

The Potential of Biological Soil Disinfestation to Manage *Fusarium* Foot and Root Rot in Asparagus

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Abstract

In a field experiment on an abandoned asparagus field we studied the effect of Biological Soil Disinfestation (BSD) on survival of buried inoculum samples of three test pathogens (*Fusarium redolens* f.sp. *asparagi* (FRA), *Rhizoctonia tuliparum* (RT) and *Verticillium dahliae* (VD)) and on the *Fusarium* infestation level. The BSD treatments involved incorporation of grass into moist soil and covering the soil with airtight plastic. The amount of grass incorporated was varied (42, 62 or 102 tons of grass/ha) as well as the depth of incorporation (40 or 80 cm). It was found that BSD greatly reduced all three pathogens in buried soil samples and that incorporation of 62 or 102 tons of grass per ha to 80 cm soil depth resulted in a significant decrease in soil infestation in the upper 40 cm; in the deeper layer the decrease was lower. Asparagus plants grown from seed in the field for one year showed a strong decrease in *Fusarium* root rot severity with all BSD treatments. The results clearly show the potential of BSD to decrease soil infestation levels of *Fusarium* pathogens and to contribute to an enhanced life span of replanted asparagus crops.

INTRODUCTION

Early decline of asparagus (*Asparagus officinalis* L.) is a phenomenon common to all asparagus producing areas, especially in replanted fields (Elmer et al., 1996). In the Netherlands, the economic life span of an asparagus crop on virgin land is about 10 years. When old asparagus land is replanted, crop establishment is generally not a problem and the first yields are satisfactorily. However, then crop vigor declines and the number of dead plants increases rapidly, resulting in an average economic life span of only five to six years. A number of factors have been identified to contribute to early decline, including soilborne fungal pathogens such as *Fusarium* and *Phytophthora* species, pathogens attacking the shoot, autotoxic compounds present in asparagus residues, viruses, insects, and unsuitable environmental conditions (Hartung, 1987; Peirce and Colby, 1987; Elmer et al., 1996). Blok and Bollen (1996a) have studied the causal factors involved in early decline under Dutch conditions. They found that asparagus plants grown in soil with an asparagus history show reduced growth compared to plants grown in virgin soil. Using selective soil treatments they identified *Fusarium oxysporum* f.sp. *asparagi*, causing foot and root rot, as the main cause of early decline of Dutch asparagus fields. Autotoxins were shown to be very persistent in asparagus residues but the concentration was considered to be too low to cause any significant direct inhibition. Also indirect effects of these autotoxins, e.g. by increasing, either directly or indirectly, *Fusarium* foot and root rot, were found to be of minor importance (Blok and Bollen, 1996b). Later it was found that part of the isolates first identified as *F. oxysporum* f.sp. *asparagi* (FOA) belong to the closely related *F. redolens* f.sp. *asparagi* (FRA) (Baayen et al., 2000).

FOA can be present on the seed, in planting material and in the soil (Blok and Bollen, 1996c). When virgin soil is planted with asparagus, the pathogen is present in relatively low amounts limited to the upper soil layer. Following planting, soil infestation builds up and spreads to the whole rootable soil layer. After asparagus production is

terminated, FOA can survive for many years without asparagus crop, which renders crop rotation ineffective. When the field is replanted plants will be infected quickly and extensively, also at greater depth. Because Fusarium foot and root rot severity is strongly correlated with plant stress (Nigh, 1990) the effect on yield will be limited in the first years if growing conditions are optimal. However, after several years plants have become weakened by the extensive colonization by FOA and yields can no longer keep up with yields of crops on virgin land.

Management options for Fusarium foot and root rot include the use of clean seed and planting material, chemical disinfestation of planting material, optimal soil preparation to ensure unrestricted root growth and prevention of water logging and plant stress. However, the most effective strategy would be to reduce the soil infestation by FOA before replanting. However, effective methods to disinfest the soil are currently not available.

