani* damping-off of radish: Effect of successive plantings, PCNB and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68, 900-909.

Herr, L. J. (1976). In field survival of *Rhizoctonia solani* in soil and in diseased sugarbeets. Can. J. Microbiol. 22, 983-988.

Hiremath, P. C. and Prasad, C. K. P. S. (1985). Vertical distribution of *Rhizoctonia solani* in soil and effect of culture filtrate on the rhizosphere mycoflora of fenugreek. J. Soil Biol. & Ecol. 5, 126-128.

Hjeljord, L. and Tronsmo, A. (1998). *Trichoderma* and *Glioclacium* in biological control: an overview. In: Harman, G. E. and Kubicek, C. P. (eds.) *Trichoderma & Gliocladium*, Vol. 2, Enzymes, Biological Control and Commercial Applications. London: Taylor & Francis Ltd., pp. 131-151.

Huissman, O. C. (1988). Colonization of field-grown cotton roots by pathogenic and saprophytic soilborne fungi. Phytopathology 78, 716-722.

Hussey, R. S. and Roncadori, R. W. (1977). Vertical distribution of soil microorganisms following subsoiling in a cotton management system. Phytopathology 67, 783-786.

Hyde, P. M. (1981). The effects on wheat of inoculation with *Puccinia striiformis* and *Septoria nodorum* with respect to possible interactions. Phytopathol. Z. 100, 111-120.

Inbar, J., Abramsky, M., Cohen, D. and Chet, I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. Eur. J. Plant Pathol. 100, 337-346.

Jenkins, P. D. and Jones, D. G. (1980). Predisposition to *Septoria nodorum* as a result of take-all (*Gaeumannomyces graminis*) infection of wheat. Ann. Appl. Biol. 95, 47-52.

Jesus Junior, W. C., Vale, F. X. R., Coelho, R. R., Hau, B., Zambolim, L., Costa, L. C. and Bergamin Filho, A. (2001). Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathology 91, 1045-53.

Johnson, K. B., Radcliffe, E. B. and Teng, P. S. (1986). Effect of interacting populations of *Alternaria solani*, *Verticillium dahliae*, and the potato leafhopper (*Empoasca fabae*) on potato yield. Phytopathology 76, 1046-1052.

Klein, D. and Eveleigh, D. E. (1998). Ecology of Trichoderma. In: Kubicek, C. P. and Harman, G. E. (eds.) *Trichoderma & Gliocladium*, Vol. 1, Enzymes, Biological Control and Commercial Applications. London: Taylor & Francis Ltd., pp. 57-74.

Knudsen, G. R. and Bin, L. (1990). Effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets. Phytopathology 80, 724-727.

Ko, W. and Hora, F. K. (1971). A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61, 707-710.

Kobriger, K. M. and Hagedorn, D. J. (1983). Determination of bean root rot potential in vegetable production fields of Wisconsin's Central Sands. Plant Dis. 67, 177-178.

Kok, C. K., Hageman, P. E. J., Maas, P. W. T., Postma, J., Roozen, N. J. M. and Van Vuurde, J. W. L. (1996). Processed manure as carrier to introduce *Trichoderma harzianum*: Population dynamics and biocontrol effect on *Rhizoctonia solani*. Bioc. Sci. Technol. 6, 147-162.

Kumar, S., Sivasithamparam, K., Gill, J. S. and Sweetingham, M. W. (1999). Temperature and water potential effects on growth and pathogenicity of *Rhizoctonia solani* to lupin. Can. J. Microbiol. 45, 389-395.

Leach, L. D. and Garber, R. H. (1970). Control of *Rhizoctonia*. In: Parmeter, J. R. Jr. (ed.) *Rhizoctonia solani*: Biology and Pathology. Berkeley: Univ. Calif. Press, pp. 189-199.

Leach, S. S., Porter, G. A., Rourke, R. V. and Clapham, W. M. (1993). Effects of moldboard plowing, chisel plowing and rotation crops on the rhizoctonia disease of white potato. Amer. Potato J. 70, 329-377.

