Effect of Mycorrhizal Inoculation and Compost Supply on Growth and Nutrient Uptake of Young Leek Plants Grown on Peat-based Substrates

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Abstract. Organic horticultural production systems often are characterized by the use of beneficial soil microorganisms because the application of soluble inorganic P or N fertilizers is not endorsed. Due to the limited supply of soluble nutrients in organic production systems, nutrient deficiency may limit plant growth and yield. The sole use of peat for pot-based cultures is also discouraged in organic production systems. Therefore, we have studied viable alternatives for highly soluble fertilizers and pure peat substrates using leek [Allium ampeloprasum L. var. Porrum] as a test plant. Plants were grown on peat-based substrates with different rates of compost additions, and with and without inoculation with arbuscular mycorrhizal (AM) fungi. Inoculation with a commercial AM fungus inoculum resulted in colonization rates of up to 70% of total root length, whereas not inoculated plants remained free of root colonization. Mycorrhizal fungus colonization increased shoot Zn and K concentrations, but did not significantly affect shoot dry matter or shoot N and P concentrations. In contrast, compost addition increased plant growth, and also increased P and K concentrations in plants. We conclude that plants with high rates of mycorrhizal colonization can be obtained on peat-based substrates, but that under these conditions plants may not consistently benefit in growth from the mycorrhizal symbiosis. In contrast, additions of compost are a possible means to improve the substrate quality in organic horticultural production.

Peat-based substrates are widely used in horticulture to produce seedlings for out-planting or to grow commercial crops. These substrates are usually supplemented with soluble fertilizers in conventional production systems to achieve optimal supply of nutrients such as N and P. The use of synthetic chemical fertilizers is discouraged in organic horticulture. The activity of soil microorganisms should contribute to the mobilization of mineral nutrients in the soil (Herrmann and Plakolm, 1991). It is sometimes assumed that conventional methods of applying highly soluble nutrients in combination with pesticides may have a negative effect on plant quality for human consumption (Asami et al., 2003).

The use of peat is also critically viewed for other reasons by organic growers. Peat is a limited natural resource and use of peat at the present rates is not sustainable (George and Eghbal, 2003; Joosten and Clarke, 2002).

Official guidelines for organic growers, presented, e.g., by the European Union (2004) and organic growers associations in many countries, mandate the use of organic or nonsoluble fertilizers and a reduction of peat amendments to growth substrates to a maximum of 70% in the next few years (George and Eghbal, 2003). This results in problems for producers, because many vegetable and ornamental plants have a high nutrient demand for satisfactory growth and yield. In addition, often only high quality vegetable products or ornamental plants without any deficiency symptoms can be marketed.

For the long-term economic success of ecological greenhouse horticulture, it is therefore important a) to reduce the amount of peat in pot cultures without loss of plant quality, and b) to define methods to improve nutrient supply from organic sources.

Various substitute materials have been tested to replace peat at least partly in growth substrates. Such substitute materials consist of bark, coconut residues (Linderman and Davis, 2003a), other biosolids (Ozores-Hampton et al., 1999), or compost (Veeken et al., 2004). Compost has been widely used in traditional agriculture and horticulture and has beneficial effects, for example, on soil structure or soil biota (Carpenter-Boggs et al., 2000; Wells et al., 2000). Compost applications were avoided in many modern greenhouse horticultural systems due to a risk of transmitting plant diseases with compost applications. However, high quality composts, e.g., produced from organic household waste, can be almost free of pathogenic micro-organisms and may even have a suppressive effect on soil born diseases (Schüler et al., 1989). High quality composts also have a high nutrient content. A substrate of 20% high quality compost mixed with peat is therefore recommended for current practice of organic horticulture in Germany and Switzerland (George and Eghbal, 2003).