Here we report on the potential of Biological Soil Disinfestation (BSD) to contribute to the effective management of Fusarium foot and root rot in asparagus. BSD is a new method, recently developed to reduce soil infestation by persistent soilborne pathogens (Blok et al., 2000). The method involves the incorporation of easily decomposable organic matter into moist soil followed by application of airtight plastic sheeting. This procedure leads to anaerobic soil conditions and fermentation of the organic matter under which soil conditions a number of persistent soilborne plant pathogens were found to be greatly reduced (Blok et al., 2000). Most pathogens are present in the upper 20-25 cm of soil so incorporation of organic matter into this layer is sufficient for a good BSD effect. However, in the case of Fusarium foot and root rot, pathogens are present to a depth of one meter or more. Therefore, in the present study, we used an adapted BSD protocol by incorporating organic matter to a depth of 40 or 80 cm into an abandoned asparagus field in an attempt to decrease Fusarium infestation in deeper soil layers. After BSD asparagus plants were grown for one year to study the effect of BSD on soil infestation levels and plant infestation.

MATERIALS AND METHODS

Experimental Set-up and Treatments

The experiment was performed on an experimental field at Horst. In this field long-term asparagus production was terminated eight years before and the soil was still heavily infested with Fusarium root rot pathogens. The experiment consisted of six treatments in four replicates (randomized complete block design). The following treatments were applied:

1. no grass incorporation, without plastic cover (C-p)
2. no grass incorporation, with plastic cover (C+p)
3. 42 tons grass/ha incorporated 40 cm deep, with plastic cover (G_{42t,40 cm})
4. 62 tons grass/ha incorporated 40 cm deep, with plastic cover (G_{62t,40 cm})
5. 62 tons grass/ha incorporated 80 cm deep, with plastic cover (G_{62t,80 cm})
6. 102 tons grass/ha incorporated 80 cm deep, with plastic cover (G_{102t,80 cm})

Plot size was 5*6 m for treatments 1-4 and 5*12 m for treatments 5 and 6. The larger plot size was needed because heavy machinery was used for incorporation of grass to greater depth in these plots. All measurements and analyses were done in the inner 3*3 m of the plots. The grass (Italian ryegrass, *Lolium multiflorum*) was grown on the plots of treatments 3-6 during two months, then mown and part of the grass from plots of treatment 3 was then redistributed on the plots of treatment 6. The amount of root material was estimated by harvesting three plots measuring 1 m², the amounts of shoot material were determined by weighing. The grass material was incorporated using two soil operations. First, the upper 25-30 cm of soil in all plots was thoroughly mixed by rototilling. Then, the plots were rototilled to a depth of 40 cm for treatments 1-4 and to a depth of 80 cm for treatments 5-6. Finally the soil of all plots was compacted by driving over it with a heavy tractor. After these soil operations equipment was installed to

measure soil oxygen levels, soil samples were collected to test soil infestation by *Fusarium* root rot pathogens, and pathogen inoculum samples were buried. Subsequently, the field was sprinkler irrigated overnight with approx. 50 mm water and then plots of treatments 2-6 were covered with Hytileen (Klerks Plastic Industrie, Noordwijkerhout, The Netherlands), an airtight ensilage plastic, 0.125 mm thick, with a black and a white side, which was applied with the black side up. The plastic was tightened by burying the edges 10 cm deep into the soil. After 12 wk, the plastic was removed, pathogen samples were retrieved and soil samples were collected. The soil was then rototilled superficially and left fallow over winter.

Soil Oxygen Levels and Redox Potential

Soil oxygen levels and redox potentials were determined according to Blok et al. (2000). Two 10-ml gas diffusion chambers and two platinum electrodes were installed at 20 and 60 cm depth in all plots of one block.

Survival of Pathogens

Duplicate inoculum samples of three test pathogens were buried in all plots at 20 and 60 cm depth. The test pathogens were: *Fusarium redolens* f.sp. *asparagi* (FRA), *Rhizoctonia tuliparum* (RT) and *Verticillium dahliae* (VD). Inoculum production and testing of survival was done as follows.

The isolate of FRA (CWB1) was the same as used in an earlier study (Blok et al., 2000) under the name *Fusarium oxysporum* f.sp. *asparagi*. In a recent taxonomic study that involved different molecular methods (Baayen et al., 2000), this strain was reclassified as *F. redolens* f.sp. *asparagi*. Inoculum was produced by growing the fungus in Erlenmeyer flasks with two times autoclaved field soils amended with oat meal (0.5 %, dw/dw), during 3 wk at 25°C. The colonized soil was dried with forced air and stored at 4°C until use. Samples consisted of 100 g soil inoculum in nylon bags. Survival was tested by plating 10-fold dilutions of the inoculum in 0.1% water agar on Komada's medium (Komada, 1975) with four replicates of 0.25-ml aliquots per dilution. The number of colonies was counted after six days of incubation (25°C, 12 h light).

