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FULL RESEARCH PAPER

# Effects of soil moisture and sowing depth on the development of bean plants grown in sterile soil infested by *Rhizoctonia solani* and *Trichoderma harzianum*

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**Abstract** The effects of soil moisture (varying from 15% to 42% (v/v)) and sowing depth (1.5–6.0 cm) on the development of bean plants grown in sterile soil infested by the pathogen *Rhizoctonia solani* and its antagonist *Trichoderma harzianum* were studied under greenhouse conditions. The four possible combinations of soil infestation with both fungi were tested. Disease severity, percentage of plants emerged, plant height and dry weight were evaluated 3 weeks after sowing. Emergence rate and growth of plants inoculated only with *R. solani* were not affected by soil moisture, but in the presence of both fungi, plant emergence, plant height and dry weight significantly decreased when soil moisture diminished. Deep sowing significantly reduced the emergence rate and growth of those plants that were inoculated with *R. solani* only. However, when the soil was infested with both fungi, 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.

**Keywords** Biological control · Integrated control · Regression analysis · Linear combinations

## Introduction

Rhizoctonia root rot caused by *Rhizoctonia solani* is a widely distributed disease of common bean (*Phaseolus vulgaris*) in the world. The fungal isolates that cause root rot on common beans usually belong to anastomosis groups (AG) 2 or 4 and more rarely to AG 1 (Abawi and Pastor-Corrales 1990). Bean production has substantially declined 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 (Vieira and Paula Júnior 2006). Although Hall (1991) affirmed that soil moisture has little effect on disease severity on beans, Abawi and Pastor-Corrales (1990) asserted that the disease might be more severe under moderate to high soil moisture conditions and moderate temperature. High root rot severity on beans has frequently been correlated with high soil moisture (Galindo et al. 1982; Kobriger and Hagedorn 1983), but also contradictory results have

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been found on beans (Van Bruggen et al. 1986; Fenille and Souza 1999) and on other crops (Huissman 1988; Teo et al. 1988; Dorrance et al. 2003).

The disease may be reduced by various cultural practices such as sowing on raised beds during the wet rainy season, delayed sowing, rotation with non-host crops, deep plowing, use of more resistant cultivars, incorporation of organic residues and shallow sowing (Abawi and Pastor-Corrales 1990). Shallow sowing can be effective in reducing the damage caused by *R. solani*, since deep sowing extends the period of seedling emergence and increases the severity of infection (Manning et al. 1967; Leach and Garber 1970). Apart from cultural practices, biological control using *Trichoderma* species can reduce the activities of *R. solani*, especially *T. harzianum* which is one of the more intensively investigated biocontrol agents (Elad et al. 1980; Chet et al. 1982). *Trichoderma* spp. are particularly prevalent in humid environments and are relatively intolerant of low moisture levels; nevertheless, they can be isolated from all climatic zones, including desert soils (Klein and Eveleigh 1998).

The integration of biological agents with additional strategies is increasingly recommended to enhance Rhizoctonia root rot control (Sweetingham 1996). However, environmental and edaphic factors, including soil moisture, as well as cultural practices used to control root rot, such as water management, may influence the antagonistic properties of *T. harzianum*. Similarly, little is known about the effects of practices such as shallow sowing on the biocontrol of root rot using *T. harzianum*. The objective of this work was to investigate the influence of soil moisture and sowing depth on the development of bean plants infected by *R. solani* in the presence of *T. harzianum*.

## Materials and methods

Two kinds of experiments were conducted under greenhouse conditions at Hanover, Germany, varying the soil moisture or the sowing depth. The fungal isolates used were taken from the collection of the Institute of Plant Diseases and Plant Protection, University of Hanover. They were maintained subcultured on potato dextrose agar (PDA) at 4°C. *Rhizoctonia solani* (AG-4) was isolated from bean and grown on autoclaved rice grains in 200 ml flasks, while *T. harzianum* (isolate T-12) was grown on autoclaved wheat bran. Two 5-mm diam. mycelial-agar (PDA) 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 open-air dried on trays for 24 h before soil infestation. Soil (Fruhstorfer Erde Typ 1, Industrie-Erdenwerk Archut, Lauterbach) was mixed with sand (2:1) and sterilized at 150–170°C for 24 h. 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 healthy seeds of the bean cv. '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.). The soil moisture was adjusted to four levels: 42%, 32%, 23% and 15% (v/v), which correspond to matric water potentials of −0.005, −0.023, −0.137 and −1.03 MPa, respectively. The pots were weighed to monitor water loss and irrigated once a day. 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 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 maintained at 23/18°C (day/night).