Lewis, J. A. and Kulik, M. M. (1996). Introduced biocontrol agents to suppress diseases caused by *Rhizoctonia*. In: Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (eds.) *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht: Kluwer Academic Publishers, pp. 507-514.

Lewis, J. A. and Papavizas, G. C. (1977). Factors affecting *Rhizoctonia solani* infection of soybeans in the greenhouse. Plant Dis. Rep. 61, 196-200.

Lewis, J. A. and Papavizas, G. C. (1980). Integrated control of Rhizoctonia fruit rot of cucumber. Phytopathology 70, 85-89.

Lewis, J. A. and Papavizas, G. C. (1987). Reduction of inoculum of *Rhizoctonia solani* in soil by germlings of *Trichoderma hamatum*. Soil Biol. Biochem. 19, 195-201.

Lewis, J. A. and Papavizas, G. C. (1991). Biocontrol of cotton damping-off caused by *Rhizoctonia solani* in the field with formulations of *Trichoderma* spp. and *Gliocladium virens*. Crop Prot. 10, 396-402.

Lewis, J. A., Fravel, D. R., Lumsden, R. D. and Shasha, B. S. (1995). Application of biocontrol fungi in granular formulations of pregelatinized starch-flour to control damping-off diseases caused by *Rhizoctonia solani*. Biol. Control 5, 397-404.

Lewis, J. A., Lumsden, R. D. and Papavizas, G. C. (1983). Integrated control of snap bean diseases caused by *Pythium* spp. and *Rhizoctonia solani*. Plant Dis. 67:1241-1244.

Lindsey, D. L and Baker, R. (1967). Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. Phytopathology 57, 1262-1263.

Liu, S. and Baker, R. (1980). Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology 70, 404-412.

Livne, A. and Daly, J. M. (1966). Translocation in healthy and rust-affected beans. Phytopathology 56, 170-175.

Lo, C. T., Nelson, E. B. and Harman, G. E. (1996). Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. 80, 736-741.

Lopes, D. B. and Berger, R. D. (2001). The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathology 91, 212-220.

Lorito, M., Hayes, C. K., Di Pietro, A., Woo, S. L. and Harman, G. E. (1994). Purification, characterization and synergistic activity of a glucan 1,3-glucosidase and N-acetyl-b-glucosaminidase from *Trichoderma harzianum*. Phytopathology 84, 398–405.

Lynch, J. M., Wilson, K. L., Ousley, M. A. and Whipps, J. M. (1991). Response of lettuce to *Trichoderma* treatment. Lett. Appl. Microbiol. 12, 59-61.

Manning, W. J., Crossan, D. F. and Morton, D. J. (1967). Effects of planting depth and asphalt mulch on Rhizoctonia root and hypocotyl rot of snapbean. Plant Dis. Rep. 51, 158-160.

Marshall, D. S. (1982). Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acidic soils. Plant Dis. 66, 788-789.

Maughan, R. D. and Barbetti, M. J. (1983). Rhizoctonia root rot of white clover. Aust. Plant Pathol. 12, 13-14.

McCarter, S. M. and Halpin, J. E. (1961). Studies on the pathogenicity of 4 species of soil fungi on white clover as affected by the presence of bean yellow mosaic virus under conditions of controlled temperature and light. Phytopathology 51, 644. (Abstr)

McIntyre, J. L. and Dodds, J. A. (1979). Induction of localized and systemic protection against *Phytophthora parasitica* var. *nicotianae* by tobacco mosaic virus infection of tobacco hypersensitive to the virus. Physiol. Plant Pathol. 15, 321-330.

Nelson, E. B. and Hoitink, H. A. J. (1983). The role of micro-organisms in the suppression of *Rhizoctonia solani* in container media amended with composted hardwood bark. Phytopathology 73, 274-278.

Nicholson, R. L., Bergeson, G. B., De Gennaro, F. P. and Viveiros, D. M. (1985). Single and combined effects of the lesion nematode and *Colletotrichum graminicola* on growth and anthracnose leaf blight of corn. Phytopathology 75, 654-661.

