

# Plant and soil response to single and mixed species of arbuscular mycorrhizal fungi under fungicide stress

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## Abstract

Defining sustainable agricultural practices requires an understanding of both above- and below-ground consequences to management strategies. While alternatives to biocides are sought for the control of weeds, insects and pathogens, biocide use will continue with the goal of reducing quantities used in an integrated approach. The impact of three fungicides on plant growth, seed yield, seed nutrient composition, and on soil aggregation as mediated by arbuscular mycorrhizal (AM) fungi was studied in a silty-clay loam soil with a high extractable P concentration. Shoot dry mass, seed yield and seed nutrient (N, P, K) contents of pea (*Pisum sativum* L.) plants were enhanced by three AM fungi and a mixture of three fungi compared with nonAM plants. Each AM fungus produced a distinct pattern of shoot responses of plants, but the mixed inoculum treatment was as good or better than each single species for all of the above-ground measures of plant performance. Soil aggregation was improved by two of the three AM fungi as individual inocula, and was further increased in the mixed inoculum treatment. Two of the three fungicides reduced shoot dry mass and seed yield, but none of the fungicides affected soil aggregation. Fungicides inhibited mycorrhiza formation least in the mixed inoculum treatment which gave the best overall plant and soil responses. Since the three fungi together were more tolerant of fungicides than each fungus alone, it appeared that as a community, AM fungi modified and alleviated fungicide stress, resulting in high levels of plant performance and soil aggregation. © 1997 Elsevier Science B.V.

*Keywords:* Mycorrhizal community; Seed yield; Soil aggregation; AM fungi; Glomales

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## 1. Introduction

Much research has been directed at identifying 'efficient' arbuscular mycorrhizal (AM) fungi (Sieverding, 1991; Secilia and Bagyaraj, 1992; Smith

et al., 1992; Tisserant et al., 1993), focusing on host-plant growth promotion and enhanced nutrient uptake as the criteria of efficiency. However, the role of AM fungi in improving soil structure (Tisdall and Oades, 1982; Miller and Jastrow, 1992) has sparked a debate to include the soil response in defining efficient strains of AM fungi (Bethlenfalvay and Schüepp, 1994).

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Different AM-fungal species and even distinct geographic isolates of the same species can affect plant development differently (Wilson, 1988; Bethlenfalvay et al., 1989). AM-fungal isolates that outperform others under one set of conditions may be used as a bioresource on a small scale for improving plant performance under similar soil and environmental conditions. However, the difficulty and cost of producing AM-fungal inocula (Jarstfer and Sylvia, 1993) and the largely unknown impact of AM-fungal inoculation under field conditions (McGonigle, 1988) has impeded the use of AM fungi on a large scale. Such limitations have advanced the idea of managing the native AM-fungal population by the use of cultural practices that benefit symbiotic expression (Miller et al., 1994). It follows that as more functions of AM fungi become known and are integrated into management practices, the value of a diverse AM-fungal community to agroecosystems will be realized.

Inoculation of soils with multiple AM fungi has been shown to promote plant growth better than single isolates in some studies (Daft and Hogarth, 1983; Koomen et al., 1987), but this is not always the case (Yocom, 1985), as some host plant/AM fungus/soil combinations will be better suited to enhance plant growth under some conditions. Soil ecosystems contain a community of AM fungi and responses of the community to cultural practices differ from those of single species. At present, however, our knowledge of AM fungi is largely based on single-fungus experiments. Emphasis on studying responses of mixed populations of AM fungi in disturbed ecosystems is required before we can integrate mycorrhizal research findings into beneficial farming techniques (Abbott and Gazey, 1994).

We tested the hypothesis that a mixed population of AM fungi responds differently than individual species to biocide stress by comparing above- and below-ground responses of pea plants inoculated with multiple vs. single AM fungi. We report how three fungicides modified the effects of three individual AM fungi and a mixture of the three fungi on host-plant growth, seed yield, and soil aggregation. The specific objectives were to compare how fungicides affected root colonization and sporulation in different AM-fungal treatments and how these effects benefitted plant development and soil structure.

## 2. Materials and methods

### 2.1. Experimental design

The experiment had a  $4 \times 5$  factorial design with four fungicide (untreated, Benomyl, PCNB, Captan) and five AM-fungal (uninoculated (nonAM), three individual fungi (Ge, Gm or Gr), mixed inoculum of three fungi (Mix)) treatments as factors. Each treatment combination was replicated five times for a total of 100 experimental units (potted plants). Statistical analysis was carried out using analysis of variance (ANOVA) procedures on Statgraphics version 5.0 (STSC, 1991). Main effects of fungicides and AM fungi on response variables were evaluated across the entire data set, while effects of fungicides on root colonization and sporulation by AM fungi were analyzed separately for each AM-fungal treatment, since the nonAM treatment biases the ANOVA. Mean contrasts were evaluated at 95% using Fisher's protected LSD method.

