European Journal of Plant Pathology (2007) 117:45–55
DOI 10.1007/s10658-006-9067-7

Are field populations of arbuscular mycorrhizal fungi able to suppress the transmission of seed-borne *Bipolaris sorokiniana* to aerial plant parts?

Johanna Sjöberg[1], Anna Mårtensson[2], and Paula Persson[1,*]
[1]*Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, Uppsala, SE-750 07, Sweden;* [2]*Department of Soil Sciences, Swedish University of Agricultural Sciences, Box 7014, Uppsala, SE-750 07, Sweden* **Author for Correspondence (Phone: +46-18-672358; Fax: +46-18-672890; E-mail: paula.persson@vpe.slu.se)*

Accepted 22 September 2006

Key words: biological control, common root rot, *Glomeromycota*, *Hordeum vulgare*, seedling blight, spot blotch

Abstract

The development of seed-borne *Bipolaris sorokiniana* in barley in the presence of arbuscular mycorrhizal fungi was studied. To exploit natural variation in their ability to control disease development, arbuscular mycorrhizal fungi from various Swedish arable soils were multiplied in trap cultures using a mixture of plant species. Six out of eight trap culture soil inocula were able to reduce transmission of *B. sorokiniana* from seeds to stem bases when grown together with infected barley seed. Based on this result two soil inocula, of different origin, from semi-natural grassland and barley respectively, were chosen for further greenhouse studies. Both soil inocula gave significant reductions in pathogen transmission from seeds to seedlings compared to the untreated control. In addition, treatment with spore inocula, collected from the different trap culture soils, showed disease suppression. Treatment with spores from the pure culture *Glomus intraradices* gave significant reduction in leaf lesion development. A treatment with the commercial inoculum Vaminoc® was included and gave some suppression of the pathogen. In conclusion this study has shown that AM soil inocula from trap cultures suppressed the transmission of seed-borne *B. sorokiniana* in the aerial parts of barley plants.

Introduction

Bipolaris sorokiniana. (syn. *Helminthosporium sativum*), teleomorph *Cochliobolus sativus* is a widespread pathogenic fungus that causes different diseases, i.e. common root rot, spot blotch, seedling blight and crown rot in wheat (*Triticum aestivum*) and barley (*Hordeum vulgarae*) (Sivanesan and Holliday, 1981). The pathogen can be transmitted by seed, be soil-borne or dispersed as conidia by air currents or rain splashes and is thus a serious problem that is difficult to control in agricultural production (Sivanesan and Holliday, 1981). Severe yield losses of up to 100% due to *B. sorokiniana* infection of wheat have been reported from sub-tropical areas (Saari, 1998). Also in temperate regions with intense barley cultivation, it has been identified as an important pathogen (Jørgensen, 1974; Whittle, 1977), with yield losses of 10–15% being reported (Stack, 1982; Kurppa, 1985; Forsberg, 2004).

In order to control common root rot in barley, a search for resistance has been initiated, but no complete resistance has so far been found (Duczek, 1984; Almgren et al., 1999). Disease control has therefore, in intensively mechanised cereal production, been achieved by seed treatment with synthetic fungicides. Attempts to find alternatives to fungicides include studies of biological control. For

example Knudsen et al. (1995) found that isolates of different saprophytic fungi inoculated on barley seeds acted as antagonists towards *B. sorokiniana*. In addition to this promising observation a field study was conducted where treatment of barley seed with *Idriella bolleyi* was found to decrease the disease level and improve yield (Duczek, 1997). Furthermore, *I. bolleyi* inoculated onto barley seeds was shown to systemically induce resistance in plants against subsequent infection by *B. sorokiniana* (Liljeroth and Bryngelsson, 2002).

Several reports have shown that arbuscular mycorrhizal (AM) fungi are promising candidates for biological disease control (Dehne, 1982; Borowicz, 2001; Whipps, 2004). For example, a number of AM fungal species have been found to suppress soil-borne pathogens, e.g. root rot caused by *Fusarium oxysporum* f. sp. *asparagi* in asparagus (*Asparagus officinalis*) (Matsubara et al., 2001) and root necroses due to *Rhizoctonia solani* in cowpea (*Vigna unguiculata*) (Abdel-Fattah and Shabana, 2002). Under field conditions, AM fungi were found to suppress the production of oospores but not the vegetative stage of *Aphanomyces euteiches* in pea, although the symptoms of the disease were not affected (Bødker et al., 2002).

Furthermore, Thompson and Wildermuth (1989) found that AM fungal root colonisation of crop and pasture species was negatively correlated with root infection by *B. sorokiniana*. This interesting observation indicates that AM fungi may reduce root infection by *B. sorokiniana*. However, so far no studies have been conducted on the influence of AM fungi on the development of *B. sorokiniana* on aerial plant parts. In this paper we report studies on the transmission of *B. sorokiniana* from barley seeds to the developing plants, after treatment with AM fungi.