Daily observations were made on emergence. The plants were removed 3 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): (1) no visible symptoms, (2) light discolouration without necrotic lesions, (3) light discolouration with one or two small lesions, (4) <10% of the hypocotyl and root tissues covered with lesions, (5) 10–25% of the hypocotyl and root tissues covered with lesions but tissues remain, (6) approximately 25% of the hypocotyl and root tissues covered with lesions and light softening, (7) 25–50% of the hypocotyl and root tissues covered with lesions combined with softening, rotting, and reduction of the root system, (8) 50–75% of the hypocotyl and root tissues covered with lesions, significant 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 (PH) and dry weight (DW) 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. Analyses of variance (ANOVA) were calculated followed by multiple range tests (Tukey, $P = 0.05$) to separate the means for each fungal combination. In addition, the effects of the moisture level (ML) on some variables $y$, for instance AUPEC, PH and DW, were investigated by means of regression analyses using the following model for the combined data of the four fungal combinations on a pot basis (total 48 pots):

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

In this equation, the $d_{ij}$ are dummy variables (0 or 1) to select the fungal combinations (rt, rT, Rt or RT), and the four parameters $y_{ij}$ represent the estimated levels of the dependent variable of the four fungal combinations for moisture level 0. The coefficient $b_1$ describes the increasing effect of ML, measured in % 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*, the following model was also fitted to the combined data to assess the effects of the pathogen and the antagonist on AUPEC, PH and DW:

$$y = y_a[1 - d_R\; b_R\; (1 - d_T\; b_T)]\; (1 + b_2\; \mathrm{ML}) \qquad (2)$$

Here again $d_R$ *and* $d_T$ are dummy variables (0 or 1) describing if *R. solani* and *T. harzianum* were added to the soil. The parameter $y_a$ is an estimate of the dependent variable at moisture level 0 (ML = 0) when *R. solani* was 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* for a given moisture level can be measured by the relative increase in $y$, calculated as $E = [b_R\, b_T/(1 - b_R)]$. If $E$ is equal to 2, the dependent variable $y$ increases by 200% due to the application of *T. harzianum*. 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 $y$ such as AUPEC, PH and DW:

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

$$y = y_a[1 - d_R\; b_R\; (1 - d_T\; b_T)]\; (1 - b_2\; \mathrm{SD}) \qquad (4)$$

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 DW, decrease with the sowing depth. The analyses of variance and the regression analyses were carried out with SAS (SAS Institute, Carey, NC) and Sigma Plot (SPSS Inc., Chicago).

## Results

As the trends in each experiment and repetitions were similar, results from the second run of experiments will be emphasised.

### 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 (Table 1). The pathogen consistently reduced the emergence so that plants in the treatment Rt emerged three days later and the final emergence rate was only between 23% and 43%