### 2.2. Biological materials

Pea (*Pisum sativum* L. cv. 'Little Marvel') plants were used as host, grown from a seed lot that originated from a single seed to minimize plant variability. Three AM fungi propagated in potculture with *Sorghum bicolor* L. were used as inoculum: *Glomus etunicatum*, Ge (Becker & Gerd.), INVAM No. UT183-1; *Glomus mosseae*, Gm (Nicol. & Gerd.) Gerd. & Trappe, Banque Européenne des Glomales (BEG) No. 46 (Dodd et al., 1994); and *Gigaspora rosea*, Gr (Nicol. & Schenck), INVAM No. FL105. The inoculum for each fungus was stored dry at 4°C for 1 year prior to use. A quantity of soil inocula of each fungus containing spores, hyphae and root fragments was thoroughly mixed into the soil of each AM-fungal treatment to yield the following number of spores per pot in the individual AM-fungal treatments: Ge, 500; Gm, 500; Gr, 1000. Plants to be colonized by a mixed inoculum (henceforth referred to as Mix) received soil inoculum yielding 250, 250 and 500 spores of each fungus, respectively. The quantity of soil inoculum of each fungus used in the Mix treatment was based on the results of two prior studies (Schreiner and Bethlenfalvay, 1996, 1997) employing the same fungi, host plant and soil and

was expected to give roughly equal development of each of our three fungi. The quantity of inoculum used for the individual AM-fungal treatments was twice that of the Mix to ensure high colonization rates for each fungus. Germination trials for each fungus conducted 1 month prior to the experiment gave mean germination rates of 72, 53 and 24%, and hyphal lengths of 12, 13 and 23 mm per germinated spore after 2 weeks in the experimental soil for Ge, Gm and Gr, respectively. The soil used was Chehalis silty-clay loam (28 mg kg<sup>-1</sup> NaHCO<sub>3</sub>-extractable P) described earlier (Schreiner and Bethlenfalvay, 1997).

### 2.3. Growth conditions and fungicide application

Five-day-old pea seedlings were transplanted from vermiculite into 1.5 L pots filled with steam-pasteurized soil (75°C, 45 min) mixed with the appropriate AM-fungal inocula. All pots received a microbial suspension collected from the three AM-fungal inocula. The suspension was prepared from AM-fungal inocula equivalent to one-third of the quantity used for each treatment by suspending in water, blending at high speed for 1 min in a Waring blender and passing it through a 45 µm sieve six times to remove AM-fungal propagules. The resulting suspension was then diluted to 500 ml and 5 ml were added to each pot in the planting hole. Three fungicides, Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, 50% WP), PCNB (pentachloronitrobenzene, 75% WP), and Captan (*N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide, 50% WP) were applied as a soil drench (200 ml per pot) at the rate of 20 mg active ingredient (a.i.) kg<sup>-1</sup> soil 1 day after transplanting the seedlings. Fungicides were applied again at the same rate 14 days after the first application. Untreated controls received 200 ml of water.

Pots were arranged in a randomized complete block design on benches and the plants were grown for 69 days in an unshaded greenhouse (December 1993 to February 1994, Corvallis, OR) until harvest. Temperatures were maintained between 20 and 28°C during the photoperiod, and were 18–24°C during the night. Supplemental lighting was provided by 1000 W, metal-halide lamps with phosphor-coated bulbs which supplied 450 µE m<sup>-2</sup> s<sup>-1</sup> photo-

synthetically active radiation at soil level for a 16 h photoperiod. One week after transplanting, each pot received 200 ml of a P-free, one-half strength Hoagland's solution (Machlis and Torrey, 1956) three times per week. Plants received supplemental watering with tap water whenever the soil on the surface became dry.

### 2.4. Assays

Seed pods were stripped from the plants as they ripened (when the stem subtending pods was yellow and dry). The pods were stored in the greenhouse in paper bags. By 69 days after transplanting, all of the pods were removed from plants, shoots were harvested, dried (70°C, 3 days), and weighed. The pods and seeds produced by each plant were counted. Seed yield, specific weight (seed weight/seed number), and N, P, and K concentrations and contents were determined for each plant (the latter by A&L Western Laboratory, Modesto, CA).

Pots were cored 42 and 69 days after transplanting to obtain root samples. Pasteurized soil was added to the holes produced by coring on day 42, and the location of the core was noted to avoid future sampling from the same site. Total and AM-colonized root lengths were determined by the grid-line intersect method (Newman, 1966) after clearing and staining the washed roots from each soil core. Root samples were cleared in 5% (w:v) KOH (20 min, 90°C), rinsed with water, acidified in 1% (v:v) HCl (10 min, 90°C), stained with trypan blue (0.05%, w:v, in lacto-glycerin, 20 min, 90°C) and de-stained overnight in lacto-glycerin. The number of spores produced by each AM fungus was determined by wet-sieving a subsample of air-dried soil from each pot onto a stack of sieves (500, 250, 125 and 75 µm) and counting the spores retrieved on each sieve or in appropriately diluted samples. Spore volumes were calculated from the number of spores by multiplying by the following specific spore volumes (based on spore diameters): Ge, 0.45 nl; Gm, 4.19 nl; Gr, 9.20 nl (see Schreiner and Bethlenfalvay, 1996).

Water-stable soil aggregation (WSA) was determined on 1–2 mm, air-dried soil aggregates collected from each pot employing the method of Kemper and Rosenau (1986). Pots were watered after shoot excision to equalize soil moisture contents.

