

# Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield

E.P. Boswell, R.T. Koide<sup>\*</sup>, D.L. Shumway<sup>1</sup>, H.D. Addy

*Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA*

Accepted 25 June 1997

## Abstract

The relationships among winter cover cropping, inoculum potential of vesicular–arbuscular mycorrhizal (VAM) fungi, and the growth and yield of a subsequent maize crop were investigated. In the first experiment, an autumn-sown winter wheat cover crop increased VAM fungal inoculum potential of a field soil as measured by an in situ maize bioassay during the following growing season. Infective extra-radical hyphal densities were significantly increased by cover cropping as interpreted from the effect of soil disturbance on infection of the maize bioassay plants. In a second experiment the following year, the winter wheat cover crop again increased VAM fungal inoculum potential as assessed by an in situ maize bioassay during the following growing season. Moreover, the degree of mycorrhizal infection of maize was correlated with maize growth and yield. This study suggests that the management of mycorrhizal fungi by cover cropping may be a useful practice in sustainable agriculture. © 1998 Elsevier Science B.V.

*Keywords:* VA mycorrhizal inoculum potential; Cover crop; Extra-radical hyphae; *Triticum aestivum* (winter wheat); Crop yield

## 1. Introduction

Currently there is some emphasis on the use of so-called ‘sustainable’ agricultural practices such as reduced tillage, diverse crop rotation, reduced chemical inputs, and management of the soil microflora. In particular, vesicular–arbuscular mycorrhizal (VAM) fungi might be profitably managed to maximize nutrient availability with reduced nutrient inputs (Miller

et al., 1994; Thompson, 1994b). VAM fungi form symbioses with many agriculturally important plants and can increase the uptake of nutrients such as P and Zn from the soil. The management of VAM fungi may be useful because VAM fungal inoculum potential can be reduced significantly during fallow periods, or when nonmycorrhizal plant species occur in the crop rotation. The consequence of reduced inoculum potential may be a significant reduction in nutrient uptake and yield of subsequent mycorrhizal crops (Black and Tinker, 1977; Thompson, 1987, 1994a).

Crop systems that include winter fallow periods may be stressful to mycorrhizal fungi. During the autumn, winter and early spring, the absence of host plants eliminates the energy available for growth of

<sup>\*</sup> Corresponding author. 102 Tyson, Department of Horticulture, Penn State University, University Park, PA 16802, USA. Tel.: +1-814-863-0710; fax: +1-814-863-6139; e-mail: rxk13@psu.edu

<sup>1</sup> Current address: Department of Biology, Frostburg State University, Frostburg, MD 21532, USA.

VAM fungi that are strictly biotrophic. Moreover, freezing and thawing of the bare soil can physically disrupt the extra-radical hyphae. Winter cover crops have been used to suppress weeds, reduce soil erosion and nitrate leaching, and increase nitrogen fixation and soil organic matter (Lal et al., 1991). Winter cover crops can also serve as mycorrhizal fungal hosts, and thus maintain or increase VAM fungal inoculum potential through the winter (Dodd and Jeffries, 1986). Indeed, mycorrhizal winter cover cropping has been shown to increase VAM fungal spore densities when compared to fallowing over the winter (France et al., 1984; Galvez et al., 1995). The density of infected root pieces must also increase as a consequence of winter cover cropping. Moreover, in the autumn and spring, cover crops may support the growth of extra-radical hyphae because the development of VAM infections and hyphal growth can occur even at temperatures close to freezing (Chilvers and Daft, 1982; Boswell and Koide, unpublished). It is generally accepted that rapid infections of emerging seedlings can be caused by contact with extra-radical hyphae (Read et al., 1976; Brundrett et al., 1985). As such, extra-radical hyphae may be particularly important for the establishment phase of seedlings, allowing them to become rapidly established into a preexisting nutrient-absorptive structure.

This study tested the hypothesis that planting a mycorrhizal winter cover crop will result in higher VAM fungal inoculum potential compared to fallowing, resulting in improved yield of a subsequent crop. In two separate experiments, winter wheat cover crop were grown, and their inoculum potential were assessed with an in situ maize bioassay during the subsequent growing seasons. The second experiment also assessed the effects of cover cropping on growth and yield of a maize crop.

## 2. Materials and methods

### 2.1. Experiment 1 (1994–1995)

On 11 May 1994 a 0.2-ha agricultural field (Field #102 of the Horticulture Farm, R.A. Larson Agricultural Research Center at Rock Springs, PA, USA) was fumigated by injection with a mixture of chloropicrin and methyl bromide (33:67) at a rate of

560 kg ha<sup>-1</sup> to destroy existing VAM fungi. The soil is a Hagerstown silty clay loam with a bicarbonate-extractable P concentration of approximately 8.0 µg P g<sup>-1</sup>. On 10 June, five 4-m<sup>2</sup> blocks were established. Each block was inoculated with soil from pot cultures containing *Glomus intraradices* Shenck and Smith (with approximately 30 spores and an unknown number of infected roots per g air dry soil) at approximately 10 cm depth at a density of 850 g m<sup>-2</sup>. The fungus had originally been supplied by Native Plants, (NPI, Salt Lake City, UT, USA). Each block was then sown with seeds of *Abutilon theophrasti* Medic. (Valley Seed Service, Fresno, CA, USA) at a density of 210 plants m<sup>-2</sup> to produce an early successional annual vegetation capable of supporting high levels of mycorrhizal infection (Stanley et al., 1993). The resulting plant density at maturity was approximately 24 shoots m<sup>-2</sup>. On 19 September, two soil cores (3.5-cm dia.) were taken randomly from each block to determine the fractional VAM infection of *Abutilon* roots. Roots were rinsed free from soil, cleared with 10% KOH, then stained with trypan blue. Fractional infection was determined using a grid intersect technique (Koide and Mooney, 1987). Standing shoots of *Abutilon* plants were removed on 5 October.