An improvement of the plant nutrient status in organic operations may require the application not only of composts, but also of other organic fertilizers. In addition, a living component, i.e., rhizosphere or soil micro-organisms, may help the plants to mobilize and acquire nutrients from the substrate. A group of soil micro-organisms that live in very intimate contact with the root are the arbuscular mycorrhizal (AM) fungi. These fungi are known to assist the plant in the uptake of nutrients and to improve plant growth (Douds et al., 2005), including growth of Allium species (Dickson et al., 1999), on soils low in phosphorus (P). They occur both in natural ecosystems and in agricultural soils (Smith and Read, 1997). AM fungus colonization often leads to increased plant uptake of P, nitrogen (N), zinc (Zn), and copper (Cu), and sometimes also of potassium (K) (George, 2000). Phosphate from organic fertilizers may be particularly accessible to AM colonized plants (Linderman and Davis, 2004). In addition, much published evidence shows disease suppression in plants due to colonization by AM fungi (Kasiamdari et al., 2002). Mycorrhizal fungi can also stabilize soil aggregates (Piotrowski et al., 2004), and some reports show that mycorrhizal plants may be more resistant to stresses such as drought (Neumann and George, 2004) or salinity (Tian et al., 2004). Plant phytohormone levels can also be affected by mycorrhizal fungus colonization (Shaul-Keiman et al., 2002).

Only a few studies have investigated the effect of compost supplements on mycorrhizal and nonmycorrhizal plant seedlings. Substrates with composts may be adequate for mycorrhizal plants (Goswani and Jamaluddin, 2001; Linderman and Davis, 2001), if the quality of the compost is sufficient (Boddington and Dodd, 2000; Raviv et al., 1998). Sainz et al. (1998), however, pointed out that compost additions may reduce mycorrhizal root length colonization and therefore the activity of AM fungi. Thus, at present it is not clear whether compost additions and mycorrhizal fungus inoculation are complementary measures to increase yield and stability in organic operations.

Therefore, we used leek as a test plant in two experiments studying whether a) commercial or specifically prepared peat-based substrates support AM fungus colonization of plants, b) AM fungus colonization is beneficial to plants on these substrates, and c) compost additions affect the contribution of AM fungi to plant growth. The aim was to increase the understanding of the role of AM fungi in plant growth on organic substrates, and to advise producers on optimal compost and AM fungi addition treatments.

Material and Methods

Overview on experimental design and cultivation. Seeds of leek (Allium ampeloprasum L. var. Porrum ‘Prelina’) were placed in a commercial potting mix (KKS Bio-Potgrond, Klasmann-Deilmann GmbH, Geeste-Grossen).
in a climate chamber. In Expt. 1, a commercial potting substrate was used. In Expt. 2, two substrates with different addition rates of compost (20% compost; 40% compost) were used (see below). The substrates were inoculated with one of three different types of AM fungus inocula (Pla, Bio, Tri) or remained without AM fungi (NAM). Five replications were used for each treatment in both experiments. The first experiment was planted in 2002, and the second experiment in a climate chamber.

Drip irrigation (40 mL min⁻¹) was used once a day in the climate chamber (total of 40 mL) and twice a day in the greenhouse (total of 40 to 80 mL depending on weather conditions) to maintain favorable water conditions in the substrate. Once a week the substrate was soaked with water to equalize the water content of the pots. The pots were standing on saucers, and nutrient loss through leaching was prevented. Experiment 1 was carried out from 30 Aug. to 23 Oct. 2002 in a greenhouse facility at Großbreiten (long. 13°19′60″E; lat. 51°22′0″N), Germany. Average air temperatures in the greenhouse during this time were 21 to 24 (maximum 30 °C) day/17 to 20 °C night and relative humidity was on average 70%. For Expt. 2, a climate chamber was used with a light period of 16 h/day 8 h night, a temperature of 22 °C day/18 °C night, and a relative humidity of 70%/day 80%/night. Light intensity provided by lamps (Agro Son T 400, Phillips, Hamburg, Germany) was between 450 and 600 μmol m⁻² s⁻¹ at different positions in the chamber. Pots were re-arranged in regular intervals in both experiments. Pots were always arranged in a completely randomised design.

Substrate preparation and characterization. All substrates used in this study were suitable for organic production. In Expt. 1, a commercial substrate (KKS Bio-Potgrond, Klaasmann-Deilmann GmbH, Geeste-Gross Hesepe, Germany) was used that contained 80% v/v sphagnum peat (black peat) and 20% v/v compost of green residues. The substrate also contained clay material, lime, horn meal and Thomas phosphate. This substrate is commonly used by organic growers in Germany. The extractable nutrients (extraction by CaCl₂, [N] and CAL [P, K]; C: Bruns, personal communication). The substrate had a salt content of 2.8 g L⁻¹ and had a pH (CaCl₂) of 6.9. The compost was mixed with sphagnum peat from the Baltic region (white peat) to obtain a compost substrate with 20% and 40% compost by volume. The substrates were limed with CaO to a pH of 6.2 and sieved to 5 mm. The compost substrate was of similar or higher horticultural quality as the commercial substrate (KKS Bio-Potgrond) used in Expt. 1.