The root ball was removed intact from each pot and air-dried until the soil was friable and could easily be shaken from the roots. The mostly root-free soil samples were then spread on paper to air-dry completely. A 200 ml sample of the dry soil was sieved through a series (2 mm, 1 mm) of soil sieves by hand-shaking at a uniform stroke length 30 times. Air-dry aggregates (1–2 mm) were collected and 4-g samples were placed on 1 mm sieves. Samples were vapor-wetted, placed in a mechanical, sieving apparatus (Kemper and Rosenau, 1986) and wet-sieved for 10 min in distilled water to obtain the water-unstable fraction that passed through the sieves. The aggregates remaining on the sieves were dispersed by wet-sieving in 0.2% (w:v) NaOH solution to obtain the water-stable fraction. A rubber spatula was gently brushed over the sieve openings to facilitate the dispersion of water-stable aggregates in the NaOH solution. The dry mass of water-unstable and water-stable fractions was determined after oven-drying at 110°C. The percentage of water stable aggregates (% WSA) was calculated by dividing the mass of the water-stable fraction by the sum of water-unstable and water-stable fractions.

### 3. Results

#### 3.1. Fungicide and AM effects on shoots

Both AM fungi and fungicides affected growth and development of pea plants. All AM fungi increased shoot dry matter and seed yield, while the fungicides PCNB and Captan decreased shoot dry mass and seed yield (Table 1). Benomyl did not influence above-ground parameters. Shoot dry masses of the Ge and Mix plants were greater than those of the Gr plants. Increases in seed yield were due to a greater number of seeds in all AM plants, but Ge and Mix plants also had greater specific seed masses than the other plants. The decrease in seed yield associated with PCNB and Captan was due to reductions in both seed number and pod number per plant. Increases in seed number in AM plants were not linked to the number of pods produced. Both fungicide and AM main effects on pea shoots were significant, except for the effect of Benomyl on shoots.

The lack of significant interactions between the two factors indicated that the plant response to either

Table 1  
AM-fungal and fungicide main effects on growth and reproduction of potted pea (*Pisum sativum* L.) plants

Treatment	Fungicide	Dry mass at 69 days (g)		Seeds per plant	Pods per plant	Specific seed mass <sup>b</sup> (mg)
		Shoot	Seed			
nonAM		3.4C	7.4B	30B	13	251B
Ge		4.2A	9.5A	36A	14	265A
Gm		4.1AB	8.9A	36A	13	252B
Gr		3.8B	8.9A	35A	14	252B
Mix		4.3A	9.0A	34A	13	265A
	Control	4.2X	9.7X	38X	15X	255
	Benomyl	4.1XY	9.1X	36X	14X	254
	PCNB	3.8YZ	8.2Y	32Y	13Y	260
	Captan	3.7Z	8.0Y	31Y	12Y	259
ANOVA <sup>c</sup>						
AM-fungal		< 0.001	< 0.001	0.015	0.298	< 0.001
Fungicide		0.004	< 0.001	< 0.001	< 0.001	0.265
Interaction		0.49	0.982	0.962	0.715	0.626

<sup>a</sup>nonAM, nonmycorrhizal; Ge, *Glomus etunicatum*, Gm, *Glomus mosseae*; Gr, *Gigaspora rosea*; Mix, mixed inocula

<sup>b</sup>Seed mass/seed number.

<sup>c</sup>Significance levels are given for the factors AM-fungal, fungicide and their interaction.

Letters A–C designate significant groups (95% LSD) between AM-fungal treatments ( $n = 20$ ). Letters X–Z designate significant groups (95% LSD) between fungicide treatments ( $n = 25$ ).

fungicides or AM fungi was similar at all levels of the other (Table 1). Plant responses to fungicides within AM treatments were similar in direction, with the order of greatest inhibition given as Captan > PCNB > Benomyl > control. Fungicide effects on above-ground parameters within each of the different AM treatments were generally not significant.

### 3.2. Fungicide and AM effects on roots and soil

Main effects of AM fungi and fungicides were significant for the below-ground variables, except for fungicide effects on WSA (Table 2). Root growth was improved by Ge, Gm and Gr at 42 days, but not by Mix. By the final harvest at 69 days, root length of the nonAM plants was the same as the AM plants colonized by individual isolates. Root length of the Mix plants, however, remained lower than that of all other treatments. Reduced root length in the Mix plants was not associated with a correspondingly small shoot mass (Table 1), suggesting that these plants relied more on AM soil hyphae for nutrient uptake. All fungicides reduced root length at 69

days, but only Benomyl did so at the earlier sampling date.

The significant interaction between AM-fungal and fungicide effects on root length at 42 days (Table 2) was due to an inhibition of root growth by Benomyl and PCNB in nonAM plants. The mean root lengths of nonAM plants at 42 days in Benomyl and PCNB treatments were 69 and 74 m, respectively, while those for the control and Captan treatments were 159 and 145 m. At harvest at 69 days, the inhibitory effects of Benomyl and PCNB on root growth of nonAM plants had disappeared. Root length was slightly reduced by fungicides within each of the four mycorrhizal treatments (Captan or PCNB having the greatest effect in various AM-fungal treatments), but such effects were not significant ( $P > 0.05$ ) when each AM-fungal treatment was examined alone.