Nitzany, F. E., Joffe, A. Z. and Palti, J. (1973). Synergism between *Fusarium* spp. and cucumber mosaic virus. Phytopathol. Z. 76, 314-318.

Papavizas, G. C, Adams, P. B., Lumsden, R. D., Lewis, J. A., Dow, R. L., Ayers, W. A. and Kantzes, J. G. (1975). Ecology and epidemiology of *Rhizoctonia solani* in field soil. Phytopathology 65, 871-877.

Papavizas, G. C. (1982). Survival of *Trichoderma harzianum* in soil and in pea and bean rhizospheres. Phytopathology 72, 121-125.

Papavizas, G. C. (1985). *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Ann. Rev. Phytopathol. 23, 23-54.

Papavizas, G. C. and Davey, C. B. (1962). Activity of *Rhizoctonia solani* in soil affected by carbon dioxide. Phytopathology 52, 759.

Papavizas, G. C. and Lewis, J. A. (1979). Integrated control of *Rhizoctonia solani*. In: Schippers, B. and Gams, W. (eds.) Soil-borne Plant Pathogens. London: Academic Press, pp. 415-424.

Pieczarka, D. J. and Zitter, T. A. (1981). Effect of interaction between two viruses and *Rhizoctonia* to pepper. Plant Dis. 65, 404-406.

Pitt, D. (1964). Studies on sharp eyespot disease of cereals. I. Disease symptoms and pathogenicity of isolates of *Rhizoctonia solani* Kühn and the influence of soil factors and temperature on disease development. Ann. Appl. Biol. 54, 77-89.

Ploetz, R. C. and Mitchell, D. J. (1985). Influence of water potential on the survival and saprophytic activity of *Rhizoctonia solani* AG-4 in natural soil. Can. J. Bot. 63, 2364-2368.

Powell, N. T. (1971). Interactions between nematodes and fungi in disease complexes. Ann. Rev. Phytopathol. 9, 253-274.

Pratt, R. G., Ellsbury, M. M., Barnett, O. W. and Knight, W. E. (1982). Interactions of bean yellow mosaic virus and an aphid vector with Phytophthora root diseases in arrowleaf clover. Phytopathology 72, 1189-1192.

Raggi, V. (1980). Correlation of CO_2 compensation point (r) with photosynthesis and respiration and CO_2-sensitive r in rust-affected bean leaves. Physiol. Plant Pathol. 16, 19-24.

Reddy, P. P., Singh, D. B. and Sharma, S. R. (1979). Interaction of *Meloidogyne incognita* and *Rhizoctonia solani* in a root rot complex of French bean. Indian Phytopathol. 32, 651-652.

Rosa, S. D. V. (1990). Efeitos das profundidades de semeadura e do molhamento do solo sobre o estabelecimento do estande e desenvolvimento da cultura do feijão (*Phaseolus vulgaris* L.). M. Sc. Thesis. Piracicaba (Brazil): ESALQ, 65p.

Roth, L. F. and Riker, A. J. (1943). Influence of temperature, moisture and soil reaction on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*. Agric. Res. 67, 173-293.

Ruppel, E. G. (1991). Survival of *Rhizoctonia solani* in fallow field and buried sugarbeet roots at three depths. J. Sugar Beet Res. 28, 141-153.

Shehata, M. A., Davis, D. W. and Anderson, N. A. (1984). Resistance to *Rhizoctonia* stem rot in peas as influenced by temperature, watering method, and period of disease development. Plant Dis. 68, 22-24.

Sikora, R. A. and Carter, W. W. (1987). Nematode interaction with fungal and bacterial plant pathogens - fact or fantasy. In: Veech, J. A. and Dickson, D. W. (eds.) Vistas on Nematology. Hyattsville (Maryland): Society of Nematologists, Inc., pp. 307-312.