Each of the five blocks were then divided into two plots each. One randomly selected plot was left fallow, referred to hereafter as the no cover crop (NCC) plot. The other was sown by hand with seeds of winter wheat (*Triticum aestivum* L. cv. 'Pennmore' [Cecil J. Irvin and Son, State College, PA, USA]) in furrows approximately 5 cm deep and 18 cm apart. The winter wheat plot is hereafter referred to as the cover crop (CC) plot. Wheat emergence was observed on 17 October 1994. The average density of wheat shoots was 392 m<sup>-2</sup>. Wheat root samples were collected on 24 October 1994, 29 November 1994, 3 February 1995 and 23 May 1995 by excavating randomly selected wheat root systems. They were washed, cleared and stained, and fractional VAM infection was determined as above. On 31 May 1995, standing winter wheat shoots were mowed, and the shoot residue was removed. Regrowth of some wheat shoots required the application of glyphosate on 5 June to all plots.

Soil temperature was measured with a bimetal soil thermometer on both plots of each block at 8 cm

depth, approximately every two weeks from October 1994 until April 1995. Two soil cores (3.5-cm dia.) were also taken randomly from both plots of each block on 24 October 1994, 3 February 1995 and 6 June 1995. VAM fungal spores were extracted by wet-sieving (Gerdemann and Nicolson, 1963) and centrifugation (Jenkins, 1964). Spores were quantified and identified as *Glomus intraradices* by color and diameter (Schenck and Smith, 1982b).

In addition to the contribution of spores to inoculum potential, the importance of extra-radical hyphae was investigated. The contribution of extra-radical hyphae to infection has been assessed by comparing the infection of bioassay plants in disturbed and undisturbed soil (O'Halloran et al., 1986; Evans and Miller, 1988; Jasper et al., 1989a,b). Therefore, on 2 June 1995, before sowing maize bioassay plants, a 1 × 2-m area randomly located in both the CC and NCC plots within each block was disturbed by turning over to a depth of 25 cm with a flat spade, and then rototilling to a depth of 20 cm. On 6 June 1995, soil cores (3.5-cm dia.) were taken from undisturbed CC and NCC plots at two depths (0–4 and 4–7 cm) for the calculation of bulk density.

Before sowing, maize seeds (*Zea mays* L. cv. 'Bodacious' [Rupp Seeds, Waliseon, OH, USA]) were rinsed in water to remove the fungicide (captan-thiram-imazilil) applied by the supplier. The seeds were sown on 5 June 1995 using a manually operated seeder in rows 25 cm apart with in-row spacing of 20 cm at a depth of 5 cm across all blocks. The resulting density of maize was much higher than in standard maize fields to have a sufficient number of bioassay plants in each plot. Emergence was observed on 11 June. To determine the effect of cover cropping on inoculum potential, two bioassay plants were randomly selected from undisturbed CC and NCC plots on 19 June. Entire root systems were excavated, and the fractional VAM infection was determined as above. Two seedlings were also removed from disturbed CC and NCC plots on 19 June for estimation of infective hyphae (disturbance-sensitive propagules, see above) by difference from undisturbed plots. Mycorrhizal infection was assessed on 19 June (8 d after emergence), because rapidly forming infections are thought to be caused primarily by extra-radical hyphae and not by spores (Brundrett et al., 1985; McGee, 1989).

The effect of cover cropping on spore density, soil bulk density, soil temperature and fractional mycorrhizal infection of bioassay plants was determined from comparisons of undisturbed CC and NCC plots using a single factor analysis of variance (STSC, 1991). Data were appropriately transformed when necessary, but tables and figures report untransformed means and errors. Treatment differences were considered statistically significant when  $P \leq 0.05$ . A measure of the amount of infective extra-radical hyphae was calculated within both CC and NCC plots by subtracting the fractional infection in disturbed soil from the fractional infection in undisturbed soil.

## 2.2. Experiment 2 (1995–1996)

This experiment was conducted on a 0.1-ha field (Field #108 of the Horticulture Farm, R.A. Larson Agricultural Research Center). The soil is a Hagerstown silty clay loam with a bicarbonate-extractable P level of approximately  $10 \mu\text{g P g}^{-1}$ . This level is generally considered to be deficient for maize (Agricultural Analytical Services Laboratory of the Pennsylvania State University). In contrast with the field in Experiment 1, Field #108 was not fumigated, so the indigenous mycorrhizal fungi were present. Although they were not systematically quantified, the majority of the spores present in this soil were of the *Glomus* type. In autumn 1994, the entire field was planted to winter wheat (*Triticum aestivum* var. Pennmore) to establish a similar level of inoculum potential across the entire field. The wheat was harvested the following summer. In August 1995, the field was plowed, disked and harrowed. On 6 September 1995, six blocks were established, each containing two 100-m<sup>2</sup> plots, and winter wheat was planted in one randomly selected plot (CC) of each block using a tractor-mounted seeder. The other plot (NCC) of each block was left fallow over the winter. On 5 June 1996, the wheat in the CC plots was cut back to stubble, and the herbicide glyphosate was applied to the entire field.