The inoculum of RT consisted of nylon bags with 100 g of potting soil in which tulips had been grown that were heavily infected by gray bulb rot caused by RT. The soil contained on average 25 sclerotia of RT per sample. After retrieval of samples from the soil, sclerotia were sieved out, externally disinfested (2 min in a 1:1 (v/v) mixture of 10% sodium hypochlorite and 96% ethanol followed by three rinses in sterile distilled water) and plated on disks (ø 12 mm, five disks per Petri dish) of malt extract agar amended with oxytetracyclin (25 mg L⁻¹). After incubation for four wk at 20°C, outgrowth of RT was scored.

Inoculum samples of VD consisted of nylon bags with 100 g of naturally infested field soil. Viability of VD was determined as follows. Samples were air dried at room temperature and 12.5 g of air-dry soil was wet-sieved through 20 and 106 µm nested sieves. The fraction retained on the 20 µm sieve was suspended in 0.08% water agar and ten replicate 0.8 ml-aliquots of the suspension were plated onto modified soil extract agar (MSEA), a semi-selective medium (Harris et al., 1993). After four wk of incubation at 20°C, soil particles were washed from the plates and colonies formed in the agar were counted under a dissecting microscope.

Just prior to the start of the experiment soil samples were collected in all plots from the depth of 0-40 and 40-80 cm using an Edelman auger. For each plot one composite sample was collected per layer, consisting of 10 randomly collected sub samples. One day after removal of the plastic, similar soil samples were collected. The infestation with *Fusarium* foot and root rot pathogens of the samples was quantified in a bioassay. For each soil sample to be tested four 1-L pots were filled and planted with four disinfested, pregerminated asparagus 'Gijnlim' seeds. Seeds were disinfested for 30 min in 0.5% NaOCl, then for 24 hr in benomyl in acetone (25 mg a.i./ml), followed by several rinses in sterile distilled water. Pots, placed on saucers, were placed in four blocks with

random placement within blocks, in a greenhouse at 20-22°C. Each pot received 100 ml of a complete nutrient solution containing 1.67 g Nutriflora-t and 2.0 g calcium nitrate per L, once every two wk. After 15 wk, plants were uprooted; roots were washed free of soil and rated for root rot severity using a disease index as described by Blok and Bollen (1996a).

Fusarium Root Rot Severity after BSD

In May of the year following the BSD treatment, asparagus 'Franklin' was sown in each plot. Per plot, three beds with each three rows were sown. After one year, the number of surviving plants was counted in the middle 3 m of the middle bed (three rows) and all plots were mechanically harvested. During harvesting of the middle 3 m of the middle bed, 30 plants were randomly collected. These plants were cleaned with a high-pressure spraying pistol and evaluated for color of the roots (1 (light, cream brown) – 3 (dark brown)), the number of storage roots, total plant fresh weight, and percentage of storage roots with one or more typical *Fusarium* root rot lesions.

Statistical Analysis

Data for survival of pathogen inoculum, disease index and percentage infected roots were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Prior to statistical analyses, data were checked for normality and homogeneity of variances, and transformed when needed. A log-transformation ($\log_{10}(x + 1)$) was applied to the data of FRA and VD, and a square root transformation ($\sqrt{(x + 0.5)}$) was applied to the data of RT. Protected LSD tests were used to evaluate the significance of pair wise comparisons.

RESULTS

Shortly after the plots were covered, the oxygen in the soil was depleted and the soil became strongly reduced (Fig. 1). Anaerobic conditions were reached even in the plots that did not receive grass. The anaerobic conditions persisted during the whole experimental period. At 20 cm depth oxygen levels were below 1-1.5 %, indicating that most of the soil was completely anoxic. At 60 cm depth, oxygen could not be measured at all (data not shown). The Eh reached a minimum of -200 mV, indicating strongly reduced soil conditions, quickly and increased to a stable level of 0 mV after five wk.