Silbernagel, M. J. (1981). Effects of cultural practices on root rot in snap beans. Phytopathology 71, 254. (Abstr.)

So, M. L. and Thrower, L. B. (1976). The host-parasite relationship between *Vigna sesqipedalis* and *Uromyces appendiculatus*. Phytopahol. Z. 86, 252-256.

Stavely, J. R. and Pastor-Corrales, M. A. (1984). Roya. In: Pastor-Corrales, M. A. and Schwartz, H. F. (eds.) Problemas de Producción del Frijol en los Trópicos. Cali: CIAT, pp. 185-225.

Strashnow, Y., Elad, Y., Sivan, A. and Chet, I. (1985). Integrated control of *Rhizoctonia solani* by methyl bromide and *Trichoderma harzianum*. Plant Pathol. 34, 146-151.

Sumner, D. R. (1996). Sclerotia formation by *Rhizoctonia solani* and their survival. In: Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (eds.) *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht: Kluwer Academic Publishers, pp. 207-215.

Sumner, D. R. and Bell, D. K. (1982). Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. Phytopathology 72, 86-91.

Sumner, D. R., Smittle, D. A., Threadgill, E. D., Johnson, A. W. and Chalfant, R. B. (1986). Interactions of tillage and soil fertility with root diseases in snap bean and lima bean in irrigated multiple-cropping systems. Plant Dis. 70, 730-735.

Sweetingham, M. W. (1996). Integrated control of *Rhizoctonia* species. In: Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (eds.) *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht: Kluwer Academic Publishers, pp. 549-558.

Teo, B. K., Yitbarek, S. M., Verma, P. R. and Morrall, R. A. A. (1988). Influence of soil moisture, seeding date and *Rhizoctonia* solani isolates (AG 2-1 and AG 4) on disease incidence and yield in canola. Can. J. Plant Pathol. 10, 151-158.

Tu, J. C. and Ford, R. E. (1971). Maize dwarf mosaic virus predisposes corn to root rot infection. Phytopathology 61, 800-803.

Van Bruggen, A. H. C., Whalen, A. H. and Arneson, P. A. (1986). Emergence, growth, and development of dry bean seedlings in response to temperature, soil moisture, and *Rhizoctonia solani*. Phytopathology 76, 568-572.

Van Schoonhoven, A. and Pastor-Corrales, M. A. (1987). Standard System for the Evaluation of Bean Germplasm. Cali: CIAT, 53p.

Vieira, R. F. and De Paula, T.J. (1998). Semente: veículo de disseminação de patógenos. In: Vieira, C., de Paula, T. J. and Borém, A. (eds.) Feijão: Aspectos Gerais e Cultura no Estado de Minas. Viçosa (Brazil): Ed. UFV, pp. 451-505.

Von Alten, H. (1983). The effect of temperature, light and leaf age on the frequency of appressoria formation and infection with *Uromyces phaseoli* (Pers.) Wint. Phytopathol. Z. 107, 327-355.

Waller, J. M. and Bridge, J. (1984). Effects of pathogen interactions on tropical crop production. In: Wood, R. H. S. and Jellis, G. L. (eds.) Plant Diseases: Infection, Damage and Loss. Oxford: Blackwell Scientific Publications, pp. 311-320.

Wester, R. E. and Goth, R. W. (1965). Pathogenicity of *Rhizoctonia solani* on lima bean seedlings. Phytopathology 55, 506. (Abstr.)

Win, H. H. and Sumner, D. R. (1988). Root rot induced in snap bean by *Rhizoctonia solani* AG-4 and AG-2 Type 2 in conservation tillage following corn. Plant Dis. 72, 1049-1053.

Windham, M. T., Elad, Y. and Baker, R. (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76, 518-521.

Wong, P. Y. O. and Thrower, L. B. (1978). Sugar metabolism and translocation in *Vigna sesquipedalis* infected by *Colletotrichum lindemuthianum*. Phytopathol. Z. 92, 102-112.

Wright, E. (1957). Influence of temperature and moisture on damping-off of American and Siberian elm, black locust, and desert willow. Phytopathology 47, 658-662.