As in Experiment 1, to estimate the amount of infective VAM extra-radical hyphae in CC and NCC plots, the difference in fractional infection of maize bioassay plants growing in undisturbed and disturbed portions of each plot was calculated. Thus, on 17

June 1996, before sowing the maize, a randomly selected half of both the CC and NCC plots in each block was disturbed to a depth of approximately 30 cm using a rototiller mounted on a tractor.

On 24 June, maize seeds (*Zea mays* cv. Bodacious) were sown using a tractor-mounted seeder in all blocks at a rate of 65 000 seeds ha<sup>-1</sup>. Nitrogen was added as NH<sub>4</sub>NO<sub>3</sub> at a rate of 280 kg ha<sup>-1</sup> (approximately 100 kg N ha<sup>-1</sup>). Shoot and root samples were taken 10 days after emergence (on 5 July) when the plants were at the 3-leaf stage. Five seedlings were selected at random from each plot, and the entire root system for each seedling was excavated from the soil. They were washed and then stored in formaldehyde–acetic acid–ethanol (FAA). The roots from the five seedlings were bulked to give one sample per plot. The root samples were cleared in 10% KOH in an autoclave for 15 min and stained with trypan blue at room temperature overnight. Fractional mycorrhizal infection was assessed by the line intersect method (Koide and Mooney, 1987). Maize shoots were washed, dried at 65°C for 48 h, and ground. The ground shoot tissue was acid-digested at 400°C for colorimetric analysis of P (Watanabe and Olsen, 1965) and N concentrations (Jensen, 1962).

Eight weeks after emergence (on 20 August 1996), the height of six plants selected at random from each plot was recorded. Three of these plants were excavated using a shovel, and a subsample of fine roots was collected from each plant. The roots were washed, bulked and stored in FAA, then cleared and stained as above. On 10 September, the flag leaves and ears were collected from seven plants selected at random in each plot. The leaves were washed, dried, ground and pooled to give one sample per plot. The fresh ears were dried at approximately 80°C to constant weight. Grain was removed from the dry cobs and weighed. Leaf samples were analyzed for N and P concentrations as above. Leaf tissue was also analyzed for other elements (see results) by the Agricultural Analytical Services Laboratory of the Pennsylvania State University using the techniques of Dahlquist and Knoll (1978).

The effects of cover cropping on fractional mycorrhizal infection of maize plants and maize growth and yield components were determined from comparisons of undisturbed CC and NCC plots using a

single factor analysis of variance (STSC, 1991). Data were appropriately transformed when necessary for statistical analysis, but tables and figures report untransformed means and errors for ease of comparison. Treatment differences were considered statistically significant when  $P \leq 0.05$ . A measure of the amount of infective extra-radical hyphae was calculated for both CC and NCC plots by subtracting the fractional infection in disturbed soil from the fractional infection in undisturbed soil.

A linear correlation analysis was performed to reveal potentially significant relationships between fractional mycorrhizal infection of maize plants (on 5 July 1996) and height (on 20 August), yield (on 10 September), shoot P concentration (on 5 July), or flag leaf P concentration (on 10 September), and between height and yield using the correlation analysis procedure of the Statgraphics Plus Programs (STSC, 1991). In these analyses, there were 24 data points (six blocks, two cover crop treatments and two disturbance treatments).

### 3. Results

#### 3.1. Experiment 1

By 19 September 1994 roots of *Abutilon* plants supported substantial fractional mycorrhizal infections (mean  $\pm$  se = 30.4%  $\pm$  2.9). The fractional infection of the subsequent winter wheat cover crop plants was initially low but was approximately 12% just before removal in the summer of 1995 (Fig. 1a). Soil temperature at 8-cm depth was significantly affected by cover crop treatment only on the last two sampling dates (4 and 25 April 1995) when average soil temperatures were higher in the NCC plots (Fig. 1b). Soil bulk density from 0–4 cm in the CC plots (1.45  $\pm$  0.06 g cm<sup>-3</sup>) was not significantly different from that in the NCC plots (1.49  $\pm$  0.05 g cm<sup>-3</sup>). Soil bulk density from 4–7 cm in the CC plots (1.64  $\pm$  0.03 g cm<sup>-3</sup>) was not significantly different from that in the NCC plots (1.61  $\pm$  0.07 g cm<sup>-3</sup>).

On 24 October 1994, the VAM fungal spore density was significantly higher in the CC plots than in the NCC plots, but by June 1995 when the maize was planted, there was no significant effect of the cover crop on spore density (Fig. 1c). Eight days