Materials and methods

Production of AM fungal inocula in trap cultures

AM fungal populations intended for interaction studies were multiplied in trap cultures by vertically placing 12 soil cores (Ø2.5 cm × 15 cm) collected from arable fields onto trays (26 cm × 36 cm × 20 cm) containing a sand/silt mixture (described below). Each tray represented soil from a particular geographical site and also represented different cultivation practices (Table 1). A mixture of Alexandrian clover (*Trifolium alexandrinum*), corn (*Zea mays*), leek (*Allium porrum*), marigold (*Tagetes erecta*), pea (*Pisum sativum*), sunflower (*Helianthus annuus*), tomato (*Lycopersicon esculentum*), wheat (*Triticum aestivum*) and white clover (*Trifolium repens*) was grown at the same density in each tray (approx. 16 plants dm^{-2}). At maturity, plants were cut 1 cm above the soil surface and new seeds were sown. The AM fungal communities that developed were collected from the rhizosphere of maturing trap plants for further use in the experiments by sampling portions of the sand/silt mixture while avoiding the original soil cores. The sand/silt mixture was collected, with a spoon, from different parts of the tray and transferred to different pots in the following experiments. This was possible since the soil cores had the same positions in all trays and in addition there was a visible difference between the soil cores and the sand/silt mixture. The inocula from the trap cultures used in Experiment 1 were wet-sieved and checked for their content of AM fungal spores. The spore densities were as follows (spores g^{-1} sand/silt mixture): >0–0.1 in inocula from a semi-natural grassland, two leys and a wheat field (Table 1; trap cultures 13, 27, 32, 43); 0.5–1 in inocula from a barley field and a semi-natural grassland (Table 1; trap cultures 6, 29); and 2–3 in inocula from a wheat field and a barley field with undersown ley (Table 1; trap cultures 38, 41). A commercial inoculum Vaminoc® (Becker Underwood, Littlehampton, UK), which consists of a mixture of AM fungal isolates, was included in Experiment 2. In Experiment 3, where mixtures of AM fungal spores from different soils were used, the trap cultures were kept dry for 6 months after the third crop of plants before AM fungal spores were collected by wet-sieving in 0.85 and 0.063 mm sieves. The spores were picked up with the plastic tip of a pipette from both sieves and collected in a test tube. All trap cultures included one dominant spore type, different for different origins (although some might have been the same species), and occasionally one or two minor spore types. The collected spores were surface-washed to reduce the amount of bacteria by rinsing five times in autoclaved tap water. The spores from each trap culture were spread onto a Petri dish and viable looking spores (not floating, not contaminated by saprophytes, etc) were selected for the trial. The commercial *in vitro* cultured AM fungal species *Glomus intraradices* (BCCM™/MUCL,

Table 1. Origin of the soils used in the development of arbuscular mycorrhizal (AM) fungal trap cultures and two commercial inocula

Trap culture no.[a]	Soil sampling location	Crop at sampling	Used in experiment
3	Öjebyn	Barley[b]	3
6	Röbäcksdalen	Barley	1
12	Ås	Barley	3
13	Nästmyren	Semi-natural grassland[c]	1
23	Lyckos gård	Wheat	3
27	Fors	Ley	1
29	Andersby ängsbackar	Semi-natural grassland[c]	1, 2
32	Vreta kloster	Wheat	1
33	Lanna	Barley	3
38	Borgholm	Wheat	1, 3
40	Solmarkagård	Triticale[b]	3
41	Solmarkagård	Barley with undersown ley[b]	1, 2, 3
42	Alnarp	Barley	3
43	Alnarp	Ley	1
Vaminoc®[d]	No information available	No information available	2
G. intraradices[e]	Québec, Canada	Tree plantation	3

The cultures were used for interaction studies with *Bipolaris sorokiniana* in barley as follows: Experiment 1 – screening of AM fungal soil inocula; Experiment 2 – testing of two selected soil inocula of AM fungal trap cultures on barley growth and transmission of *B. sorokiniana* to seedlings; Experiment 3 – influence of spore mixtures of AM fungi on barley growth.
[a]The numbers refer to sampling sites in Sweden as described by Sjöberg et al. (2004).
[b]Organically managed field.
[c]Pastures or cutting fields with self-seeding, no disturbance by ploughing and no external inputs of fertilisers or pesticides.
[d]Commercial Inoculum, Becker Underwood, Littlehampton, UK. Inert expanded clay granules are carriers for the AM fungal units (spores, mycelia and colonised root fragments).
[e]Commercial inoculum, BCCM™/MUCL, Belgium, culture no. 43194.

Belgium, culture no. 43194) was also included in Experiment 3.