Wu, W. S. (1982). Seed treatment by applying *Trichoderma* spp. to increase the emergence of soybeans. Seed Sci. Technol. 10, 557-563.

Yarwood, C. E. (1969). Association of rust and halo blight on beans. Phytopathology 59, 1302-1305.

Yarwood, C. E. (1977). *Pseudoperonospora cubensis* in rust-infected bean. Phytopathology 67, 1021-1022.

Zaki, A. I. and Durbin, R. D. (1965). The effect of bean rust on the translocation of photosynthetic products from diseased leaves. Phytopathology 55, 528-529.

Zaumeyer, W. J. and Thomas, H. R. (1957). A monographic study of bean diseases and methods for their control. Washington: USDA, 255p. (Tech. Bull. 868)

Zimand, G., Elad, Y. and Chet, I. (1996). Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology 86, 1255-1260.

Lebenslauf

Name:	Trazilbo José de Paula Júnior
Wohnort:	Lohgrund 3 in 30453 Hannover Rua A, Quadra A, Cidade Jardim in 36570-000 Viçosa (MG), Brasilien
Staatsangehörigkeit:	brasilianisch
Familienstand:	verheiratet (Maria Eugênia de Paula), 2 Kinder (Lis und Júlia de Paula)
Geburtsdatum:	23.05.1966
1972-1976	Besuch der Grundschule in Resplendor (MG), Brasilien
1977-1983	Besuch des Gymnasiums in Resplendor (MG) und Colatina (ES), Brasilien
1983	Erwerb der Allgemeinen Hochschulreife am Colégio Marista in Colatina (ES), Brasilien
Februar 1984 – Juli 1988:	Studium der Agrarwissenschaft an der Universidade Federal de Viçosa – UFV – (MG), Brasilien
Februar 1989 – Juli 1992:	Master in Phytopathology an der UFV, Brasilien
Seit Mai 1992:	Wissenschaftlicher Mitarbeiter bei EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais), Brasilien
Seit April 1999:	Doktorand am Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover

Nachwort

Gott danke ich für seine Gegenwart. Meiner Frau Maria Eugênia de Paula und meinen Töchtern Lis und Júlia de Paula danke ich für die Liebe und Unterstützung während der Zeit in Deutschland. Familie Nückel danke ich für die herzliche und offene Freundschaft.

Herrn Prof. Dr. B. Hau danke ich besonders für das mir gewährte Vertrauen während der gesamten Zeit sowie seine Unterstützung bei der Erstellung dieser Arbeit.

Herrn Prof. Dr. Francisco Xavier Ribeiro do Vale danke ich für die freundliche Übernahme des Korreferates. Herrn Prof. Dr. Laércio Zambolim danke ich für die wertvollen Tipps.

Bei dem DAAD (Deutscher Akademischer Austauchdienst), EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais) und CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) bedanke ich mich für die finanzielle Unterstützung.

Weiterhin danke ich allen beteiligten Personen, besonders Frau Dipl. Ing. agr. Claudia Rotter, Frau Nathalie Röder, Herrn MSc Belayneh Admassu, Herrn Dr. Waldir Cintra de Jesus Júnior, Herrn W. Arndt und Herrn H. Seelbinder, für ihre Unterstützung bei der Durchführung der Versuche.

Allen Mitarbeitern des Institutes für Pflanzenkrankheiten und Pflanzenschutz der Universität Hannover sei an dieser Stelle herzlich gedankt.

Eidesstattliche Erklärung

Hiermit versichere ich an Eides Statt, dass ich die vorliegende Dissertation eigenständig verfasst habe und sie weder an der Universität Hannover noch an anderen Universitäten als Diplomarbeit oder andere Prüfungsarbeit verwendet oder eingereichet habe. Die zur Erstellung der Arbeit benutzten Hilfen oder Hilfsmittel habe ich vollständig aufgeführt.

Hannover, den 20. April 2002

(Trazilbo de Paula)