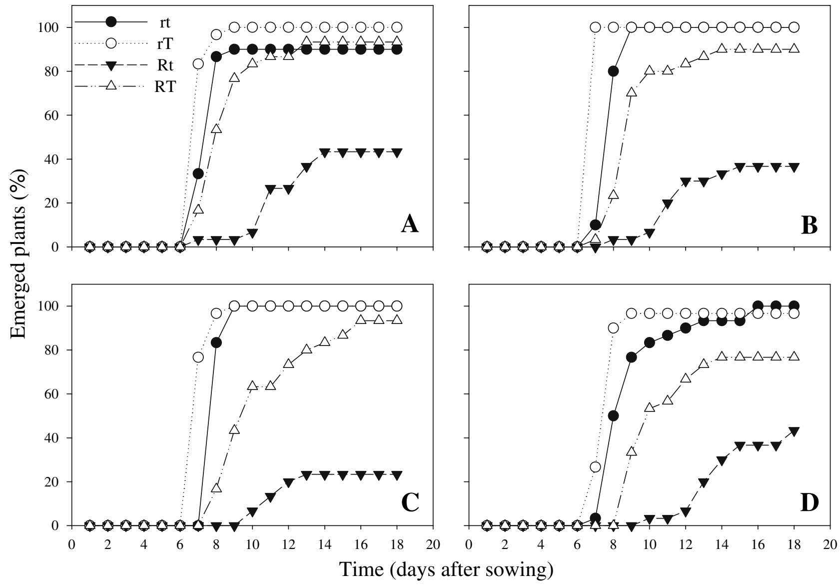


**Fig. 1** Effect of four soil moisture levels (v/v) (**A** = 42%, **B** = 32%, **C** = 23%, and **D** = 15%) on emergence of bean seedlings inoculated with *R. solani* and on biological control with *T. harzianum*. (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

**Table 1** Area under plant emergence curve (AUPEC, %-days) and root rot severity (1–9 scale) at 21 DAS for each fungal combination and soil moisture level (%, v/v)

| Soil Moisture | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | rt | | rT | | Rt | | RT | |
| | AUPEC | Severity | AUPEC | Severity | AUPEC | Severity | AUPEC | Severity |
| 42 | 104.8 a[1] | 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 |

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

(Fig. 1). Differences in emergence rate between treatments Rt and RT were dramatic, indicating that the antagonist protected the seedlings from pre-emergence damping-off in the presence of *R. solani* (Fig. 1). The % of emerged plants 18 days after sowing in the treatment RT varied from 77% to 93%. Root rot and hypocotyl symptoms were observed in all plants inoculated with *R. solani* (treatments Rt and RT). In spite of the biological control provided by *T. harzianum*, the severity indexes were always >7 for plants inoculated with both fungi (Table 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 fungal combinations were 108%, 117%, 27% and 87%-days for treatments rt, rT, Rt and RT, respectively (Table 1). The effect of the biological control resulted from the comparison of the treatments Rt and RT (Table 1 and Fig. 2). Due to the antagonist, AUPEC increased by 192%, 215%, 336% and 195%, PH by 504%, 827%, 613% and 175%, and DW by 221%, 539%, 270% and 80%, respectively for moisture levels of 42%, 32%, 23% and 15%. The statistical analyses done for each fungal combination separately showed that different moisture levels did not influence the emergence rate

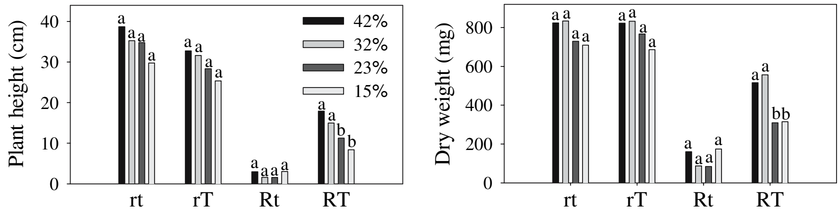


**Fig. 2** Effect of four soil moisture levels (%, v/v) on plant height and dry weight. Values of plant height and dry weight are means of three replicates. For each fungal combination, means followed by the same letter are not significantly different (Tukey, $P = 0.05$). (rt = without both fungi; rT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

and plant growth in treatment Rt (Table 1, Fig. 2). In this treatment (and also in RT) disease severity did not differ among the moisture levels.