There were significant AM effects on WSA: Gr and Gm soils were more highly aggregated than nonAM and Ge soils, while the Mix soil had an even higher level of WSA (Table 2). Fungicide effects on WSA were not significant, and the lack of interaction

Table 2  
AM-fungal and fungicide main effects on total root length, AM-colonized root length, and soil aggregation in potted pea (*Pisum sativum* L.) plants

Treatment	Root L 42 days	AM-root L <sup>b</sup> 42 days	Root L 69 days	AM-root L 69 days	% WSA <sup>c</sup> 1–2 mm	
AM-fungal <sup>a</sup> Fungicide	(m)	(m)	(m)	(m)		
nonAM	112B	0C	213A	0D	81.6C	
Ge	151A	71A	195A	127A	83.6BC	
Gm	154A	45B	186A	73C	84.5B	
Gr	166A	45B	191A	81BC	85.4B	
Mix	127B	63A	149B	91B	88.0A	
	Control	52X	214X	98X	84.9	
	Benomyl	128Y	44XY	188Y	77Y	84.0
	PCNB	147X	39Y	172Y	57Z	83.9
	Captan	138XY	45XY	174Y	66YZ	85.7
ANOVA <sup>d</sup>						
AM-fungal	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Fungicide	0.037	0.037	0.006	< 0.001	0.186	
Interaction	< 0.001	< 0.001	0.740	< 0.001	0.716	

<sup>a</sup>nonAM, nonmycorrhizal; Ge, *Glomus etunicatum*, Gm, *Glomus mosseae*; Gr, *Gigaspora rosea*; Mix, mixed inocula.

<sup>b</sup>Root length colonized by arbuscular mycorrhizal fungi.

<sup>c</sup>Water-stable soil aggregates, 1–2 mm in diameter.

<sup>d</sup>Significance levels are given for the factors AM-fungal, fungicide and their interaction.

Letters A–C designate significant groups (95% LSD) between AM-fungal treatments ( $n = 20$ ). Letters X–Z designate significant groups (95% LSD) between fungicide treatments ( $n = 25$ ).

between the factors indicated that the magnitude of AM effects was independent of the fungicide levels.

### 3.3. Fungicide effects on AM fungi

A significant interaction between fungicide and AM-fungal treatments occurred for the total root length colonized by mycorrhizal fungi at both sampling times (Table 2). Each of the individual fungi and the Mix treatments developed similar levels of total root length colonized (AM-root lengths in the control treatment were not significantly different between Ge, Gm, Gr or Mix) and similar levels of percent root colonized over the course of the experiment (Fig. 1). Therefore, the effects of fungicides on different AM-fungal treatments could be compared. Fungicides reduced AM-fungal colonization more in Gm and Gr roots than in Ge or Mix roots. In Ge and Mix plants, fungicides did not effect percent root colonization (Fig. 1) nor total root length colonized at 42 days (data not shown). Although, Captan reduced the total AM root length at the final harvest from 150 to 109 m in Ge plants and from 107 to 71 m in Mix plants. All three fungicides reduced AM root length at 69 days in Gm and Gr plants, but PCNB depressed total root colonization more than Benomyl or Captan in Gm and Gr plants. The mean values for root length colonized at 69 days in the control, Benomyl, PCNB, and Captan treatments were 110, 76, 33, and 75 m for Gm and 122, 81, 43, and 78 m for Gr plants, respectively. PCNB also inhibited total colonized root length in Gm and Gr plants at 42 days, while Benomyl and Captan did not (data not shown). The strong inhibition by PCNB in the Gm and Gr treatments was reflected by a similar reduction in percent root colonization, while Benomyl and Captan did not significantly reduce percent colonization (Fig. 1). Interactions between AM-fungal and fungicide treatments on colonized root length were significant (Table 2) because Ge and Mix plants reacted differently to PCNB and Benomyl than Gm and Gr plants. The response patterns of Ge and Mix plants were alike in direction and magnitude, and so were those of Gm and Gr. These data suggest that Ge was functionally dominant in the Mix, and that Ge characteristics were expressed in Mix roots in response to fungicide stress.

The effects of fungicides on AM-fungal spore

formation (Fig. 2) were similar to those found on root colonization (Fig. 1) for Gm and Gr. There were large reductions in spore density in the PCNB treatment, and to a lesser extent in the Benomyl and Captan treatments. Spore density in the Ge soil was reduced by PCNB, but not by Benomyl or Captan (Fig. 2). This was not consistent with the colonization response, where none of the fungicides reduced the percent Ge colonization. The fungicides affected spore density in the Mix soil least (Fig. 2A), reflecting weak fungicide effects on root colonization (Fig. 1). However, when expressed as spore volume (a more exact reflection of fungal biomass), the data