Conditions for growth of plant material

In all three experiments, seeds of barley (cv. Bartok) were used. After germinating 100 seeds in a humid chamber for 1 week, the degree of natural infection by *B. sorokiniana* was determined to be 95%. After hot humid air treatment of the seeds (Forsberg, 2004), the infection level was reduced to 50%. Different levels of seed infection were created by mixing the hot humid air-treated seeds with untreated seeds, thus creating infection levels of 54%, 72% and 95%. This was done in order to detect possible differences in the effect of AM fungi due to the infection level of the seeds.

Barley plants were grown in a greenhouse (12/12 h day/night artificial light in addition to natural light and 20/15 °C day/night temperature cycle; light source Osram HQI-T 400 W, Philips HPI-T, 400 W). A sand/silt mixture was used in proportions of 2:1 (v:v) as growth medium. The silt originated from an arable field (Sala, Sweden) and was sterilised by β-irradiation (2×26.0 kGy) by Gammaster® Sweden AB and the sand was pure silica (Silversand 90, Askania, Gothenburg, Sweden). Pots were randomly rearranged twice a week and watered regularly and carefully to prevent spores or fungal hyphae from splashing between pots. Nutrients were added as quarter strength Hoagland's solution with the following elements; N, P, K, Ca, Mg, S, B, Na, Cl, Mn, Fe, Cu, Zn and Mo. The height of the plants was measured from the seed to the top of the tallest leaf.

Experiment 1: Screening of AM fungal soil inocula from AM fungal trap cultures on barley growth and transmission of B. sorokiniana *to seedlings*

Soil inocula from trap cultures of eight AM fungal field populations multiplied in the greenhouse and representing different geographical sites and cultivation practices were included in a screening (Table 1). Ten barley seeds with seed-borne

B. sorokiniana (infection level 95%) were sown in pots (Ø12 cm × 9 cm) filled with 0.12 l sand at the bottom and 0.3 l of the sand/silt mixture (soil inoculum) from the AM fungal trap cultures added on top (six replications). After 3 weeks in the greenhouse, the plants in half of the pots were harvested. The remaining plants were harvested after 5 weeks. Nutrients (see above) were added regularly to correspond to the increasing demands as the plants were growing. At harvest, the number of living plants in each pot was noted, as well as the height of the plants. The stem bases were incubated in a moist chamber for identification of *B. sorokiniana* and the result expressed as the proportion of healthy stem bases of living plants.

Experiment 2: Further testing of two selected soil inocula of AM fungal trap cultures on barley growth and transmission of B. sorokiniana *to seedlings*

Ten seeds of barley were sown in pots (Ø12 cm × 9 cm) with eight replications for each of two AM fungal field populations and a non-AM fungal control. A commercial inoculum (Vaminoc®, Becker Underwood, Littlehampton, UK) with a non-AM fungal control was tested in a separate experiment with the same number of seeds, pots and replications. All five treatments were carried out with three different levels of *B. sorokiniana* seed infection (54%, 72% and 95%). The AM fungal field populations used were fungi from semi-natural grassland and from a barley field with undersown ley (Table 1; trap cultures 29 and 41), multiplied in trap cultures in the greenhouse. The reason for choosing these two inocula from the trap cultures were that in the screening (Experiment 1) the former resulted in a high number of living plants at harvest and the latter resulted in a high proportion of healthy stem bases. The base of each pot was covered with 0.3 l light expanded clay aggregates (12–20 mm; AB Svensk Leca, Linköping, Sweden) mixed with 0.2 l sand. For AM fungi from the field populations, 0.2 l of the sand/silt mixture (soil inoculum) from the trap cultures was added on top of the clay aggregate/sand mixture. The control for these treatments (Control I) consisted of trays of the sand/silt mixture in which plant mixtures were grown, but without added soil cores with AM fungi. For the Vaminoc® treatment, 0.025 l of Vaminoc® were mixed with 0.175 l of the sand/silt mixture and added on top of the clay aggregate/sand mixture. The control for this treatment (Control II) consisted of additional sand/silt mixture instead of Vaminoc®. The plants were incubated in the greenhouse with nutrients added as in Experiment 1. After 6 weeks, the plants in three pots per treatment were harvested. The plants in the remaining five pots were harvested when plants were 12 weeks old. At both harvests, the number of living plants in each pot was noted, as well as the height of the plants. The stem bases (2 cm above the kernel), nodes and leaf lesions were placed in a humid chamber for identification of *B. sorokiniana* infection.