Inoculum samples of all three test pathogens buried at 20 cm depth showed significant and strong decreases in population density or viability due to BSD (Fig. 2). The differences between the four BSD treatments were only minor and generally not significant. The treatment with only plastic also showed a reduction but not as much as the BSD treatment, except for RT. For RT and VD, the results for the inoculum samples buried at 60 cm were similar to those for 20 cm. For FRA, however, the results were different. There was a strong and significant reduction for treatments $G_{62t-40cm}$ and $G_{102t-80cm}$ but, surprisingly, not for $G_{62t-80cm}$.

The results of the bioassay showed that all plots had the same infestation level before the experiment (data not shown). After the experiment, the soil infestation of all BSD treatments was clearly reduced in the upper 40 cm (Fig. 3). However, due to the limited discriminative power of the bioassay, the reduction caused by $G_{42t-40cm}$ and $G_{62t-40cm}$ was not significant. Treatments $G_{62t-80cm}$ and $G_{102t-80cm}$ caused an almost complete elimination of the pathogens in the upper layer. These treatments caused also a reduction in infestation level of the 40-80 cm layer, although this was not significant.

The reduction of soil infestation resulted in a strong reduction of root rot severity of asparagus plants grown for one year in the treated soil (Table 1). Root rot severity was significantly reduced for all BSD treatments with the highest reduction in the plots where the grass was incorporated to 80 cm and a slightly lower reduction when the grass was incorporated to 40 cm. The number of storage roots per plant and plant weight was not significantly different among the treatments. The color of the root system was strongly correlated with the number of lesions.

DISCUSSION

The dynamics of soil oxygen concentration and redox potential observed in this experiment was similar to what was found in other field experiments with BSD in which organic matter was incorporated into the upper 25-30 cm of soil (Blok et al., 2000; Blok et al., unpublished). Strong reductions were found for the buried inoculum samples of the three test pathogens, confirming the potential of BSD to control a range of persistent soilborne plant pathogens. From the results for the samples buried at 60 cm it can be concluded that BSD has the potential to also inactivate pathogens in deeper soil layers, which is relevant for a number of deep rooting crops, including asparagus, perennial fruit crops and woody ornamentals. The results for the samples buried at 60 cm also show that the test pathogens differ in sensitivity to BSD; RT being most sensitive followed by VD and FRA. The high survival of FRA at 60 cm in treatment G_{62t-80cm} was unexpected and is hard to explain as survival of the other two pathogens was not higher in these plots and also the bioassays did not show a lower BSD effect on *Fusarium* infestation for the 40-80 cm layer.

The results for the inactivation of the buried inoculum samples of FRA correspond very well with the results of the bioassay. This indicates that the type of inoculum used in the buried samples was relevant for testing the capacity of FRA to survive in the soil under field conditions. The results of the bioassay show that incorporating 60-100 tons grass to a depth of 80 cm results in an almost complete disinfestation of the upper 40 cm. The pathogens were reduced in the 40-80 cm layer as well but here the effect was much less. This is related to the fact that in this layer less grass was present than in the upper layer. The rototilling brought part of the grass to the deeper layer but the distribution was by far not homogeneous as we observed after digging some profile pits. If one would be able to incorporate more easily decomposable organic matter into the deeper layer, the disinfestation effect in this layer could be improved. This is probably hard to achieve with grass and using the standard farm equipment. However, we are not bound to grass. All easily decomposable organic materials will produce anaerobic conditions with BSD and we could try to find a way to introduce liquid organic wastes homogeneously into the whole soil profile.

A further improvement could be to use other types of plastic that are more oxygen tight and can more easily be applied by machines. We have obtained some promising results with special barrier films that result in a higher efficacy of BSD as compared to the ensilage plastic used in this study (Lamers et al., 2004).