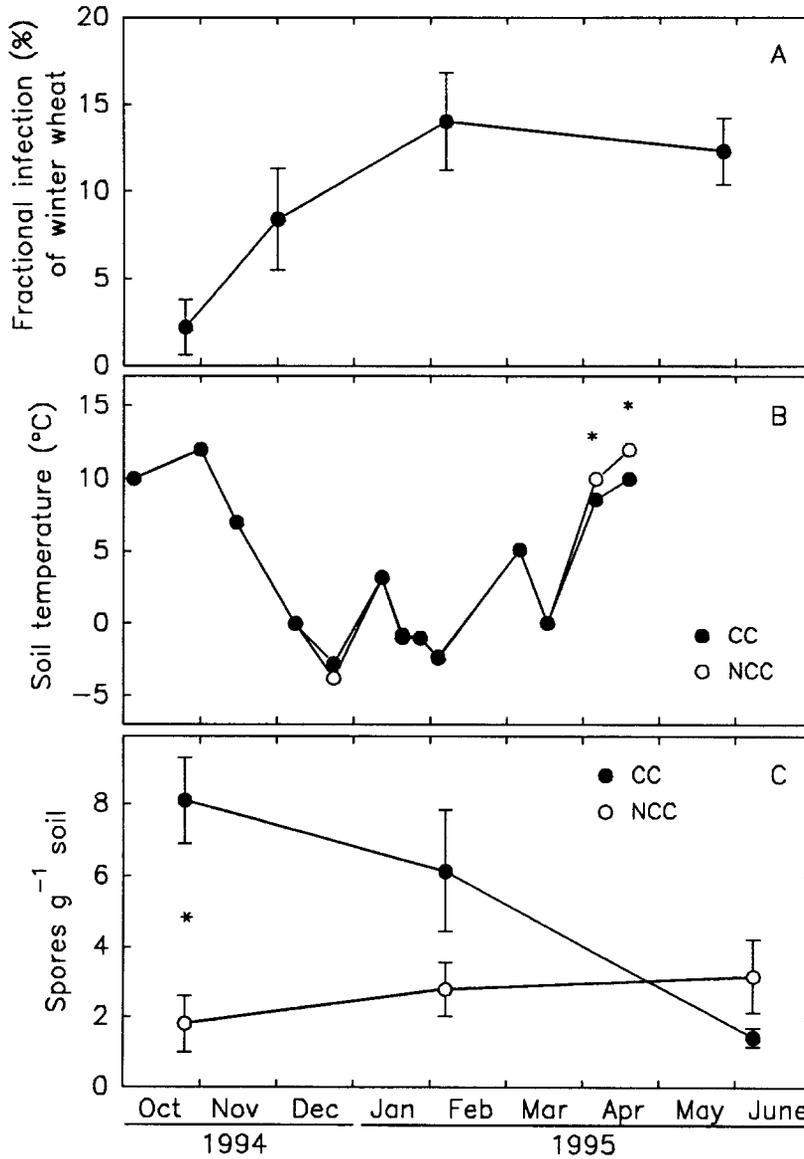


Fig. 1. (a) Mean percent mycorrhizal infection of winter wheat cover crop plants in Experiment 1. (b) Mean soil temperature at 8-cm depth in cover crop (CC) and control (NCC) plots in Experiment 1. (c) Mean vesicular-arbuscular mycorrhizal fungal spores g<sup>-1</sup> dry soil in cover crop and control plots in Experiment 1. Asterisks indicate that treatment had a significant ( $p < 0.05$ ) effect. Vertical bars are  $\pm 1$  se. Vertical bars are hidden by the symbol in (b).  $n = 5$ .

after emergence of the maize, total root length of the seedlings in the CC plots was not significantly different from that in the NCC plots (Table 1). However, the fractional mycorrhizal infection was significantly higher in the CC plots than in the NCC plots

(Table 1). Thus, cover cropping significantly increased VAM inoculum potential.

To estimate the contribution to inoculum potential by extra-radical hyphae, the difference between the fractional infections of corn growing in disturbed

Table 1

Mean ( $\pm$ se) of calculated variables in Experiment 1 eight days after emergence of maize bioassay plants

| Treatment | Mycorrhizal colonization (%) | Total root length (cm) | U – D (% infection)          |
|-----------|------------------------------|------------------------|------------------------------|
| CC        | 83.4 $\pm$ 3.2 <sup>a</sup>  | 108.3 $\pm$ 12.1       | 45.6 $\pm$ 7.4 <sup>a</sup>  |
| NCC       | 32.4 $\pm$ 5.2 <sup>b</sup>  | 99.1 $\pm$ 11.3        | -1.6 $\pm$ 11.6 <sup>b</sup> |

Different superscripts indicate a significant ( $p \leq 0.05$ ) difference between treatment means.

U – D indicates the difference in fractional mycorrhizal infection between maize plants in the undisturbed and disturbed portions of each plot.

 $n = 5$ .

Table 2

Mean ( $\pm$ se) of calculated variables in Experiment 2 ten days after emergence of maize plants

| Treatment | Mycorrhizal colonization (%) | Total root length (cm) | U – D (% infection)         |
|-----------|------------------------------|------------------------|-----------------------------|
| CC        | 81.7 $\pm$ 1.5 <sup>a</sup>  | 167.5 $\pm$ 13.1       | 32.5 $\pm$ 2.6 <sup>a</sup> |
| NCC       | 21.3 $\pm$ 3.3 <sup>b</sup>  | 211.9 $\pm$ 19.4       | 11.5 $\pm$ 2.4 <sup>b</sup> |

U – D indicates the difference in fractional mycorrhizal infection between maize plants in the undisturbed and disturbed portions of each plot.

 $n = 6$ .Different superscripts indicate a significant ( $p \leq 0.05$ ) difference between treatment means.

Table 3

Mean ( $\pm$ se) of growth and yield variables for maize plants grown in Experiment 2

| Treatment | Height (cm)              | Ears plant <sup>-1</sup>     | Grain dry weight ear <sup>-1</sup> (g) | Grain dry weight plant <sup>-1</sup> (g) |
|-----------|--------------------------|------------------------------|--|--|
| CC        | 192 $\pm$ 3 <sup>a</sup> | 1.55 $\pm$ 0.07 <sup>a</sup> | 11.8 $\pm$ 1.5 <sup>a</sup>            | 18.4 $\pm$ 2.5 <sup>a</sup>              |
| NCC       | 139 $\pm$ 5 <sup>b</sup> | 1.29 $\pm$ 0.06 <sup>b</sup> | 7.1 $\pm$ 0.8 <sup>b</sup>             | 9.0 $\pm$ 0.9 <sup>b</sup>               |

Different superscripts indicate a significant ( $p \leq 0.05$ ) difference between treatment means.