Experiment 3: Influence of spore mixtures of AM fungi on barley growth and transmission of B. sorokiniana *to seedlings*

To avoid possible interference from other soil micro-organisms, an experiment was set up with spore mixtures as inoculum collected from the AM fungal field populations multiplied in trap cultures. The AM fungal field populations used were fungi from a barley field with undersown ley (Table 1; trap culture 41) and in addition AM fungi from seven other cereal fields from different geographical locations were tested (Table 1). In addition, *in vitro* multiplied spores of *Glomus intraradices* were included. Barley seeds were pre-germinated on moist filter paper for 4–6 days (Munktell Filter AB, Grycksbo, Sweden). Seedlings (10 replications per AM fungal source) with slight brownish coleoptiles caused by *B. sorokiniana* infection were placed in individual plastic trays (5 ml, 31 mm × 53 mm × 5.3 mm) to allow for close contact between the roots and the spores. The roots were simultaneously inoculated with 20 randomly picked, individual AM fungal spores, representing one soil, in this study termed spore mixture inoculum. In trap cultures with few spores, all viable-looking spores were selected. The roots were covered with the sand/silt mixture and watered. Each plastic tray was then placed on the surface of a pot (Ø16 cm × 12 cm) filled with 1.2 l of waterlogged light expanded clay aggregates and covered with a 2 cm layer of the light expanded clay aggregates. The pots were placed in the

greenhouse and the roots were allowed to emerge from the plastic tray into the surrounding clay aggregates. Nutrients (as before) were added twice a week, in increasing amounts as the plants were growing. Six weeks after transplanting, the plants were harvested. Leaf parts, 3 mm at the base of each leaf (referred to as leaf base) and the upper half of each leaf were placed separately in a humid chamber for identification of *B. sorokiniana*. The leaf bases, instead of the nodes as in Experiment 2, were chosen to monitor the transmission of *B. sorokiniana* upwards in the plant, as the nodes were not developed on the young plants. At harvest the height of the plants was measured as before.

Quantification of AM fungal colonisation

The roots were cold-stained according to Koske and Gemma (1989) in order to study the growth of the AM fungi and the expansion of root necrosis due to the pathogen down in the root system. Roots from each plant were first cut by scissors at three levels to divide the root systems into four parts according to their position relative to the seed. The roots were carefully rinsed in water and placed in process and embedding cassettes (Histolab Products AB, Västra Frölunda, Sweden). The roots were immersed in 20% potassium hydroxide solution for 1 day, washed in tap water and acidified with 1% hydrochloric acid. Following this, roots were stained in 0.05% trypan blue in a destaining solution (14:1:1 lactic acid:glycerol:water) for 1 day, followed by destaining in a solution without trypan blue until visual observations were made. For examination, the roots were spread onto Petri dishes with destaining solution and the root length colonised by AM fungi was visually estimated under a binocular microscope. Results are expressed as percent-colonised root length out of the total root system (Giovannetti and Mosse, 1980).

Quantification of B. sorokiniana *infection*

For quantification of *B. sorokiniana* infection, barley seeds and parts of stems and leaves were placed on moist filter paper (Munktell Filter AB, Grycksbo, Sweden) in a bowl covered with glass. The samples were irradiated with a mixture of NUV light (360 nm) and fluorescent light, 12 h/12 h light at 24 °C (humid chamber). After 1 week, the plant parts were studied using a binocular microscope. The presence of *B. sorokiniana* was determined by registration of the development of typical black, shiny conidia (Kurppa, 1984). In Experiment 3, the extent of necrosis on the roots due to the pathogen was also recorded. This was done by scoring the percentage of the root tissue that remained dark blue after destaining (see method below) into the following categories; 0–5%, 6–25%, 26–50%, 51–75% and 76–100%. The scoring was done using a binocular microscope.

Statistical analyses

Statistical analysis of the data was performed using the SAS Statistical Programmes, release 8 (SAS Institute Inc., USA). The data assumed to follow a normal distribution was analysed by PROC GLM and data assumed to follow a binomial distribution was analysed by PROC GENMOD (logistic regression). Means from PROC GLM were separated by LSD-values, and for logistic regression, odds ratios were calculated. Hypotheses were rejected at the 5% level.

Results

Experiment 1: Screening of AM fungal soil inocula from AM fungal trap cultures

The screening of AM fungal soil inocula from eight AM fungal trap cultures revealed differences between cultures with soil originating from different fields (Table 2). Soil inocula from six trap cultures resulted in significantly healthier stem bases compared to the control. The two soil inocula, which gave the healthiest stem bases, originated from trap cultures with a soil from an organically managed barley field with undersown ley and a soil from a conventionally managed barley field (Table 1; trap cultures 41 and 43). Soil inoculum from trap culture 29 resulted in a higher proportion of living plants compared to the control and all other soil inocula (Table 2). This soil inoculum came from a trap culture with soil originating from semi-natural grassland (Table 1). When using plant height as a criterion for choosing candidates for further studies, an average of

Table 2. Influence of soil inocula from different arbuscular mycorrhizal (AM) fungal trap cultures on seed-borne *Bipolaris sorokiniana* in barley