The fact that the upper soil layer was disinfested almost completely but that the pathogen survived rather well in the deeper soil layer brings up the question whether inoculum in the deeper layers will not recolonize the upper layer quickly thus nullifying the disinfestation effect in a short time. The root rot severity of the asparagus plants grown in the plots after BSD correlated very well with the infestation levels found in the bioassay, which indicates that in the first year following BSD no rapid recolonization by *Fusarium* root rot pathogens took place. In an earlier study (Blok et al., 2000) we showed already that the disease suppressiveness against FRA of soil collected immediately after BSD treatment is not affected by BSD. In yet another study (Goud et al., 2004) we followed the *Verticillium* wilt incidence in two tree species during four year following BSD treatment. Also in this study we found no indication of an enhanced recolonization of the pathogen in BSD soil. The explanation for this is probably that in contrast to soil steaming and some chemical soil disinfestants, BSD does not create a biological vacuum but rather a shift from an aerobic microflora to an anaerobic microflora and back again thus maintaining the natural microbial buffering of the soil against pathogen invasions.

Although we produced one-year-old asparagus plants in this study, we do not want to suggest that planting materials should be produced on BSD treated old asparagus land. As we have shown, *Fusarium* pathogens are not completely inactivated and, therefore, clean planting material can not be produced on old asparagus land, even not after BSD. We propose that BSD is further tested for its effect on the economic life of asparagus crops on former asparagus land. We have shown that the *Fusarium* infestation of the

upper layer can be well reduced and may expect that this will result in significantly lower infection levels of stem bases, rhizomes and a large part of the storage roots during at least the first years after planting, and ultimately in an extended economic crop life.

It might be good to realize that after a successful BSD treatment the *Fusarium* populations will be significantly decreased but will build up again after planting the next asparagus crop. This indicates that for a sustainable management of Fusarium foot and root rot we need to do more than just decreasing the soil infestation levels from time to time. We need to find ways to restrict or prevent the build-up of pathogenic *Fusarium* species to damaging levels. There are many studies that show that by regular additions of good quality composts and the introduction of biocontrol agents the capacity of the soil to restrict pathogen activity can be greatly enforced (Hoitink and Boehm, 1999). Also for the control of Fusarium pathogens in asparagus promising results have been obtained (Blok et al., 1997; Elmer, 2004; He et al., 2002) and this is certainly a strategy that is worth to be developed further, in combination with BSD.

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Literature Cited

- Baayen, R.P., Van den Boogert, P.H.J.F., Bonants, P.J.M., Poll, J.T.K., Blok, W.J. and Waalwijk, C. 2000. *Fusarium redolens* f.sp. *asparagi*, causal agent of asparagus root rot, crown rot and spear rot. Eur. J. Plant Pathol. 106:907-912.
- Blok, W.J. and Bollen, G.J. 1996a. Etiology of asparagus replant-bound early decline. Eur. J. Plant Pathol. 102:87-98.
- Blok, W.J. and Bollen, G.J. 1996b. Interactions of asparagus root tissue with soil microorganisms as a factor in early decline of asparagus. Plant Pathol. 45:809-822.
- Blok, W.J. and Bollen, G.J. 1996c. Inoculum sources *Fusarium oxysporum* f.sp. *asparagi* in asparagus production. Annals of Applied Biology 128:219-231.
- Blok, W.J., Zwankhuizen, M.J. and Bollen, G.J. 1997. Biological control of *Fusarium oxysporum* f.sp.*asparagi* by applying nonpathogenic isolates of *F. oxysporum*. Biocontrol Science and Technology 7:527-541.
- Blok, W.J., Lamers, J.G., Termorshuizen, A.J. and Bollen, G.J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253-259.
- Elmer, W.H. 2004. Combining nonpathogenic strains of *Fusarium oxysporum* with sodium chloride to suppress *Fusarium* crown rot of asparagus in replanted fields. Plant Pathol. 53:751-758.
- Elmer, W.H., Johnson, D.A. and Mink, G.I. 1996. Epidemiology and management of the diseases causal to asparagus decline. Plant Dis. 80:117-125.
- Goud, J.K., Termorshuizen, A.J., Blok, W.J. and Bruggen, A.H.C. van. 2004. Effect of biological soil disinfestation on verticillium wilt in tree nurseries and on inoculum density-disease incidence relationships. Plant Dis. 88:688-694.
- Harris, D.C., Yang, J.R. and Ridout, M.S. 1993. The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathol. 42:238-250.
- Hartung, A.C. 1987. Allelopathic potential of asparagus (*Asparagus officinalis* L.). PhD thesis Michigan State University, Michigan, USA.
- He, C.Y., Hsiang, T. and Wolyn, D.J. 2002. Induction of systemic disease resistance and pathogen defence responses in *Asparagus officinalis* inoculated with nonpathogenic strains of *Fusarium oxysporum*. Plant Pathol. 51:225-230.
- Hoitink, H.A.J. and Boehm, M.J. 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu. Rev. Phytopathol. 37:427-447.

- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.
- Lamers, J.G., Wanten, P. and Blok, W.J. 2004. Biological soil disinfestation: a safe and effective approach for controlling soilborne pests and diseases. Agroindustria 3:289-291.
- Nigh, E.L. 1990. Stress factors influencing *Fusarium* infection in asparagus. Acta Hort. 271:315-322.
- Peirce, L.C. and Colby, L.W. 1987. Interaction of asparagus root filtrate with *Fusarium oxysporum* f.sp. *asparagi*. J. Amer. Soc. Hort. Sci. 112:35-40.

Tables

Table 1. Severity of *Fusarium* root rot of one-year-old asparagus plants grown from seed in all plots of the experimental field after application of BSD. Means with similar letters do not differ significantly from each other (Protected LSD test; $P < 0.05$).

BSD treatment	Grass incorporation		Plastic cover	<i>Fusarium</i> severity (% root with lesions)
	amount (t/ha)	depth (cm)		
C-p	0	-	No	60.6 a
C+p	0	-	Yes	43.2 ab
G _{42t-40cm}	42	40	Yes	27.0 bc
G _{62t-40cm}	62	40	Yes	25.3 bc
G _{62t-80cm}	62	80	Yes	14.8 c
G _{102t-80cm}	102	80	Yes	13.8 c

Figures

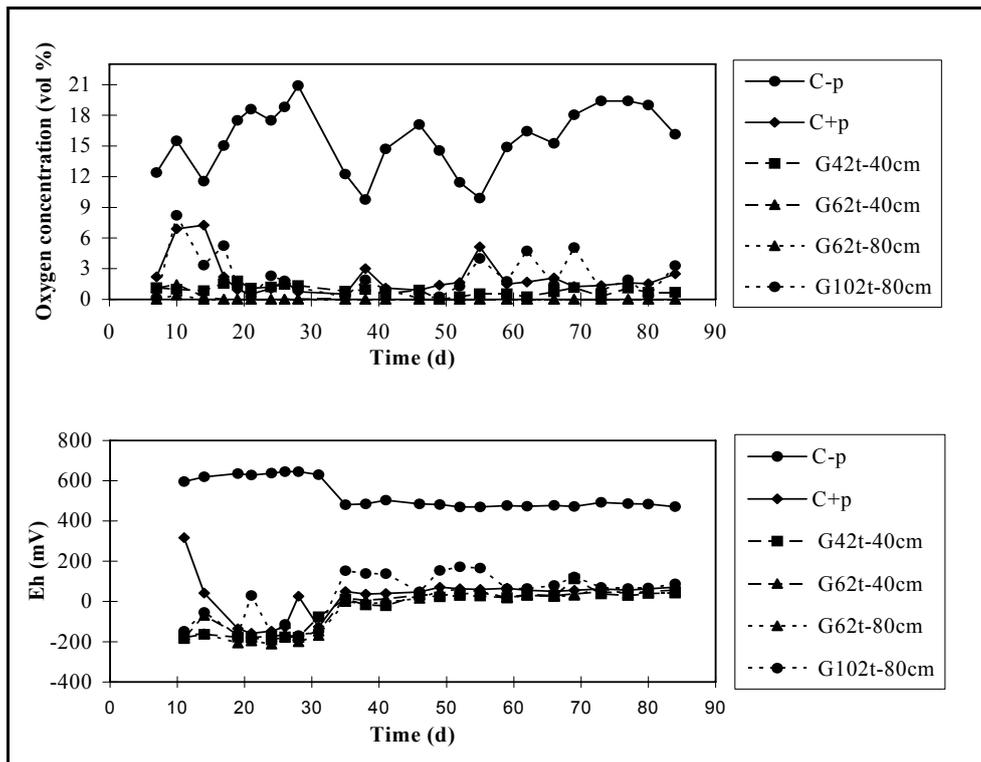


Fig. 1. Oxygen concentrations (upper figure) and redox potential (Eh) (lower figure) in time, measured at 20 cm soil depth in one plot of each treatment.