Addition of 20% compost supplemented N at 5 mg L⁻¹, P at 67 mg L⁻¹, and K at 347 mg L⁻¹ to the substrate (C. Bruns, personal communication). In addition, N fertilizer was added to the substrate 1 d before the start of the experiment. The N fertilizer (a mixture of 33% horn meal 0 to 2 mm, containing 10% N, and 66% horn meal 2 to 6 mm, containing 14% N) was uniformly mixed into the substrate (7600 mg L⁻¹). Previous experience (C. Bruns, personal communication) suggests that two weeks after planting 25% of the added N was available to the plants, and that 8 weeks after planting 85% of the added N was available. Therefore, the plant available N content of the compost substrate together with the horn meal fertilizer added up to 200 mg L⁻¹ (50 mg pot) in the first 2 weeks after planting. The 40% compost substrate was fertilized with less N fertilizer (7400 mg L⁻¹), to account for the higher input of nutrients by the increased compost addition.

Water-holding capacity. The maximum water-holding capacities of all the substrates were evaluated following the method of Schaller (1988): 50 g of the substrate was filled into a glass tube that was closed with fine gauze, and left soaking in water over night to absorb water through capillary rise. Shortly before the end of incubation time, water was raised in the surrounding vessel until water was visible at the soil surface. The surplus water dripped out when the tubes were allowed to stand on moist sand, allowing for the measurement of the maximum water-holding capacity (WC in g). The maximum water-holding capacity (WC in %) was calculated with the weight of the dried substrate (DW in g) at 150 °C for 12 h on a DUMAS method (Elementar Vario EL, Hanau, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar, Hanau, Germany).

Mycorrhizal fungus colonization of roots was determined following the method of Koske and Gemma (1989) with slight modifications. Roots were cleared with 10% KOH, acidified with 2 N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization was determined with a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 50× using the grid line intersection method (Giovannetti and Mose, 1980).

Statistics. Data in Expt. 1 were subjected to a one-way analysis of variance, with inoculum type as treatment levels (n = 5). Mean separation was carried out with the Newman–Keuls method (P < 0.05). In Expt. 2, data were analyzed by a two-factorial analysis of variance, with compost addition rates and mycorrhizal inoculation as experimental factors (n = 5). Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, Okla.) software.

Results

Experiment 1. Roots were colonized by AM fungi in treatments with live mycorrhizal inoculum (Fig. 1). The percentages of colonized root length in AM plants were between 20 and 30%, but were not significantly different between the three different mycorrhizal inocula. The treatment without live mycorrhizal inoculum (NAM) remained free of mycorrhizal root colonization.

Shoot (Table 1) and root (data not shown) dry weights were not significantly affected by the inoculation treatments. Similarly, AM fungus root colonization had no significant effect on shoot N, P, and Zn concentrations. In
contrast, K shoot concentrations were increased in mycorrhizal plants (Table 1). The highest K concentration was measured in shoots of the Pla treatment.

Experiment 2. The percentage root length colonization with AM fungi in Expt. 2 was higher (t test; *P* < 0.05) in 20% compost than in 40% compost (Fig. 1). Highest root colonization rates were observed in 20% compost, in the Bio treatment. However, root colonization was not significantly different between the three live mycorrhizal inocula in 40% compost. As in Expt. 1, NAM plant roots remained free of AM fungi.

Shoot (Table 2) and root (data not shown) dry weights were not significantly affected by the treatments. Shoot dry weight was much higher in Expt. 2 (Table 2) than in Expt. 1 (Table 1). Shoot N, Zn and Cu concentrations were not significantly affected by the compost treatments. Shoot P and K concentrations were increased in the 40% compost treatment compared to the 20% compost treatment. Shoot Zn concentrations were significantly increased in mycorrhizal compared to nonmycorrhizal plants at 20% compost supply (Table 2). At 40% compost supply, shoot of plants in the Bio treatment had the highest Zn and Cu concentrations.