AM soil inocula culture no.[a]	Living plants[b] (%)	AM soil inocula culture no.[a]								
		Healthy stem bases[c] (%)								
		Control[d]	06	13	27	29	32	38	41	43
		20	58	53	45	30	57	32	71	64
Control[d]	73		***	**	*	NS	***	NS	***	***
6	76	NS		NS	NS	**	NS	*	NS	NS
13	62	NS	NS		NS	*	NS	NS	NS	NS
27	72	NS	NS	NS		NS	NS	NS	*	NS
29	95	*	*	**	*		*	NS	***	**
32	62	NS	NS	NS	NS	**		*	NS	NS
38	69	NS	NS	NS	NS	**	NS		***	**
41	63	NS	NS	NS	NS	**	NS	NS		NS
43	60	NS	NS	NS	NS	**	NS	NS	NS	

The figure shows matrices of statistical analyses of all pair-wise comparisons between percent healthy stembases (above the diagonal) and percent living plants at harvest (average of two harvest times) below the diagonal. Data from Experiment 1: Screening of AM fungal soil inocula.

[a]The soil inoculum numbers refer to the origins given in Table 1.

[b]For the number of living plants, the level of significance between the different treatments is presented *below* the diagonal (logistic regression, proc GENMOD).

[c]For the percentage of living plants, the level of significance between the different treatments is presented above the diagonal (logistic regression, proc GENMOD).

[d]Control treatment lacking soil inocula.

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS = not significant.

the two harvesting times was used. All AM fungi gave significantly shorter plants compared to the control, except for trap cultures 29, 32 and 41 (Table 3). Based on these results, trap cultures 29 (origin: semi-natural grass land) and 41 (origin: organic farm, barley at sampling time) were selected for use in Experiment 2.

Experiment 2: Testing of two selected soil inocula of AM fungal trap cultures on barley growth and transmission of B. sorokiniana *to seedlings*

Development of seed-borne *B. sorokiniana* in the presence of two different field AM inocula, resulted in significantly taller plants and a higher number of living plants for treatment 29 compared to the control, at both harvest times (Table 4). The number of registered leaf lesions per plant was significantly lower for both field inocula and Vaminoc® treatment at the second harvest time, compared to the control. Both field inocula also resulted in significantly lower proportions of infected barley plant nodes and stem bases, compared to the control at both harvesting times. The nodes were without symptoms even when *B. sorokiniana* was detected in the humid chamber. Conidial growth was observed from the lower nodes upwards, i.e. if two nodes of a plant were infected, these were the two lowest nodes. The Vaminoc® treatment gave a significantly lower percentage of registered node infections at the second harvest compared to the control (Table 4). However, the Vaminoc® treatment did not affect the number of living plants or the stem base infections.

Experiment 3: Influence of spore mixtures of AM fungi on barley growth and transmission of B. sorokiniana *to seedlings*

In seven of the eight AM spore mixture treatments and the pure AM culture, barley plants had significantly less leaf infections of *B. sorokiniana* compared to the control (Table 5). Two of the spore mixtures, inocula 23 and 38, gave significantly taller plants compared to the control. The examination for AM fungal colonisation (data not shown) showed that approximately 0.5–2% of the uppermost part of the root was colonised. The necrotic tissues (confirmed as *B. sorokiniana*) were

Table 3. Effect of soil inocula from different arbuscular mycorrhizal (AM) fungal trap cultures on growth of barley plants naturally infected with seed-borne *Bipolaris sorokiniana*

AM oil inocula culture[a]	Plant height[b] (cm)
Control	30
6	22*
13	26*
27	18*
29	28
32	28
38	23*
41	27
43[c]	24*
LSD	4

Data from Experiment 1: Screening of AM fungal soil inocula.
[a]The oil inocula numbers refer to the origins given in Table 1.
[b]Mean value per pot.
[c]proc GLM.
*$P < 0.05$.

stained dark blue and in plants with no added AM fungi, 26–75% necrotic roots were registered. The plants treated with spore mixture inoculum from a barley field had 26–50% necrotic roots. The rest of the treatments, including the pure culture *G. intraradices*, varied between 5% and 50%, in necrotic root rot development.