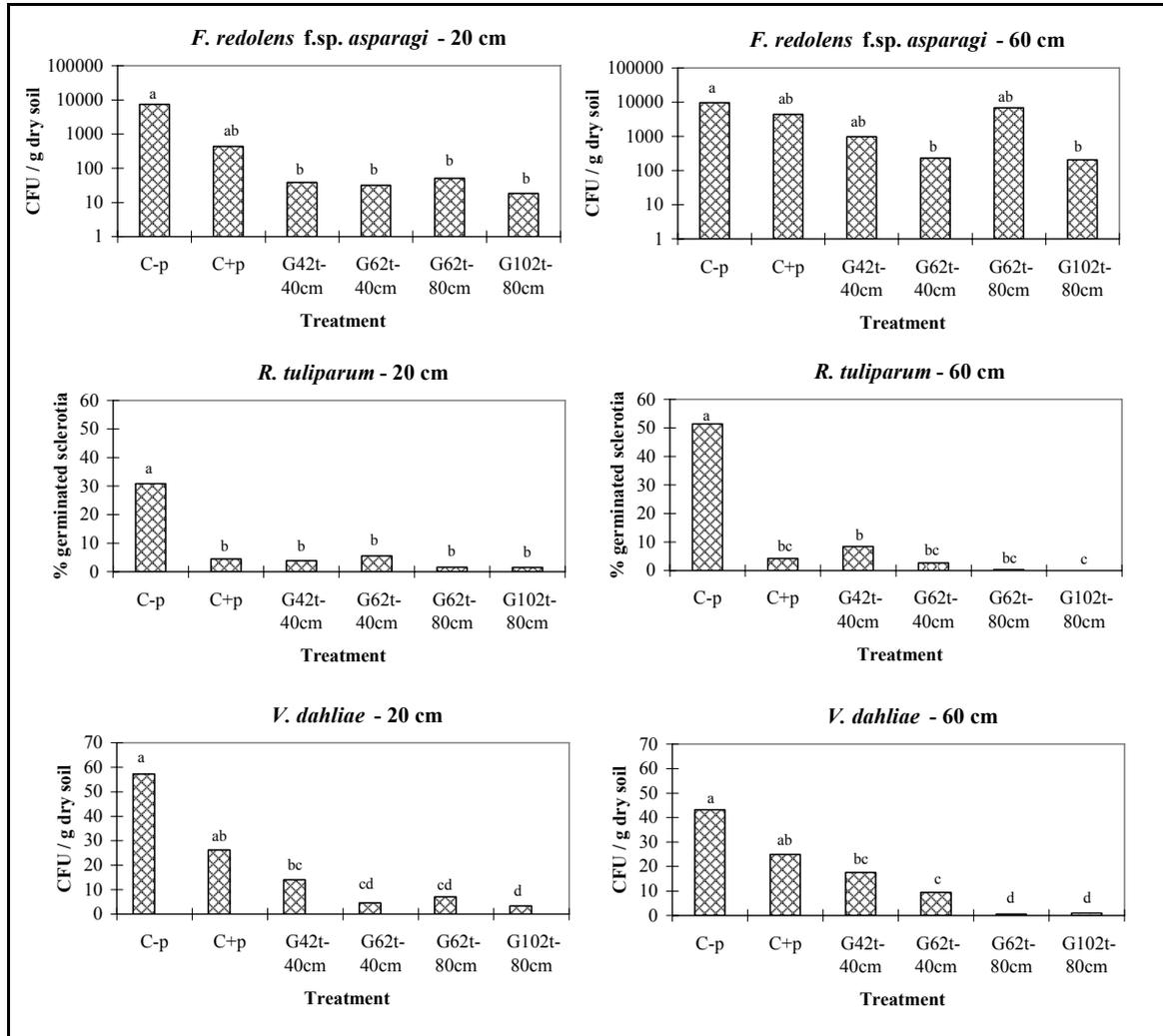


Fig. 2. Population density (*Fusarium oxysporum* f.sp. *asparagi* and *Verticillium dahliae*) and percentage germinated sclerotia (*Rhizoctonia tuliparum*) of inoculum samples that had been buried at 20 or 60 cm depth in soil. The height of the bars represents backtransformed means. Treatments with similar letters do not differ significantly from each other (Protected LSD test; $P < 0.05$).