A general trend could be observed for the effect of soil moisture levels on emergence and plant growth: at decreasing moisture levels, AUPEC and plant growth were reduced, although not always significantly. Results of ANOVA indicated that the % of plants emerged (Fig. 1) and plant growth expressed in PH and DW (Fig. 2) were not significantly affected by soil moisture in the treatment Rt. Values observed for disease severity were also similar in this treatment (Table 1). On the other hand, in the presence of *T. harzianum*, AUPEC (Table 1), PH and DW (Fig. 2) were more reduced by *R. solani* in drier soils. At decreasing soil moisture levels, AUPEC (Table 1) and plant growth (Fig. 2) were more reduced in treatment RT than in treatment rT: reductions of 16%, 23%, 29% and 37% for AUPEC, 45%, 53%, 60% and 67% for PH, and 37%, 33%, 59% and 54% for DW were observed for soil moisture contents of 42%, 32%, 23% and 15%, respectively. PH and DW were highly positively correlated with each other ($r > 0.92$; $P < 0.01$), but negatively correlated with severity ($r > -0.909$; $P < 0.01$). In the first run of the soil moisture experiment, the antagonist also improved emergence of seedlings, PH and DW of plants non-inoculated with *R. solani*.

The application of model 1 resulted in the Eqs. 5–7, that estimated AUPEC, PH and 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 % of moisture content, AUPEC, PH and DW increased by 0.48%, 1.47% and 1.1%, respectively.

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

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

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

Model 2 was also applied for the variables AUPEC, PH and DW, resulting in Eqs. 8–10. 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 showed that *R. solani* reduced AUPEC, PH and DW by 76%, 93% and 84%, respectively. However, in the presence of *T. harzianum*, the reduction of AUPEC, PH and DW by the pathogen was only 22.8%, 58.6% and 44.5%, respectively. Looking at the relative increase of the variables, the antagonistic effects are given by the values $E = 2.21$, 4.51 and 2.47 for AUPEC, PH and DW, respectively.

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

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

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

## Effect of sowing depth

The final emergence rate was again similar for treatments rt and rT (Fig. 3). Emergence took longer and pre-emergence damping-off was more severe, the deeper the seeds were sown in the treatment Rt (Fig. 3). However, deep sowing did not reduce AUPEC (Table 2) and plant growth (Fig. 4) in treatment RT. At 1.5 cm, plants in treatment Rt emerged only one day later than plants in treatment RT, and the final number of plants emerged was similar. At 6.0 cm, plants in treatment Rt emerged 3 days later than plants in treatment RT. The corresponding disease severity ratings for treatments RT and Rt were on average 8.15 and 8.7, respectively. The mean values of AUPEC in the four treatments rt, rT, Rt and RT were 119.7%, 123.9%, 26.4% and 66.2%-days, respectively (Table 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 (Table 2). Emergence and PH were in general reduced at deep sowing, but not in all treatments. In treatment Rt, plant height (Fig. 4) and DW of aerial parts of plants (data not shown) were higher and disease severity (Table 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 (Table 2) although the differences among the four sowing depths were not significant.

Model 3 applied to AUPEC, PH and DW resulted in Eqs. 11–13, which estimated the general effects of the treatments and the effect of sowing depth. The estimated parameter values for AUPEC and PH 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 significant negative effect on AUPEC and on PH leading to a reduction of 2.2% and 5.07%, respectively, for each cm depth. On the other hand, the effect of sowing depth on DW was not significant.