Discussion

Our results showed that the presence of AM fungi was able to suppress transmission of seed-borne *B. sorokiniana* to the aerial parts of barley plants. This is important in preventing the seed-borne inoculum from being transmitted to the new kernels and the following crop. This supplements findings by Dehn and Dehne (1986), who studied grass roots with an increased protection against Bipolaris common root in the presence of AM fungi. The pathogen growth in our study was suppressed although the infection level of the seeds for some treatments was as high as 95%. Seed infection means that the pathogen is already present when the AM fungi are introduced. In most other studies such as the one reported by Dehn and Dehne (1986), the barley plants were simultaneously inoculated with AM fungi and the pathogen. In the present study, using soil AM fungal inocula (Experiment 2), a reduction in transmission of *B. sorokiniana* to the stem bases increased over time. In addition, there was a suppression of the further transmission of *B. sorokiniana* to the nodes. This is important for preventing the spread of the pathogen up to the kernels. Reduced transmission of *B. sorokiniana* to the aerial plant parts probably also decreases the production of conidia, preventing spread by rain splashes. An increased reduction of *B. sorokiniana* on leaves over time was also revealed in our study. Six weeks after initiation of the experiment, the reduction was not significantly different from the control, lacking AM fungi, while there was a significant difference after 12 weeks. This might be due to enhanced AM fungal colonisation or to activation of plant defence responses by mycorrhizal formation, as shown by Benhamou et al. (1994) in a study comparing the responses of AM fungi and non-mycorrhizal transgenic carrot roots to infection by *Fusarium oxysporum*. In this study we also found that the commercial AM fungal inoculum Vaminoc® suppressed the development of *B. sorokiniana* to the nodes. Vaminoc® has previously been reported to suppress plant disease development in potato (Duffy and Cassells, 2000). However, in our study Vaminoc® did not suppress the *B. sorokiniana* infections on stem bases and leaves, nor did it increase the number of living plants. These different observations indicate the necessity of screening AM fungi with respect to their ability to suppress a specific pathogen.

The possibility that the suppression of plant disease development can be due to increased nutrient uptake by the plants colonised by AM fungi was suggested by Dehne (1982). Results from the present study do not show a consistent response when comparing plant growth and disease incidence. Jacobsen et al. (2005) showed in experiments using seven different AM fungi and barley as a host that plant phosphorous uptake and root biomass production differed considerably between the AM fungi tested. Further studies are needed to show if phosphorous or any other macro- or micro-nutrient element is involved in the induction of plant disease resistance.

The low colonisation by AM fungi in the roots observed in the present study might be explained by an inhibition of AM fungi by *B. sorokiniana*. Spores of the AM fungus *Glomus intraradices* grown in *in vitro* co-cultures with *B. sorokiniana* have been shown to have a reduced germination (Sjöberg et al., unpublished) and the toxin prehelminthosporol produced by *B. sorokiniana* has

Table 4. Effect of two selected AM soil inocula and a commercial AM fungal inoculum on plant growth and transmission of *Bipolaris sorokiniana* from seeds to plants in barley at two harvest times

AM fungal soil inocula	Mean plant height (cm)		Living plants				Occurrence of *B. sorokiniana* on different plant parts[a]							
	6 weeks[b]	12 weeks[b]	6 weeks		12 weeks		Stem bases				Leaf lesions		Nodes	
							6 weeks		12 weeks		6 weeks	12 weeks	12 weeks	
			%	Odds ratio[c]	%	Odds ratio[c]	%	Odds ratio[c]	%	Odds ratio[c]	Mean number per plant[b]	Mean number per plant[b]	%	Odds ratio[c]
Control I[d]	22	29	58	1.00	52	1.00	38	1.00	54	1.00	0.8	0.8	11	1.00
29 Semi-natural grassland	25*	39*	91	7.37***	87	6.42***	22	0.39*	29	0.22***	0.5 NS	0.3*	3	0.16***
41 Barley field	23 NS	29 NS	65	1.32 NS	65	1.76*	12	0.20**	41	0.09***	0.3*	0.1*	2	0.12***
LSD[b]	2	4	–	–	–	–	–	–	–	–	0.4	0.4	–	–
Control II[d]	25	35	77	1.00	74	1.00	71	1.00	69	1.00	1.4	1.8	17	1.00
Vaminoc®[e]	24 NS	34 NS	80	1.27 NS	78	1.29 NS	81	1.15 NS	74	1.33 NS	1.6 NS	0.7*	2	0.09***

[a]Registered after incubation in humid chamber.
[b]Analysis of variance (proc GLM).
[c]Logistic regression (proc GENMOD).
[d]Control I = similar sand/silt mixture as used for raising the trap cultures but with uninoculated plant mixtures without additions of field soil. Control II = sand/silt mixture without the Vaminoc® substrate.
[e]Commercial Inoculum, Becker Underwood, Littlehampton, UK.
$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS = not significant.

Table 5. Effects of arbuscular mycorrhizal (AM) fungal spore mixtures on plant height and occurrence of *Bipolaris sorokiniana* in barley leaves

AM fungal spore Inocula[a]	Mean plant height[b] (cm)	Occurrence of *B. sorokiniana*[c]			
		Leaf base[d]		Upper half of leaf	
		%	Odds ratio[e]	%	Odds ratio[e]
Control[f]	26	66	1.00	50	1.00
3	28	13	0.08***	13	0.14***
12	26	41	0.36 NS	33	0.45 NS
23	33*	14	0.09***	7	0.07***
33	26	3	0.02***	8	0.08***
38	33*	22	0.15***	17	0.18**
40	29	13	0.08***	27	0.34*
41	25	37	0.30*	22	0.25**
42	27	14	0.09***	26	0.31*
Glomus intraradices[g]	30*	21	0.14***	14	0.14***
LSD	5				