Heights were measured on 20 August 1996 and yield components were assessed on 10 September 1996.

 $n = 6$ .

and undisturbed areas of each plot was calculated. This difference was significantly greater in the CC plot than in the NCC plot, indicating that the contribution of disturbance-sensitive propagules (which presumably includes hyphae) was significantly greater in CC plots (Table 1). Indeed, the value for NCC plots was not significantly different from zero, indicating that there was not a significant amount of disturbance-sensitive propagules in the NCC plots.

### 3.2. Experiment 2

Ten days after emergence, total root length of the maize seedlings in the CC plots was not significantly different from that in the NCC plots (Table 2). However, the fractional mycorrhizal infection was

significantly higher in the CC plots than in the NCC plots (Table 2). Thus, as in Experiment 1, cover cropping did significantly increase inoculum potential.

To estimate the contribution to inoculum potential by extra-radical hyphae, the difference between the fractional infections of corn growing in disturbed and undisturbed areas of each plot was again calculated. This difference was significantly greater in the CC plot than in the NCC plot, indicating that the contribution of disturbance-sensitive propagules was significantly greater in CC plots (Table 2). In this experiment, the values for both CC and NCC plots were significantly greater than zero, indicating that both plots contained significant amounts of disturbance-sensitive propagules. Eight weeks after emer-

Table 4  
Mean ( $\pm$ se) of nutrients concentrations of flag leaves harvested 10 September 1996 (Experiment 2)

| Treatment | N (%)         | P (%)                        | K (%)           | Ca (%)          | Mg (%)                       | Fe ( $\mu\text{g g}^{-1}$ ) | Mn ( $\mu\text{g g}^{-1}$ ) | Zn ( $\mu\text{g g}^{-1}$ ) | B ( $\mu\text{g g}^{-1}$ ) | Cu ( $\mu\text{g g}^{-1}$ ) | Al ( $\mu\text{g g}^{-1}$ ) |
|-----------|---------------|------------------------------|-----------------|-----------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|
| CC        | 2.2 $\pm$ 0.0 | 0.31 $\pm$ 0.01 <sup>a</sup> | 1.42 $\pm$ 0.02 | 0.74 $\pm$ 0.02 | 0.22 $\pm$ 0.02 <sup>a</sup> | 45.0 $\pm$ 0.9 <sup>b</sup> | 55.7 $\pm$ 4.1              | 51.8 $\pm$ 1.4              | 9.3 $\pm$ 0.3              | 4.5 $\pm$ 0.3               | 3.0 $\pm$ 0.4               |
| NCC       | 2.4 $\pm$ 0.1 | 0.26 $\pm$ 0.01 <sup>b</sup> | 1.44 $\pm$ 0.04 | 0.76 $\pm$ 0.01 | 0.16 $\pm$ 0.01 <sup>b</sup> | 51.0 $\pm$ 1.7 <sup>a</sup> | 61.3 $\pm$ 6.1              | 44.7 $\pm$ 4.0              | 9.3 $\pm$ 0.2              | 5.0 $\pm$ 0.5               | 3.7 $\pm$ 0.9               |

Different letters indicate a significant ( $p \leq 0.05$ ) difference between treatment means.  
 $n = 6$ .