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

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

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

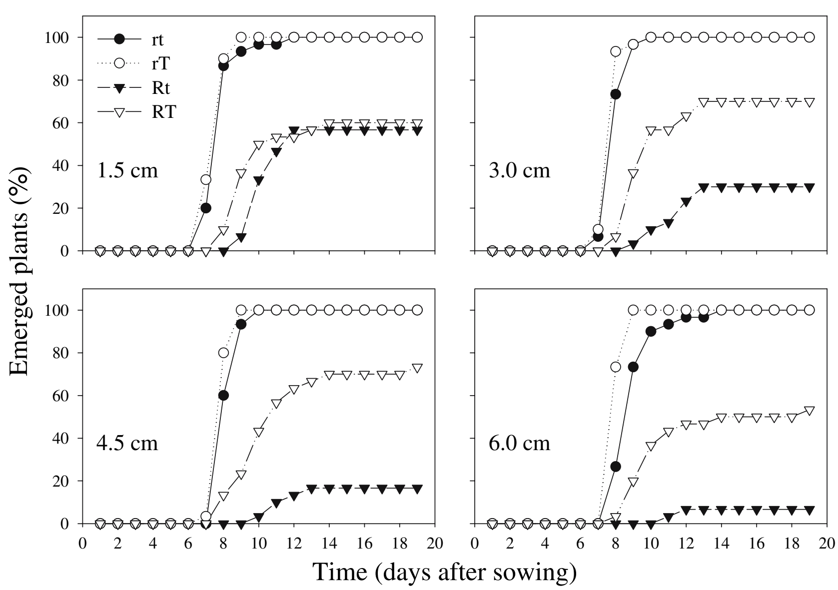


**Fig. 3** Effect of sowing depth (cm) on emergence of bean inoculated with *R. solani* and on biological control with *T. harzianum*. (rt = without both fungi; rT = without *R. solani*/with *T. harzianum*; Rt = with *R. solani*/without *T. harzianum*; and RT = with both fungi)

**Table 2** Area under plant emergence curve (AUPEC, %-days) and root rot severity (1–9 scale) at 21 DAS for each fungal combination and sowing depth (cm)

| Sowing Depth | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | rt | | rT | | Rt | | RT | |
| | AUPEC | Severity | AUPEC | Severity | AUPEC | Severity | AUPEC | Severity |
| 1.5 | 123.3 a[1] | 1.0 | 125.7 a | 1.0 | 56.8 a | 8.33 | 65.0 a | 7.97 |
| 3.0 | 122.3 a | 1.0 | 124.5 a | 1.0 | 27.5 b | 8.77 | 74.5 a | 8.07 |
| 4.5 | 120.3 a | 1.0 | 123.2 a | 1.0 | 15.2 bc | 8.80 | 72.7 a | 8.10 |
| 6.0 | 112.7 a | 1.0 | 122.3 a | 1.0 | 6.0 c | 8.93 | 52.7 a | 8.47 |

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

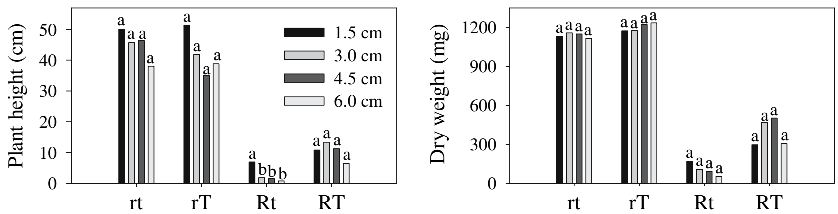


**Fig. 4** Effect of sowing depth (cm) on plant height and dry weight. Values of plant height and dry weight are means of three replicates. For each fungal combination, means followed by the same letter are not significantly different (Tukey, $P = 0.05$). (rt = without both fungi; rT = without *R. solani*/with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

In the same way, Eqs. 14–16 resulted from the application of model 4 to AUPEC, PH and DW. The estimated parameter values for AUPEC and PH 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, PH and DW by 78%, 93% and 91%, respectively. However, in the presence of the antagonist, the reduction of AUPEC, PH and DW by *R. solani* was only 46.0%, 75.3% and 66.4%, respectively. Regarding again the relative increase of AUPEC, PH and DW, the antagonistic effects are given by $E = 1.45$, 2.52 and 2.73, respectively.

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

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

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

## Discussion

The influence of soil moisture on incidence and severity of diseases caused by *R. solani* on different crops has been studied (Lewis and Papavizas 1977; Shehata et al. 1984; Teo et al. 1988; Huissman 1988; Dorrance et al. 2003), but there is no general agreement whether high or low soil moisture favours the fungus. For beans, contradictory results have been published. 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 decreased in the field, when early irrigation was reduced. Abawi and Pastor-Corrales (1990) 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) and Fenille and Souza (1999) found that soil moisture levels had negligible effects on Rhizoctonia root rot in field experiments. Van Bruggen et al. (1986) reported that disease incidence did not depend on soil moisture; however, the largest lesions developed at lower moisture levels (−0.95 MPa), but the time period available for lesion expansion was longer. They also found that *R. solani* delayed emergence and reduced plant growth rate, particularly at low soil moisture levels. It has even been demonstrated that incidence and severity of root diseases caused by *R. solani* (Kumar et al. 1999) and the survival of the pathogen (Paula Júnior 2002) may decrease at very high soil moisture levels, apparently due to the lack of aeration (Ploetz and Mitchell 1985). Our results, especially based on Eqs. 5–10, indicated that increasing soil moisture in general enhanced AUPEC and plant growth, although different moisture levels did not influence the emergence rate and plant growth in treatment Rt for each fungal combination separately.