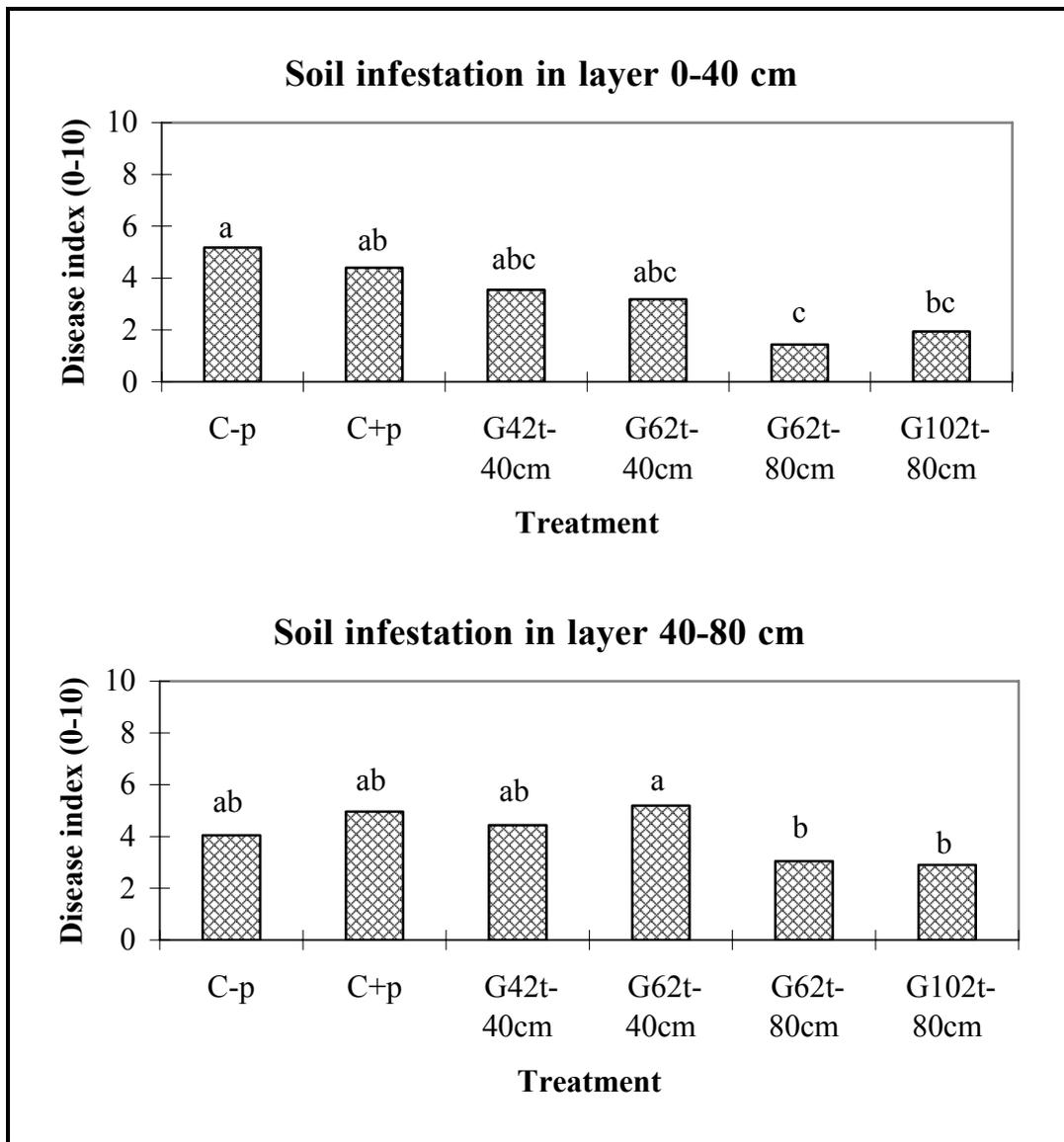


Fig. 3. Infestation of the soil after application of BSD as quantified in a bioassay. Treatments with a common letter do not differ significantly (Protected LSD test; $P < 0.05$).