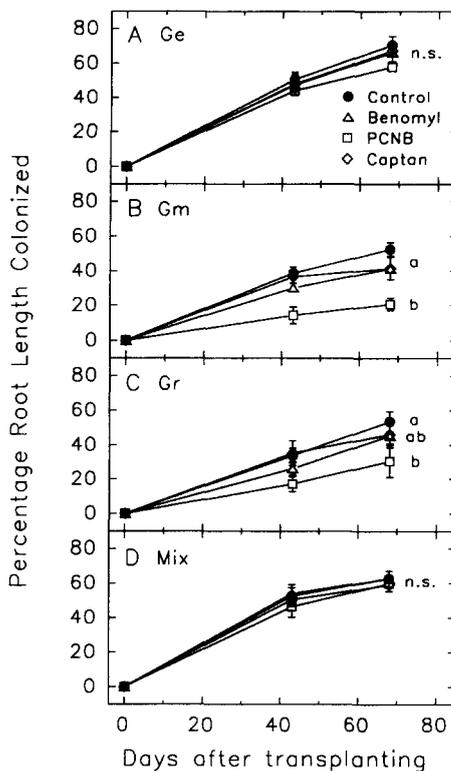


Fig. 1. Effects of fungicides on the colonization of pea roots by three arbuscular mycorrhizal fungi inoculated alone or together in a mix. Data points represent the means ( $\pm$ SE) of five replicate plants for each treatment. Letters designate significant groups (95% LSD) within each AM-fungal treatment at harvest 69 days after transplanting. Fungicides were applied as a soil drench at 20 mg a.i.  $\text{kg}^{-1}$  soil 1 and 15 days after transplanting pea seedlings. Ge, *Glomus etunicatum*; Gm, *Glomus mosseae*; Gr, *Gigaspora rosea*; Mix, mixed inocula.

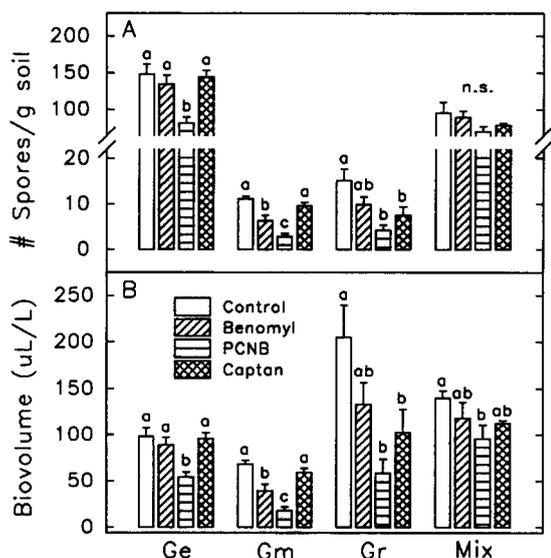


Fig. 2. Effects of fungicides on the sporulation of three arbuscular mycorrhizal fungi inoculated alone or together in a mix in association with peas. Data points represent the means ( $\pm$ SE) of five replicates for each treatment combination. Letters designate significant groups (95% LSD) within each AM-fungal treatment. Fungicides were applied as a soil drench at 20 mg a.i.  $\text{kg}^{-1}$  soil 1 and 15 days after transplanting pea seedlings. Ge, *Glomus etunicatum*; Gm, *Glomus mosseae*; Gr, *Gigaspora rosea*; Mix, mixed inocula.

did reveal inhibition by PCNB in the Mix compared with the untreated control (Fig. 2B).

The observation that fungicides affected spore formation less in the Mix soil than in the soils of the individual AM fungi (Fig. 2A) was confirmed by comparing the number of spores produced by each fungal isolate as individual inocula to those produced by each isolate in the Mix treatment (Table 3). In all cases, fungicide effects on spore numbers were greater in the individual AM treatments. In addition, the number of spores produced by each isolate in the Mix soil was lower than comparable ones in the Ge, Gm and Gr soils, indicating inter-isolate competition in the Mix (Table 3). This was particularly true for Gr.

Species composition of AM fungi in the Mix as estimated by spore formation was not altered ( $P > 0.05$ ) by fungicides in relation to untreated controls. However, the small shifts in the relative spore volume's of these fungi that occurred were similar to trends found previously (Schreiner and Bethlenfal-

vay, 1996). The relative spore volume (percent of total spore volume, mean  $\pm$  SE) of each AM fungi shifted under the influence of fungicides as follows. For Ge: control,  $39.3 \pm 4.9$ ; Benomyl,  $47.4 \pm 4.1$ ; PCNB,  $46.5 \pm 5.4$ ; Captan,  $41.1 \pm 2.4$ . For Gm: control,  $35.9 \pm 3.0$ ; Benomyl,  $35.1 \pm 2.6$ ; PCNB,  $32.5 \pm 2.8$ ; Captan,  $43.9 \pm 3.6$ . For Gr: control,  $24.8 \pm 7.2$ ; Benomyl,  $17.5 \pm 5.0$ ; PCNB,  $21.0 \pm 5.4$ ; Captan,  $14.8 \pm 5.4$ . The relative abundance of Ge spores was, however, much greater than its relative volume, accounting for 88–91% of the total number of spores produced in the Mix.