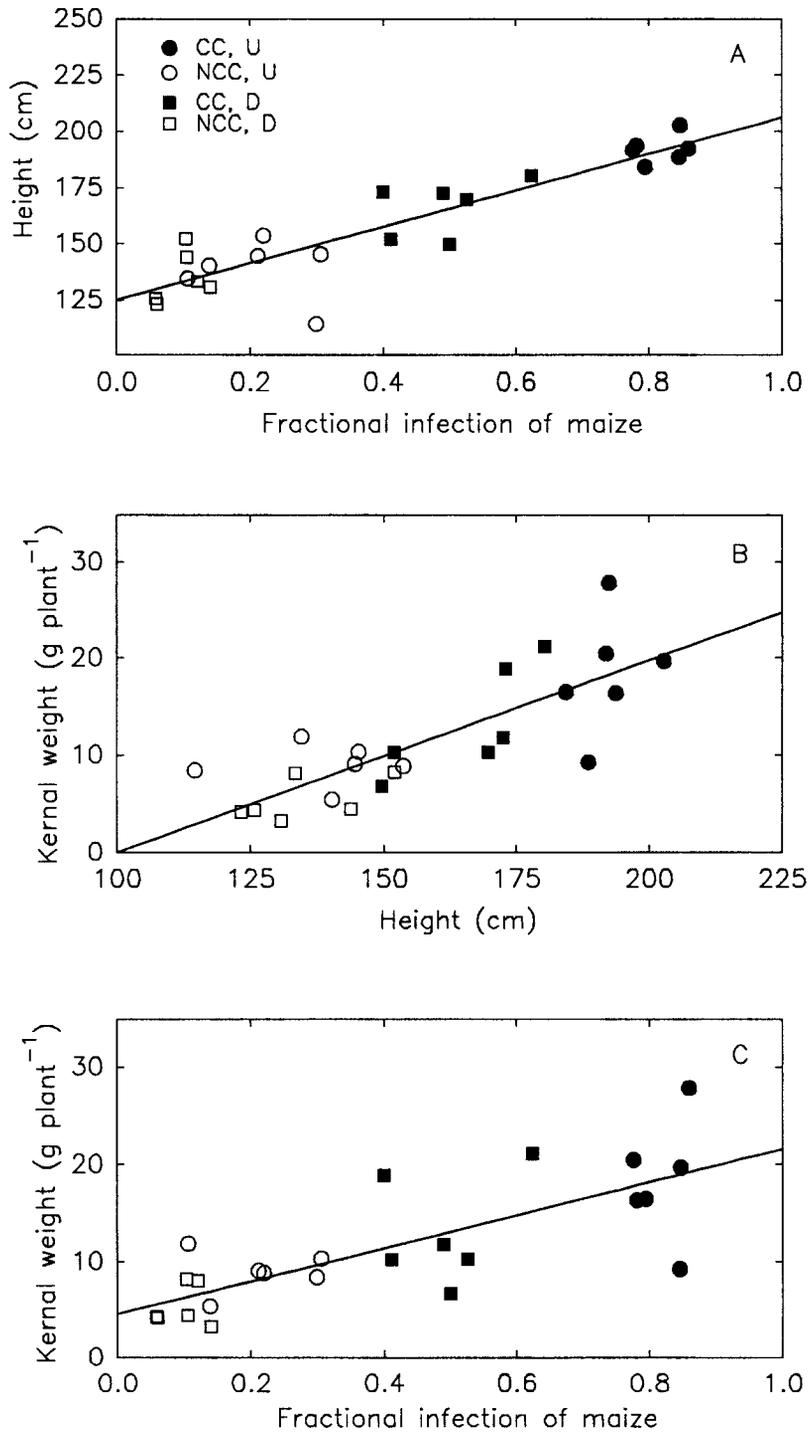


Fig. 2. (a) Significant linear relationship between fractional infection of maize on 5 July 1996 and height on 20 August 1996 (Experiment 2);  $r = 0.9072$ . (b) Significant linear relationship between height and kernel weight per plant (Experiment 2);  $r = 0.7962$ . (c) Significant linear relationship between fractional infection of maize on 5 July 1996 and kernel weight per plant (Experiment 2);  $r = 0.7676$ . For a, b and c,  $n = 24$  (6 replicate blocks, 4 treatment combination plots per block including undisturbed cover crop (CC, U) and control (NCC, U) plots and rototilled cover crop (CC, D) and control (NCC, D) plots).

gence (20 August), the fractional infection of maize was still significantly higher in CC plots ( $64\% \pm 5$ ) than in NCC plots ( $47\% \pm 5$ ).

Maize growth was significantly greater on CC plots as indicated by height (Table 3). Yield was also increased on CC plots (Table 3). Cover cropping significantly increased the number of ears per plant (by 20%), the grain dry weight per ear (by 66%) and thus significantly increased the grain dry weight per plant (by 104%). Although the flag leaf N, K, Ca, Mg, Mn, Zn, B Cu, and Al concentrations for the CC plots were not significantly different from those in the NCC plots, the P and Mg concentrations were significantly higher in the CC plots, and the Fe concentrations were significantly higher in the NCC plots (Table 4).

Fractional mycorrhizal infection of maize plants on 5 July 1996 was significantly correlated with the height of mature plants (measured 20 August; Fig. 2a). Height was significantly correlated with yield (kernel dry weight per plant, Fig. 2b). Thus, fractional mycorrhizal infection was significantly correlated with yield (Fig. 2c). Fractional mycorrhizal infection of 5 July, however, was not significantly correlated with either shoot P concentration (on 5 July) or flag leaf P concentration (on 10 September).

#### 4. Discussion

These experiments show that a winter wheat cover crop sown in the autumn can increase the VAM fungal inoculum potential in the following growing season. Similar results were given in Galvez et al. (1995). Galvez et al. (1995), however, showed an increase in spore density because of cover cropping. Here, Experiment 1 showed that there was an initial increase in spore density as a consequence of some aspect of cover cropping; apparently, this was not the cause of the increased inoculum potential the following spring because the effect was only transient and did not persist until planting of the maize. It is possible that the increase in spore density in the CC plots was merely a consequence of soil disturbance associated with the sowing of the cover crop. A similar disturbance effect on spore production was reported by Krucklemann (1975).

In the present experiments, the increase in inoculum potential from cover cropping appeared to be

caused by an increase in the contribution of extra-radical hyphae, at least in part. The effect of cover cropping on the density of infected root pieces was not assessed. Of all mycorrhizal fungal propagules in the soil, extra-radical hyphae are generally considered to be the most sensitive to physical disturbance (Evans and Miller, 1988, 1990; McGonigle et al., 1990). A significant difference in infection between bioassay plants in disturbed and undisturbed soils, therefore, is consistent with the presence of infective hyphae. These differences were significantly greater in CC plots than in NCC plots in both Experiments 1 and 2. Cover cropping, therefore, appears to have significantly increased the density and/or infectivity of extra-radical hyphae. In Experiment 1, the fungus involved was *Glomus intraradices*. Although the fungi in Experiment 2 were predominately *Glomus* spp., they were not identified to species. Thus, although it is likely that there was a different VAM fungal species composition in the two experiments, the results were fundamentally similar. The fact that there was a significant amount of disturbance-sensitive VAM fungal propagules in the NCC plots in Experiment 2 but not in Experiment 1 in the spring, may have been because the prewinter host plants varied (*Abutilon* in Experiment 1, wheat in Experiment 2); thus, the amount of hyphae before the winter may also have varied.