The reasons for the contradictory results could be the use of ambiguous terms to quantify the soil water status (Ploetz and Mitchell 1985) and the failure to distinguish between the effects of moisture tension and aeration (Shehata et al. 1984). Moreover, the results may differ depending on the *R. solani* anastomosis group (Teo et al. 1988). Chemical, physical and biological characteristics of the soil and different methods of experimentation may drastically affect results of experiments involving soil moisture and activities of soilborne pathogens.

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). 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 (Van Bruggen et al. 1986; Teo et al. 1988), since bean plants emerge more rapidly at high temperatures and thus escape infection.

There is a general agreement in the literature that 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 seeds 2.5 cm deep compared to 7.5 cm (Manning et al. 1967). In California, a 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 >6 cm (Sweetingham 1996). The results presented here also support the promoting effect of deeper sowing on bean root rot, which can be explained by constraints in seedling emergence. Rosa (1990) found no effects of different depths on % 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. The effect of sowing depth on DW was not significant because the root system of plants from deeply-sown seeds was longer. PH and DW of aerial parts of plants (data not shown) in treatment Rt were highest at 1.5 cm, and disease severity lowest. Severity of root rot increased with higher sowing depth. Deep sowing extends the period of seedling emergence, which favours seedling contact with *R. solani* and increases the probability of damping-off and root rot. It is known that colonization by *R. solani* is limited or slow in older hypocotyls (Manning et al. 1967). Thus, deep sowing increases the exposure of the growing hypocotyl to the pathogen.

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

The significant effect of soil moisture observed in treatment RT indicated that AUPEC and plant growth increased at high soil moisture. 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 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* spp. seemed 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. Paula Júnior (2002) observed that the antagonistic ability and survival of *T. harzianum* were affected by soil moisture, but were apparently 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 moist soil and favour *T. harzianum*. However, at very high soil moisture conditions the establishment of *T. harzianum* seemed to be reduced (Paula Júnior 2002). The combined use of *T. harzianum* and moderate soil moisture to reduce *R. solani* infection on beans under field conditions is feasible, but recommendations for moisture levels should be done for each particular situation. 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 pathogens (Silbernagel 1981).

Deep sowing did not reduce AUPEC and plant growth in the treatment RT; additionally, at 1.5 cm, % of emerged plants and PH were similar in treatments Rt and RT. However, differences between both treatments were dramatic at 3, 4.5 and 6 cm. 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 emergence and plant growth in the presence of *R. solani* even if seeds were sown 6 cm deep. These results show that integration of *T. harzianum* with shallow sowing may improve root rot control in the field.

The antagonist increased the emergence rate of plants not inoculated with *R. solani* in the moisture experiment. This effect suggests a direct effect of *T. harzianum* on the seedlings, which improves emergence. Plant growth promotion by *Trichoderma* spp. has been reported (Elad et al. 1980). Promotion of plant development by *T. harzianum* may be more relevant under stress situations that delay plant emergence, such as deep sowing. Plants not inoculated with *R. solani* emerged more rapidly in the presence of *T. harzianum*, when seeds were sown at 6 cm.

The results presented here demonstrate the potential of *T. harzianum* for use as a component of an integrated disease management programme 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 adverse conditions for soilborne pathogens emphasizes the potential of integrating various control measures such as moldboard ploughing (Lewis and Papavizas 1980), solarization (Chet et al. 1982) and sublethal doses of pentachloronitrobenzene (PCNB) (Hadar et al. 1979). 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. Moreover, *Trichoderma* species do not appear to be very competitive in non-sterilized soil (Adams 1990). For a successful application, 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 high quality seeds and the adjustment of soil water content to permit a rapid emergence of seedlings and to favour *T. harzianum*.

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