#### 3.4. Fungicide and AM effects on seed nutrient composition

Seed N and P concentrations increased in Ge, Gr and Mix plants relative to nonAM plants (Table 4). Seed P concentrations were lower in Gm plants than in all other treatments. Seed K concentrations were lower in some AM plants compared with nonAM plants. However, seed N, P and K contents were higher in all AM plants, corresponding to the increase in seed yield in AM plants. Differences in seed nutrient contents were found among the AM plants. In general, Ge, Gr and Mix plants outperformed Gm plants, particularly in enhancing seed P content (Table 4). There were no fungicide effects on seed nutrient concentrations (except for the positive

Table 3

Comparison of fungicide effects on sporulation of three AM fungi inoculated individually or together in a mix. Values represent the number of spores per gram of soil, and are the means of five replicates

	Fungicide treatment				P-value <sup>a</sup>
	Control	Benomyl	PCNB	Captan	
Ge alone	148a	134a	81b	144a	0.0026
Ge in mix	85	81	63	69	0.3119
Gm alone	11a	6.4b	2.9c	9.6a	<0.0001
Gm in mix	8.2a	6.6ab	5.0b	8.0a	0.0410
Gr alone	15a	9.8ab	4.3b	7.6b	0.0075
Gr in mix	2.5	1.8	1.7	1.3	0.6035
Total spores in mix	95	89	70	79	0.2495

<sup>a</sup>Significance of fungicide treatments by ANOVA employing data for each individual fungus.

Values in rows followed by different letters are significantly different at 95% confidence level (LSD).

Table 4

AM-fungal and fungicide main effects on seed nutrient concentrations and contents of potted pea (*Pisum sativum* L.) plants

Treatment		N conc.	N content	P conc.	P content	K conc.	K content (mg)
AM-fungal <sup>d</sup>	Fungicide	(mg g <sup>-1</sup> )	(mg)	(mg g <sup>-1</sup> )	(mg)	(mg g <sup>-1</sup> )	
nonAM		39.0B	289C	3.14B	23.2C	11.7A	87 B
Ge		40.4AB	383A	3.37A	31.9A	11.3B	107 A
Gm		39.0B	345B	2.98C	26.5B	11.0C	98 A
Gr		41.1A	360AB	3.36A	29.6A	11.6A	103 A
Mix		41.8A	376A	3.45A	31.1A	11.4AB	103 A
	Control	39.9	384X	3.21Y	31.1X	11.4	110 X
	Benomyl	40.0	363X	3.22Y	29.2XY	11.3	103 X
	PCNB	41.1	336 Y	3.24 XY	26.7Y	11.3	94 Y
	Captan	40.1	319 Y	3.36 X	26.7Y	11.4	91 Y
ANOVA <sup>b</sup>							
AM-fungal		0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003
Fungicide		0.289	< 0.001	0.122	0.002	0.796	< 0.001
Interaction		0.697	0.966	0.363	0.837	0.910	0.991

<sup>a</sup>nonAM, nonmycorrhizal; Ge, *Glomus etunicatum*, Gm, *Glomus mosseae*; Gr, *Gigaspora rosea*; Mix, mixed inocula.

<sup>b</sup>Significance levels are given for the factors AM-fungal, fungicide and their interaction.

Letters A–C designate significant groups (95% LSD) between AM-fungal treatments ( $n = 20$ ). Letters X–Z designate significant groups (95% LSD) between fungicide treatments ( $n = 25$ ).

effect of Captan on P), indicating that the reductions in nutrient contents caused by PCNB and Captan were also due to effects on seed yield.

#### 4. Discussion

Numerous studies have shown that AM fungi improve plant performance in soils of low fertility, and that fungicides can reduce plant growth as a result of inhibition of AM fungi. However, the complexity of fungicide effects on AM fungi known to arise from variations in the specific fungitoxicity of different chemicals (Trappe et al., 1984; Johnson and Pflieger, 1992), variable tolerance of different AM-fungal species (Dodd and Jeffries, 1989; Schreiner and Bethlenfalvay, 1997), and to variations in host plant species and soils used (Spokes et al., 1981) has led to many contradictions in the literature and does not allow us to predict whether a particular fungicide will reduce mycorrhizae formation in a given soil. For example, Benomyl is considered to be particularly toxic to AM fungi, given its potent effect on AM spore germination in agar (Carr and Hinkley, 1985) and in soil (Schreiner and Bethlenfalvay, 1997) and on root colonization in numerous pot and field

studies (Bailey and Safir, 1978; Carey et al., 1992; Perrin and Plenchette, 1993). In this study, however, Benomyl was less detrimental to AM fungi than was Captan, a fungicide with less consistent effects towards AM fungi (Trappe et al., 1984). Although our best host-plant responses occurred in the two AM associations least affected by fungicides, there was a general lack of correspondence between shoot responses and those of root colonization by AM fungi.

This lack of a strict relationship between inhibition of the endophyte and growth depression of the host plant did not permit a clear separation of fungicide effects on the components of the AM symbiosis. Captan was more detrimental to the host plant than PCNB, even though it was less inhibitory to the fungi. Such findings suggest that the host plant itself may mediate fungicide effects differently for given fungus–fungicide combinations (Ocampo, 1993), while in other cases, as here with PCNB in the Gm and Gr treatments, direct inhibition of AM fungi prevails. Some AM fungi, like Ge in our study, may be less sensitive to fungicides than others. However, one cannot generalize such findings as they may only apply to the host plant and the host soil of a specific experiment.