Discussion

Compost addition to peat can be a source of plant nutrients and at the same time contribute to the protection of global peat resources. Compost addition rates of 20% [v/v] to a peat based substrate are now in use for commercial substrates. The present results show that a compost addition rate of 40% can also be recommended. Plants had increased P and K uptake on these substrates, and plant element concentrations did not indicate any risk of toxicity. Compost used for this purpose must be low in salt content, and of course should also be free of contamination with heavy metals or organic toxins. Compared to standard solutions, the present results show that more N from horn meal became available during this time.

Table 1. Experiment 1 (commercial substrate): Shoot dry weight and shoot element (N, P, K, and Zn) concentrations of leek plants 8 weeks after planting. Plants were either noninoculated with mycorrhizal fungi (NAM), or were inoculated with one of three mycorrhizal inocula (Pla, Bio, Tri). Effects of the compost addition rate = C; mycorrhizal inoculation = M) were tested with a two-way ANOVA. Different letters denote significant differences between means as determined by the Student-Newman-Keuls test (*P* < 0.05). Values are means of five observations ± SE (I).

| Dry wt (g/pot) | Element concn (mg·kg⁻¹ DW) | N | P | K | Zn |
|---------------|-----------------|---|---|---|---|
| **NAM**       | 1.1 ± 0.1       | 13.0 ± 0.3 | 2.9 ± 0.1 | 16.3 ± 1.2 | 32.2 ± 2.6 |
| **Pla**       | 1.2 ± 0.0       | 13.6 ± 0.6 | 2.7 ± 0.1 | 35.8 ± 2.1 | 38.4 ± 2.3 |
| **Bio**       | 0.9 ± 0.1       | 12.2 ± 0.5 | 3.1 ± 0.0 | 25.9 ± 0.6 | 35.4 ± 1.6 |
| **Tri**       | 1.0 ± 0.2       | 14.1 ± 0.5 | 3.2 ± 0.2 | 23.8 ± 1.6 | 38.4 ± 3.9 |
| **P** (myc)   | 0.225           | 0.070       | 0.104       | <0.001      | 0.350       |

Table 2. Experiment 2 (peat substrate): Shoot dry weight and shoot element (N, P, K, and Zn) concentrations of leek plants 8 weeks after planting. Plants were either noninoculated with mycorrhizal fungi (NAM), or were inoculated with one of three mycorrhizal inocula (Pla, Bio, Tri). Effects of the compost addition rate = C; mycorrhizal inoculation = M) were tested with a two-way ANOVA. Different letters denote significant differences between means as determined by the Student-Newman-Keuls test (*P* < 0.05). Values are means of five observations ± SE (I).

| Dry wt (DW) | Element concn (mg·kg⁻¹ DW) | N | P | K | Zn |
|-------------|-----------------|---|---|---|---|
| **NAM**     | 5.6 ± 0.3       | 9.2 ± 0.3 | 0.9 ± 0.0 | 11.9 ± 0.5 | 14.2 ± 0.7a | 2.3 ± 0.2a |
| **Pla**     | 5.3 ± 0.3       | 10.8 ± 0.7 | 0.9 ± 0.0 | 13.1 ± 0.7 | 21.4 ± 2.3b | 2.4 ± 0.4a |
| **Bio**     | 5.8 ± 0.4       | 9.4 ± 0.7 | 1.0 ± 0.1 | 10.8 ± 0.4 | 23.2 ± 1.6b | 2.8 ± 0.4a |
| **Tri**     | 6.0 ± 0.8       | 10.1 ± 0.6 | 1.1 ± 0.0 | 11.0 ± 1.5 | 26.4 ± 2.2b | 2.2 ± 0.2a |
| **P** (myc) | 0.157           | 0.174       | <0.001       | 0.572       | <0.001      | 0.018       |
| **P** (M)   | 0.108           | 0.295       | 0.279       | 0.573       | <0.001      | 0.018       |
| **P** (C × M) | 0.061        | 0.578       | 0.024       | 0.183       | 0.102       | 0.669       |
material used for compost preparation, and high temperatures during composting further reduce the number of live mycorrhizal propagules. It is likely, that this finding applies in general to peat–compost substrates. If producers plan to use mycorrhizal plants on organic potting substrates, for example because of superiority of mycorrhizal plants in disease resistance or flowering ability, the application of mycorrhizal inoculum is necessary.