[a]The inocula numbers refer to the origins given in Table 1.
[b]Analysis of variance (proc GLM).
[c]Recorded after incubation in humid chamber.
[d]3 mm.
[e]Odds ratio and level of significance compared to the control, logistic regression (proc GENMOD).
[f]No added AM fungal inocula.
[g]Commercial inoculum, BCCM™/MUCL, Belgium, culture no. 43194.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant.

been shown to inhibit the spore germination of different fungal species (Åkesson, 1995). Nevertheless, AM fungi treatment reduced the necrosis caused by *B. sorokiniana* on the upper root parts in present study. Likewise, Caron et al. (1986) showed that both the population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and necrosis caused by this pathogen was reduced even at low levels of AM fungal colonisation.

Our study has shown that AM fungi reduce the development of *B. sorokiniana* in barley, although the AM fungal root colonisation was low. Dehn and Dehne (1986) also found that the presence of AM fungi reduced the root necrosis due to *B. sorokiniana*, and that there was no correlation between the degree of AM fungal colonisation and the degree of pathogen suppression. In addition they found that *B. sorokiniana* did not infect arbuscular-containing cells. Therefore AM fungal root colonisation does not appear to be an appropriate parameter to evaluate disease suppressiveness by AM fungi. With seed-borne inoculum of *B. sorokiniana* being present before introduction of AM fungi, as in the present study, there is probably less time for the AM fungi to form arbuscules before a possible invasion of the cell by the pathogenic fungus.

Despite a low detected degree of AM fungal colonisation in the present study, the presence of AM fungi suppressed the transmission of the pathogen to aerial plant parts. Therefore the efficacy of the mycorrhiza–plant-pathogen interaction does not primarily appear to be linked to root colonisation of the AM fungi. Volpin et al. (1994) also showed that the presence of AM fungi in the rhizosphere induced defence responses in alfalfa roots, but that no AM fungal colonisation of the root tissue was required.

In our study, suppressed transmission of *B. sorokiniana* to the above ground plant parts was combined with a tendency for fewer necrotic roots when AM fungi were added. This observation might indicate that local competition occurred (Larsen and Bødker, 2001) and/or that resistance was induced. Cordier et al. (1996) showed that *Phytophthora nicotianae* var. *parasitica* development was reduced in AM fungal-colonised and adjacent uncolonised regions of AM tomato root systems, and that in the former situation, the pathogen did not penetrate arbuscule-containing

cells. When multiplying AM fungi from field soil, an undesirable incidental increase in other microorganisms may be the result. In order to minimise this factor in our study, a 'trap culture-control' was set up based on a substrate where plants were grown along with the trap cultures in the greenhouse, but with no added soil cores with AM fungi. In the control used for the Vaminoc® treatment, a pure sand/silt mixture was used instead to simulate the Vaminoc® substrate. The results from experiments using soil inocula from AM fungal trap cultures showed a clear decrease in the transmission of *B. sorokiniana* compared to both controls.

Our results indicate that the degree of suppression of pathogen development also varies with the AM fungal population. There might also be differences between the responses of barley cultivars to AM fungi with respect to suppression of *B. sorokiniana*. Dehn and Dehne (1986) found that the disease intensity of common root rot could be reduced by AM fungal symbiosis in all seven cultivars of barley tested, but the degree of host plant protection varied with the genotype of host and pathogen. For biological control purposes, an interesting application would be to select for barley cultivars with the ability to benefit from tailor-made AM fungal isolates to reduce *B. sorokiniana* infection. Another interesting challenge for practical applications would be to inoculate efficient AM fungal isolates directly onto the soil and promote these by cultivation methods. For selection, screening and development of AM fungal isolates for control purposes, it is important to take into account the environmental conditions. By screening AM fungi collected in arable fields, as in the present study, the AM fungi are probably adapted for crop production.

In conclusion, this study has shown that AM soil inocula from trap cultures suppressed the transmission of seed-borne *B. sorokiniana* to the aerial parts of barley plants. The extent of the effect varied depending on AM fungal origin. The results indicate a potential use in biological control, either by seed coating or by cultivation methods favouring indigenous AM fungi.

Acknowledgements

This study was financially supported by the Swedish Farmers Foundation for Agricultural Research (SLF). The authors would like to thank Jonathan Yuen for help during the statistical analyses and Gustaf Forsberg for assistance with the hot humid air seed treatment of barley.

References

Abdel-Fattah GM and Shabana YM (2002) Efficacy of the cowpea plants against root rot pathogen *Rhizoctonia solani*. Journal of Plant Disease and Protection 109: 207–215.

Åkesson H (1995) Infection of barley by *Bipolaris sorokiniana*: Toxin production and ultrastructure. Doctoral thesis. Lund University, Sweden.