Host, endophyte and soil responses to fungicides in our multiple species treatment compared with the

individual fungus treatments were a salient feature of our results. Similarities between the host plant and AM-fungal responses in the Mix and Ge treatments, coupled with the large number of Ge spores produced in the Mix indicated that Ge was dominant among the colonists of the fungus-root in the Mix treatment. As in the Ge treatment, the fungi in the Mix treatment as a whole were more tolerant of fungicides than Gm or Gr. Importantly, however, the spore densities of each isolate (including Ge) were less affected by fungicides when grown together than in isolation. As a 'community' the fungi appeared to withstand fungicide stress better than each isolate alone. This is consistent with the ecological axiom that stability depends on diversity (Tilman and Downing, 1994; Naeem et al., 1994), such that increases in species richness can provide the necessary flexibility to withstand greater stress. While we cannot provide a specific mechanism why our mixed inoculum of AM fungi was better equipped to handle the negative effect of fungicides, others have found more consistent plant growth benefits with multiple species inocula of AM fungi than single isolates when tested under a variety of conditions with different inherent stresses (Daft and Hogarth, 1983; Koomen et al., 1987). This finding serves to warn against inferences drawn from single-fungus experiments to field conditions where fungal communities are the rule.

Mycorrhizal fungi are arguably the most important component of the soil microflora in developing sustainable agricultural practices, because they enhance plant growth and nutrient uptake while at the same time stabilizing soil aggregates, making the soil less susceptible to erosion (Schreiner and Bethlenfalvay, 1995). Unlike with plant responses, where Ge and Mix effects were similar, Mix effects on soil aggregate stability were enhanced above the levels produced by Gm or Gr, the better individual soil-aggregating fungi. This finding suggests that soil colonization (Jakobsen et al., 1992) and exudation (Tisdall, 1994) patterns and characteristics of different AM fungi can act in concert in the binding or stabilizing of soil particles into aggregates (Miller and Jastrow, 1992). When our experiment is evaluated by both host plant and soil structure criteria, the Mix treatment (which was equal to Ge in plant responses alone) was superior to the other treatments

investigated. Integrating both above- and below-ground responses to AM fungi provides a basis to rethink our definition of endophyte efficiency.

Our findings show that different AM fungi affect their host plant and host soil differently, and further, that a community of fungi acting in concert is likely to provide greater benefit to the plant-soil system than a single species. Under our conditions, Ge and Gr were effective symbionts for enhancing plant growth and nutrient uptake, but Ge was not effective in stabilizing soil aggregates, while Gm and Gr were effective soil stabilizing fungi, but Gm was not effective in enhancing nutrient uptake. However, when all three fungi were present, a high level of plant growth promotion and nutrient uptake was maintained with the added benefit of greater soil aggregate stabilization. The fact that plant growth and nutrient uptake were not reduced in the Mix as a consequence of greater aggregate stabilization, suggests that a community of AM fungi was more efficient than single isolates under conditions of fungicide stress. Whether the same result would have occurred had fungicides not been used in this experiment is unclear, but our sporulation data support the idea that it was the added tolerance to fungicides within the community of fungi that allowed for greater soil aggregate stability without a concomitant loss in plant growth promotion.

## References

- Abbott, L.K., Gazey, C., 1994. An ecological view of the formation of VA mycorrhizas. *Plant Soil* 159, 69–78.
- Bailey, J.E., Safir, G.R., 1978. Effect of benomyl on soybean endomycorrhizae. *Phytopathology* 68, 1810–1812.
- Bethlenfalvay, G.J., Schüepp, H., 1994. Arbuscular mycorrhizae and agrosystem stability. In: S. Gianinazzi, H. Schüepp (Eds.), *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Birkhäuser, Basel, pp. 117–131.
- Bethlenfalvay, G.J., Brown, M.S., Franson, R.L., Mihara, K.L., 1989. The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae*. *Physiol. Plant.* 76, 226–232.
- Carey, P.D., Fitter, A.H., Watkinson, A.R., 1992. A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. *Oecologia* 90, 550–555.
- Carr, G.R., Hinkley, M.A., 1985. Germination and hyphal growth of *Glomus caledonicum* on water agar containing benomyl. *Soil Biol. Biochem.* 17, 313–316.