All three commercial inocula used successfully colonized the roots. The extent of root colonization was different between the inocula only in Expt. 2 (20% compost), and this difference was not clearly related to different effects of the inocula on plant activity. For example, although Bio inoculum caused the highest colonization rate in this case, shoot Zn concentrations were not higher in Bio plants than in Pla or Tri plants. Thus, the present data indicate that the use of all three types of inocula can be recommended, but further tests with more inocula and under various environmental conditions are required to generalize this result.

Root colonization by Bio and Tri inocula was greater in 20% compost versus 40% compost (Fig. 1), very likely because of the lower level of nutrients (especially P) supplied in the 20% compost treatment versus 40%. Only the supply of N, but not of other nutrients, was equilibrated between 20% and 40% compost addition rate. A decrease of colonization with increased supply of mineral nutrients, especially of P (Boddington and Dodd, 2000; Douds et al., 2003), or with addition of certain types of compost or peat (Linderman and Davis, 2003b; Sänz et al., 1998; Wang et al., 1993), has often been observed. It is also possible that the higher water capacity of the 40% compost substrate had some effect on mycorrhizal colonization. Some AM fungi show a lower hyphal growth in moist soils (Smith and Read, 1997).

Under the conditions of both experiments, plant dry weight was not affected by mycorrhizal fungus colonization, although colonization rates were considerable. Decreases of shoot growth upon mycorrhizal colonization are sometimes observed, in particular at sub-optimal light conditions for plant growth (Smith and Gianinazzi-Pearson, 1990). In the present experiments, light supply was probably sufficient to allow for carbon fixation to sustain carbon expenses for mycorrhizal fungus colonization without negative impact on plant growth. Increases in shoot growth upon mycorrhizal colonization are often observed on substrates with low nutrient availability, in particular on substrates with low P availability. In the present experiment, plants had low N shoot concentrations (Tables 1 and 2) and probably were limited in growth mainly by low N availability in the substrate. AM fungus hyphae can transport N to the plant, but not to an extent that N deficiency of fast-growing plant species can be overcome (Hawkins and George, 1999). It is surprising, however, that P concentrations were not increased in mycorrhizal plants compared to nonmycorrhizal counterparts. Most evidence of increased P uptake in mycorrhizal plants comes from experiments and observations on mineral soils. Some evidence indicates that also freshly applied organic P sources can be used by AM fungi (Feng et al., 2003). However, it is possible that plant P uptake from organic substrates such as peat or compost is less dependent on AM fungus colonization than P uptake from soils with mineral P sources. Compost may contain P sources that are either readily accessible to plants, or are inaccessible to plants and AM fungi alike through physico-chemical fixation in form of condensed calcium phosphates such as apatites or octacalcium phosphates (Frossard et al., 2002; Grey and Henry, 1999).

Alternatively, the AM fungi contained in the commercial inocula used in this study may be specifically adapted to P supply conditions in mineral soil.

Hyphae of AM fungi can transport not only N and P (George et al., 1992), but also Cu (Li et al., 1991), Zn and probably K (George, 2000). This can lead to increased K and Zn concentrations in mycorrhizal plants. The present data confirm increased Zn uptake in mycorrhizal compared to nonmycorrhizal plants (Table 2) when the Zn status of nonmycorrhizal plants was relatively low (Expt. 2). When the Zn status of nonmycorrhizal plants was higher (Expt. 1), the effect of AM fungi colonization on Zn uptake was less.

In conclusion this experiment indicated that a) a compost addition rate of 40% in a peat based substrate can produce a growth substrate of high quality for ecological production, b) peat-compost organic substrates did not contain live AM fungus propagules, c) commercial inocula were used successfully to obtain high AM fungus colonization rates of potted plants, d) AM fungus colonization can actively support plant Zn or K uptake on these substrates, and e) plant P uptake and growth were not increased by AM colonization.

Perhaps, P bound in substrates in organic form is less available to many AM fungi than P bound to soil minerals.

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