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## YIELD AND ARBUSCULAR MYCORRHIZA FUNGAL ROOT COLONIZATION OF ORGANICALLY OR MINERALLY FERTILIZED WHEAT GROWN ON A DRY, SANDY SOIL

A.-M. Jarosch<sup>1</sup>, E. Neumann<sup>2</sup>, M. Oltmanns<sup>1</sup> and J. Raupp<sup>1</sup>

<sup>1</sup>IBDF – Institute for Biodynamic Research, Brandschneise 5, D-64295 Darmstadt (Germany), [info@ibdf.de](mailto:info@ibdf.de), [raupp@ibdf.de](mailto:raupp@ibdf.de), +49-6155-8421-0 phone, -25 fax

<sup>2</sup>IGZ – Institute of Vegetable and Ornamental Crops, Theodor-Echtermeyer-Weg 1, D-14979 Großbeeren (Germany), [Neumann@igzev.de](mailto:Neumann@igzev.de), +49-33701-78131 phone, -55391 fax

### **Abstract :**

We investigated wheat yield and arbuscular mycorrhiza (AM) fungal root colonization and spore formation in response to long-term mineral or organic soil fertilization. Plots were either fertilized with high or low amounts of mineral fertilizer or cattle manure since 27 years. At low fertilization levels there was no difference in yield between minerally and organically fertilized plants. High levels of mineral fertilizer application reduced wheat yields. Probable reasons can be that either the high nitrogen availability was less effectively used for grain development of minerally fertilized plants, or the mineral fertilization rendered the plants more sensitive towards drought stress. The extent of AM fungal root colonization appeared to depend on the plant P nutritional status rather than the type of fertilizer applied. The low fertilization treatments showed higher rates of AM root colonization. The number of AM spores, however, was higher in organically compared with minerally fertilized soil, indicating that organic fertilization increased reproduction of these beneficial microbes under dry soil conditions.

### **Introduction :**

The mobility of mineral elements in the soil solution usually decreases with increasing soil dryness (Gahoonia *et al.*, 1994; Marschner, 1995). Moreover, microbial activities and thus mineralization and release of mineral elements from organic resources may be severely retarded in dry soil. In consequence, nutrient supply to organically fertilized plants may be particularly insufficient when the soil is dry. The abundance of beneficial soil microbes like AM fungi can contribute to plant performance under conditions of soil dryness (Augé, 2001). However, not much is known about how different types of fertilizer affect the establishment of the AM symbiosis under dry soil conditions.

The objective of this investigation was, therefore, to compare the effects of farmyard manure and mineral fertilization at different rates on spring wheat performance as well as on AM fungal root colonization and spore formation in a long-term field trial on a dry sandy soil.

### **Materials and Methods :**

The investigations were undertaken on a long-term field experiment in Darmstadt, Germany (49E 50' N, 8E 34' E; 100 m a.s.l.). Field plots with spring wheat (*Triticum aestivum* L., var. Passat) were sampled in 2007. The plots had been either fertilized with composted farmyard manure and biodynamic preparations (CMBD) or with mineral fertilizers (calcium ammonium nitrate, super phosphate, potassium magnesia) (MIN) since 1980. The respective fertilizers were applied at two different rates ('High' and 'Low'), based on their total nitrogen content (Table 1). All fertilization treatments, comprising four replicates, were arranged in a split block design. The soil was a Haplic Cambisol (Table 2). The long-term averages of annual air temperature and precipitation were 9.5 EC and 590 mm, respectively. Weather data of the growth period 2007 are given in Table 3. Spring 2007 was extremely dry. Crops preceding spring wheat were berseem clover (*Trifolium alexandrinum* L., var. Alex) in 2006 and winter rye (*Secale cereale* L., var. Amilo) in 2005. Composted cattle manure was ploughed in the CMBD plots two weeks before sowing. Mineral fertilizers were distributed one week before sowing. At tillering, the 'High' treatments received a second nitrogen rate of 40 kg ha<sup>-1</sup> N, applied as cattle urine (CMBD) or as calcium ammonium nitrate (MIN). Except for fertilization, all other cultivation techniques were the same for all treatments and followed normal organic farming practises. In May and June 23 mm water were spread on the plots. Further details of the experiment were published earlier (Raupp & Oltmanns, 2006).