Cover cropping can reduce soil temperature (Galvez et al., 1995) by reducing radiative heating of the soil surface. This certainly has the potential to reduce mycorrhizal infection of subsequent crops because infection often proceeds more quickly at higher temperatures (Furlan and Fortin, 1973; Smith and Bowen, 1979; Shenck and Smith, 1982a; Smith and Roncadori, 1986). Despite the slight, but significant decrease in soil temperature associated with cover cropping in Experiment 1, in both experiments mycorrhizal infection of maize plants was increased by cover cropping. Thus, the positive effects of cover cropping outweighed the potential negative effect of decreased soil temperature as far as inoculum potential was concerned. Soil bulk density might also be influenced by cover cropping which may, in turn, influence mycorrhizal infection (Sylvia and Williams, 1992). However, in Experiment 1, there were no significant effects of cover cropping on soil bulk density.

It was expected that any increase in mycorrhizal infection would be translated into an increase in maize growth and yield because the soil was considered to be P-deficient for maize growth, and because mycorrhizal infection can increase P uptake. Indeed, the significant correlations between infection and growth or yield suggest a causal relationship between infection and growth or yield. Although flag leaf P concentrations were significantly higher in maize plants from the CC plots, there was no significant correlation between fractional mycorrhizal infection, and either shoot P concentration (on 5 July) or flag leaf P concentration (on 20 September). Unfortunately, the shoot P concentrations were assessed very early (10 d after emergence, 5 July) and very late (at harvest, 20 September), and it may have been that the most relevant time to assess the P status of the maize was at some intermediate time. The relationship between mycorrhizal infection and growth or yield in this study, however, was not necessarily mediated through P uptake. There are other potentially important effects of mycorrhizal fungi. For example, mycorrhizal hyphae can have strong effects on soil structure (Miller and Jastrow, 1992; Bethlenfalvay and Schüepp, 1994), the loss of which can severely restrict yield (Doyle and Hamlyn, 1960; De Boodt et al., 1961). Others have also suggested that mycorrhizal infection can affect plant performance in a manner not related to nutrient uptake, perhaps in some cases because of a reduction in disease or positive interactions with other soil microorganisms (Fitter and Garbaye, 1994). Although significant differences between maize plants growing in CC and NCC plots were recorded for leaf Mg and Fe concentrations, these elements are not likely to be involved in the effects on growth and yield. Values of both elements for CC and NCC plants were well within the adequate range as determined by the Agricultural Analytical Service Laboratory of the Pennsylvania State University.

Although the mechanism is presently unresolved, the results show a strong and significant relationship between the amount of rapidly forming mycorrhizal infection (10 d after emergence) and subsequent growth and yield. This suggests that the maintenance of a viable mycelium by practices such as cover cropping may be usefully incorporated into soil management practices.

## Acknowledgements

The authors acknowledge the financial support of the US National Science Foundation and the A.W. Mellon Foundation. Thanks are expressed to the staff of the Horticulture Farm of the R.E. Larson Agricultural Center, Rock Springs, PA for their assistance, and Professor Julie Whitbeck for helpful discussion.

## References

- Bethlenfalvay, G.J., Schüepp, H., 1994. Arbuscular mycorrhizas and agrosystem stability. In: Gianinazzi, S., Schüepp, H. (Eds.), *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Birkhäuser Verlag, Basel, 226 pp.
- Black, R.L.B., Tinker, P.B., 1977. Interactions between effects of vesicular–arbuscular mycorrhiza and fertilizer phosphorus on yields of potatoes in the field. *Nature* 267, 510–511.
- Brundrett, M.C., Piché, Y., Peterson, R.L., 1985. A developmental study of the early stages in vesicular–arbuscular mycorrhiza formation. *Can. J. Bot.* 63, 184–194.
- Chilvers, M.T., Daft, M.J.F., 1982. Effects of low temperature on development of the vesicular–arbuscular mycorrhizal association between *Glomus caledonium* and *Allium cepa*. *Trans. Brit. Mycol. Soc.* 79, 153–157.
- Dahlquist, R.L., Knoll, J.W., 1978. Inductively coupled plasma atomic emission spectrometer: analysis of biological materials and major, trace and ultratrace elements. *Appl. Spectrosc.* 39, 1–29.
- De Boodt, M., De Leenheer, L., Kirkham, D., 1961. Soil aggregate stability indexes and crop yields. *Soil Sci.* 91, 138–146.
- Dodd, J.C., Jeffries, P., 1986. Early development of vesicular–arbuscular mycorrhizas in autumn sown cereals. *Soil Biol. Biochem.* 8, 149–154.
- Doyle, J.J., Hamlyn, F.G., 1960. Effects of different cropping systems and of a soil conditioner (VAMA) on some soil physical properties and on growth of tomatoes. *Can. J. Soil Sci.* 40, 89–98.
- Evans, D.G., Miller, M.H., 1988. Vesicular–arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize: I. Causal relations. *New Phytol.* 110, 67–74.
- Evans, D.G., Miller, M.H., 1990. The role of the extra-radical mycelial network in the effect of soil disturbance upon vesicular–arbuscular mycorrhizal colonization of maize. *New Phytol.* 114, 65–71.
- Fitter, A.H., Garbaye, J., 1994. Interactions between mycorrhizal fungi and other soil organisms. In: Robson, A.D., Abbott, L.K., Malajczuk, N. (Eds.), *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*. Kluwer Academic Publishers, Dordrecht, 238 pp.
- France, R.C., Coleman, M.D., Cline, M.L., 1984. Cover crops to increase inoculum in the field. In: Molina, R. (Ed.), *Proc. of*