Almgren I, Gustafsson M, Fält A-S, Lindgren H and Liljeroth E (1999) Interaction between root and leaf disease development in barley cultivars after inoculation with different isolates of *Bipolaris sorokiniana*. Journal of Phytopathology 147: 331–337.

Benhamou N, Fortin JA, Hamel C, St-Arnaud M and Shatilla A (1994) Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 84: 958–968.

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations?. Ecology 82: 3057–3068.

Bødker L, Kjøller R, Kristiansen K and Rosendahl S (2002) Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza 12: 7–12.

Caron M, Richard C and Fortin JA (1986) Effect of preinfestation of the soil by a vesicular-arbuscular mycorrhizal fungus, *Glomus intraradices*, on fusarium crown and root rot of tomatoes. Phytoprotection 67: 15–19.

Cordier C, Gianinazzi S and Gianinazzi-Pearson V (1996) Colonisation patterns of root tissues by *Phytophtora nicotianae* var. parasitica related to reduced disease in mycorrhizal tomato. Plant and Soil 185: 223–232.

Dehne HW (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115–1119.

Dehn B and Dehne HW (1986) Development of VA mycorrhizal fungi and interactions with *Cochliobolus sativus* in roots of gramineae. Physiological and genetical aspects of mycorrhizae. Proceedings of the 1st European symposium on mycorrhizae, Dijon, pp. 773–779.

Duffy E and Cassells A (2000) The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Applied Soil Ecology 15: 137–144.

Duczek L (1984) Comparison of the common root rot reaction of barley lines and cultivars in northwestern Alberta and central Saskatchewan. Canadian Journal of Plant Pathology 6: 81–89.

Duczek LJ (1997) Biological control of common root rot in barley by *Idriella bolleyi*. Canadian Journal of Plant Pathology 19: 402–405.

Forsberg G (2004) Control of cereal seed borne diseases by hot humid air seed treatment. Doctoral thesis, SLU. Acta Universitatis Agriculturae Suecia.

Giovannetti M and Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytologist 84: 489–500.

Jakobsen I, Chen B, Munkvold L, Lundsgaard T and Zhu Y-G (2005) Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant. Plant, Cell and Environment 28: 928–938.

Jørgensen J (1974) Occurrence and importance of seed borne inoculum of *Cochliobolus sativus* in Denmark. Acta Agriculture Scandinavica 24: 49–54.

Knudsen IMB, Hockenhull J and Jensen DF (1995) Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. Plant Pathology 44: 467–477.

Koske RE and Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92: 486–505.

Kurppa A (1984) *Bipolaris sorokiniana* on barley seed in Finland. Journal of Agricultural Science in Finland 56: 175–182.

Kurppa A (1985) The response of some spring barley cultivars grown in Finland to air-borne secondary infection by *Bipolaris sorokiniana*. Journal of Agricultural Science in Finland 57: 97–105.

Larsen J and Bødker L (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytologist 149: 487–493.

Liljeroth E and Bryngelsson T (2002) Seed treatment of barley with *Idriella bolleyi* causes systematically enhanced defence against root and leaf infection by *Bipolaris sorokiniana*. Biocontrol Science and Technology 12: 235–249.

Matsubara Y, Ohba N and Fukui H (2001) Effect of arbuscular mycorrhizal fungus infection on the incidence of Fusarium root rot in asparagus seedlings. Journal of Japanese Society of Horticultural Science 70: 202–206.

Saari EE (1998) Leaf Blight diseases and associated soilborne fungal pathogens of wheat in South and Southeast Asia. In: Helminthosporium blight of wheat: Spot Blotch and Tan Spot. In: Duveiller E, Dubin HJ, Reeves J and McNab A (eds.) Proceedings of an international workshop held at CIMMYT, El Batan, Mexico, pp. 37–51.

Sivanesan A, Holliday P (1981) *Cochliobolus sativus*. CMI Descriptions of Pathogenic Fungi and Bacteria 71: 701.

Sjöberg J, Persson P, Mårtensson A, Mattsson L, Adholeya A and Alström S (2004) Occurrence of *Glomeromycota* spores and some arbuscular mycorrhiza fungal species in arable fields in Sweden. Acta Agriculturae Scandinavica Section B Soil & Plant Science 54: 202–212.

Stack RW (1982) Yield losses in spring barley due to common root rot in Eastern North Dakota. Phytopathology 72: 1139–1140.

Thompson JP and Wildermuth GB (1989) Colonization of crop and pasture species with vesicular-arbuscular mycorrhizal fungi and negative correlation with root infection by *Bipolaris sorokiniana*. Canadian Journal of Botany 67: 687–693.

Volpin H, Elkind Y, Okon Y and Kapulnik Y (1994) A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defence response in alfalfa roots. Plant Physiology 104: 683–689.

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany 82: 1198–1227.

Whittle AM (1977) *Cochliobolus sativus* on barley in Scotland. Plant Pathology 26: 67–74.