- Daft, M.J., Hogarth, B.G., 1983. Competitive interactions amongst four species of *Glomus* on maize and onion. *Trans. Br. Mycol. Soc.* 80, 339–345.
- Dodd, J.C., Jeffries, P., 1989. Effect of fungicides on three vesicular–arbuscular mycorrhizal fungi associated with winter wheat (*Triticum aestivum* L.). *Biol. Fertil. Soils* 7, 120–128.
- Dodd, J.C., Gianinazzi-Pearson, V., Rosendahl, S., Walker, C., 1994. Banque Européenne des Glomales: a necessary tool for mycorrhizal research. In: S. Gianinazzi, H. Schüepp (Eds.), *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Birkhäuser, Basel, pp. 41–60.
- Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytol.* 120, 371–380.
- Jarstfer, A.G., Sylvia, D.M., 1993. Inoculum production and inoculation strategies for vesicular–arbuscular mycorrhizal fungi. In: F.B. Metting, Jr. (Ed.), *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*. Marcel Dekker, New York, pp. 349–377.
- Johnson, N.C., Pflieger, F.L., 1992. Vesicular–arbuscular mycorrhizae and cultural stresses. In: G.J. Bethlenfalvy, R.G. Linderman (Eds.), *Mycorrhizae in Sustainable Agriculture*. ASA Spec. Publ. No. 54, American Society of Agronomy, Madison, WI, pp. 71–99.
- Kemper, W.D., Rosenau, R.C., 1986. Aggregate stability and size distribution. In: A. Klute (Ed.), *Methods of Soil Analysis*. Part 1. Physical and Mineralogical Methods, 2nd edn. American Society of Agronomy, Madison, WI, pp. 425–442.
- Koomen, I., Grace, C., Hayman, D.S., 1987. Effectiveness of single and multiple mycorrhizal inocula on growth of clover and strawberry plants at two soil pHs. *Soil. Biol. Biochem.* 19, 539–544.
- Machlis, L., Torrey, J.G., 1956. *Plants in Action*. Freeman, San Francisco, CA.
- McGonigle, T.P., 1988. A numerical analysis of published field trials with vesicular–arbuscular mycorrhizal fungi. *Funct. Ecol.* 2, 473–478.
- Miller, M., McGonigle, T., Addy, H., 1994. An economic approach to evaluate the role of mycorrhizas in managed ecosystems. *Plant Soil* 159, 27–35.
- Miller, R.M., Jastrow, J.D., 1992. The role of mycorrhizal fungi in soil conservation. In: G.J. Bethlenfalvy, R.G. Linderman (Eds.), *Mycorrhizae in Sustainable Agriculture*. ASA Spec. Publ. No. 54, American Society of Agronomy, Madison, WI, pp. 29–44.
- Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H., Woodfin, R.M., 1994. Declining biodiversity can alter the performance of ecosystems. *Nature* 368, 734–737.
- Newman, E.I., 1966. A method of estimating the total length of root in a sample. *J. Appl. Ecol.* 3, 139–145.
- Ocampo, J.A., 1993. Influence of pesticides on VA mycorrhizae. In: J. Altman (Ed.), *Pesticide Interactions in Crop Production*. CRC Press, Boca Raton, FL, pp. 213–226.
- Perrin, R., Plenchette, C., 1993. Effect of some fungicides applied as soil drenches on the mycorrhizal infectivity of two cultivated soils and their receptiveness to *Glomus intraradices*. *Crop Prot.* 12, 127–132.
- Schreiner, R.P., Bethlenfalvy, G.J., 1995. Mycorrhizal interactions in sustainable agriculture. In: A. Varma (Ed.), *Arbuscular Mycorrhizae: Biotechnological Applications: An Environmental Sustainable Biological Agent*. Crit. Rev. Biotechnol. 15, 271–285.
- Schreiner, R.P., Bethlenfalvy, G.J., 1997. Mycorrhizae, biocides, and biocontrol 3. Effects of three fungicides on developmental stages of three AM fungi. *Biol. Fertil. Soils* 24, 18–26.
- Schreiner, R.P., Bethlenfalvy, G.J., 1996. Mycorrhizae, biocides, and biocontrol 4. Response of a mixed culture of arbuscular mycorrhizal fungi and host-plant to three fungicides. *Biol. Fertil. Soils* 23, 189–195.
- Secilia, J., Bagyaraj, D.J., 1992. Selection of efficient vesicular–arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.). *Biol. Fertil. Soils* 13, 108–111.
- Sieverding, E., 1991. Vesicular–Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn.
- Smith, S.E., Robson, A.D., Abbott, L.K., 1992. The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. *Plant Soil* 146, 169–179.
- Spokes, J.R., MacDonald, R.M., Hayman, D.S., 1981. Effects of plant protection chemicals on vesicular–arbuscular mycorrhizas. *Pestic. Sci.* 12, 346–350.
- STSC, 1991. *Statgraphics Statistical Graphics System*. Version 5.0. STSC Inc., Rockville, MD.
- Tilman, D., Downing, J.A., 1994. Biodiversity and stability in grasslands. *Nature* 367, 363–365.
- Tisdall, J.M., 1994. Possible role of soil microorganisms in aggregation of soils. *Plant Soil* 159, 115–121.
- Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. *J. Soil Sci.* 33, 141–163.
- Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S., Gollotte, A., 1993. In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal fungi. *Mycol. Res.* 97, 245–250.
- Trappe, J.M., Molina, R., Castellano, M., 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. *Annu. Rev. Phytopathol.* 22, 331–359.
- Wilson, D.O., 1988. Differential plant response to inoculation with two VA mycorrhizal fungi isolated from a low-pH soil. *Plant Soil* 110, 69–75.
- Yocom, D.H., 1985. VA mycorrhizae and host plant reproduction: a study with green peppers. In: R. Molina (Ed.), *Proc. of the 6th North American Conference on Mycorrhizae*, Forest Research Laboratory, Oregon State University, Corvallis, OR, pp. 298–299.