- the 6th North American Conf. on Mycorrhizas, Oregon State Univ., Corvallis, pp. 92–94.
- Furlan, V., Fortin, J.-A., 1973. Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three temperature regimes. *Naturaliste Can.* 100, 467–477.
- Galvez, L., Douds, D.D. Jr., Wagoner, P., Longnecker, L.R., Drinkwater, L.E., Janke, R.R., 1995. An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. *Am. J. Altern. Agric.* 10, 152–156.
- Gerdemann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal endogone species extracted by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* 46, 235–244.
- Jasper, D.A., Abbott, L., Robson, A., 1989a. Soil disturbance reduces the infectivity of external hyphae of vesicular–arbuscular mycorrhizal fungi. *New Phytol.* 112, 93–99.
- Jasper, D.A., Abbott, L., Robson, A., 1989b. Hyphae of a vesicular–arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. *New Phytol.* 112, 101–107.
- Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Rep.* 73, 288–300.
- Jensen, W.A., 1962. *Botanical Histochemistry*. Freeman, San Francisco, CA, 408 pp.
- Koide, R.T., Mooney, H.A., 1987. Spatial variation in inoculum potential of vesicular–arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytol.* 107, 173–182.
- Krucklemann, H.W., 1975. Effect of fertilizers, soils, soil tillage and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infections in arable soils. In: Sanders, F.E., Mosse, B., Tinker, P.B. (Eds.), *Endomycorrhizas*. Academic Press, London, pp. 511–525.
- Lal, R., Regnier, E., Eckert, D.J., Edwards, W.M., Hammond, R., 1991. Expectations of cover crops for sustainable agriculture. In: Hargrove, W.L. (Ed.), *Cover Crops for Clean Water*. Soil and Water Conservation Soc. Ankeny, pp. 1–11.
- McGee, P.A., 1989. Variation in propagule numbers of vesicular–arbuscular mycorrhizal fungi in a semiarid soil. *Mycol. Res.* 92, 28–33.
- McGonigle, T.P., Evans, D.G., Miller, M.H., 1990. Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. *New Phytol.* 116, 629–636.
- Miller, M., McGonigle, T., Addy, H., 1994. An economic approach to evaluate the role of mycorrhizas in managed ecosystems. In: Robson, A.D., Abbott, L.K., Malajczuk, N. (Eds.), *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*. Kluwer, Dordrecht, pp. 27–35.
- Miller, R.M., Jastrow, J.D., 1992. The role of mycorrhizal fungi in soil conservation. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), *Mycorrhizae in Sustainable Agriculture*. ASA Special Publication 54, Am. Soc. of Agronomy, Madison, 124 pp.
- O'Halloran, I.P., Miller, M.H., Arnold, G., 1986. Absorption of P by corn (*Zea mays* L.) as influenced by soil disturbance. *Can. J. Soil Sci.* 66, 287–302.
- Read, D.J., Koucheki, H.K., Hodgson, J., 1976. Vesicular–arbuscular mycorrhizas in natural vegetation systems: I. The occurrence of infection. *New Phytol.* 77, 641–653.
- Shenck, N.C., Smith, G.S., 1982a. Responses of six species of vesicular–arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. *New Phytol.* 92, 193–201.
- Schenck, N.C., Smith, G.S., 1982b. Additional new and unreported species of mycorrhizal fungi (*Endogonaceae*) from FL. *Mycologia* 74, 77–92.
- Smith, G.S., Roncadori, R.W., 1986. Responses of three vesicular–arbuscular mycorrhizal fungi at four soil temperatures and their effects on cotton growth. *New Phytol.* 104, 89–95.
- Smith, S.E., Bowen, G.D., 1979. Soil temperature, mycorrhizal infection and nodulation of *Medicago truncatula* and *Trifolium subterraneum*. *Soil Biol. Biochem.* 11, 469–473.
- Stanley, M.R., Koide, R.T., Shumway, D.L., 1993. Mycorrhizal symbiosis increases growth, reproduction and recruitment of *Abutilon theophrasti* Medic. in the field. *Oecologia* 94, 30–35.
- STSC, 1991. *Statgraphics Statistical Graphics System Version 5.1*. STSC, Rockville, MD.
- Sylvia, D.M., Williams, S.E., 1992. Vesicular–arbuscular mycorrhizae and environmental stress. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), *Mycorrhizae in Sustainable Agriculture*. ASA Special Publication 54, Am. Soc. of Agronomy, Madison, 124 pp.
- Thompson, J.P., 1987. Decline of vesicular–arbuscular mycorrhizae in long-fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. *Aust. J. Agric. Res.* 38, 847–867.
- Thompson, J.P., 1994a. Inoculation with vesicular–arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. *Soil Biol. Biochem.* 26, 1133–1143.
- Thompson, J.P., 1994b. What is the potential for management of mycorrhizas in agriculture? In: Robson, A.D., Abbott, L.K., Malajczuk, N. (Eds.), *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*. Kluwer, Dordrecht, pp. 191–200.
- Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and  $\text{NaHCO}_3$  extracts from soil. *Soil Sci. Soc. Proc.* 29, 677.