**Table 1:** Amounts of nitrogen, phosphorus and potassium (kg ha<sup>-1</sup>) applied with manure (CMBD) and with mineral fertilizer (MIN) at different rates

|      |                             | Low rate |    |     | High rate |     |     |
|------|-----------------------------|----------|----|-----|-----------|-----|-----|
|      |                             | N        | P  | K   | N         | P   | K   |
| CMBD | Cattle manure               | 60       | 16 | 115 | 100       | 26  | 191 |
|      | Liquid manure               | -        | -  | -   | 40        | < 1 | 56  |
| MIN  | 1 <sup>st</sup> application | 60       | 22 | 62  | 100       | 44  | 104 |
|      | 2 <sup>nd</sup> application | -        | -  | -   | 40        | -   | -   |

**Table 2:** Main soil characteristics in 0-25 cm depth: organic carbon (% C<sub>org</sub>), pH value (in CaCl<sub>2</sub>), phosphorus and potassium (mg 100 g<sup>-1</sup>)

|      |  | Low rate         |     |     |     | High rate        |     |     |     |
|------|--|------------------|-----|-----|-----|------------------|-----|-----|-----|
|      |  | C <sub>org</sub> | pH  | P   | K   | C <sub>org</sub> | pH  | P   | K   |
| CMBD |  | 0.93             | 6.4 | 4.6 | 5.5 | 0.94             | 6.4 | 5.7 | 9.4 |
| MIN  |  | 0.74             | 6.3 | 3.5 | 4.5 | 0.81             | 6.2 | 6.0 | 6.8 |

According to the usual marketing standards, the grain samples were cleaned after harvest, sieved for a minimum diameter of 2.2 mm and dried until 14% relative humidity. Therefore, shrunk grain is not included in the yield results given below.

Soil samples for the assessment of AM spore abundance and AM fungal root colonization were taken on July 10<sup>th</sup>, 2007, with a bore tube. The soil in 0-5 cm and 5-10 cm depth was analysed separately. Between 0.7 and 1.0 g fresh fine roots were washed from each sample and cut into pieces. The samples were stained with trypan blue in lactic acid (Koske & Gemma, 1989) to assess the colonized root length by a modified intersection method (Tennant, 1975; Kormanik and McGraw, 1982). Spores were isolated from samples of 80 g air dried soil by the wet sieving and decanting method and subsequent sucrose gradient centrifugation (Ianson & Allen, 1986). The spores were counted under a stereoscope on a Petri dish after fixation by the agar film technique (Thomas *et al.*, 1965).

**Table 3:** Air temperature (°C) and sum of precipitation (mm) in the growth period in 2007.

| Month | °C   | mm    |
|-------|------|-------|
| March | 7.3  | 55.8  |
| April | 13.5 | 1.0   |
| May   | 15.5 | 154.5 |
| June  | 18.7 | 86.3  |
| July  | 18.3 | 91.0  |

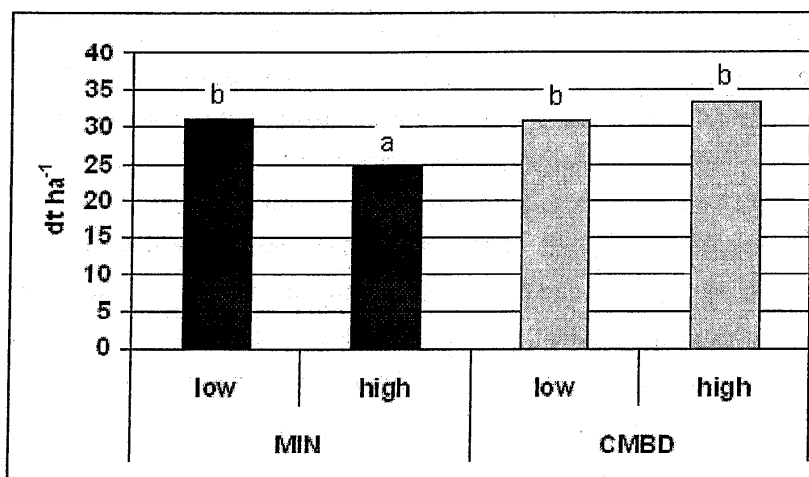
Analytical data were checked for normal distribution and processed by an analysis of variance using the split block model of Federer (1975), example 7.3, as this model represents our experiment exactly. Mean values with different letters are statistically different ( $p < 0.05$ ).

### **Results and Discussion :**

#### *Effect of fertilization on wheat yield and mineral element concentrations in wheat straw*

In 2007 the yield level was approx. 7 dt ha<sup>-1</sup> lower than the long-term average of spring wheat in this experiment, probably because of the low precipitation in March and April (Table 3). Irrigation could not yet be used in this time for technical reasons. As a result of the dry growth conditions crop density was insufficient for achieving better yields than 31-33 dt ha<sup>-1</sup> (Fig. 1). At the high rate of mineral fertilization the yield was even significantly lower than in all other treatments, because this treatment produced a relatively high amount of shrunk grain (data not shown), which is not included in the standardized yield results. The poor grain development of the mineral treatment is also shown by the thousand seed weight. It was lowest with the high rate of MIN, 34 g instead of 38-41 g in the other treatments. Nitrogen

concentration in wheat straw was significantly higher with mineral than with organic fertilization, at both application rates (Table 4). Phosphorus and potassium contents in straw have been increased by the high application rate of both fertilizers.



**Fig. 1:** Grain yield of spring wheat (without broken and shrunk grain in dt ha<sup>-1</sup>) with mineral fertilization (MIN) and composted manure with biodynamic preparations (CMBD), each at 2 rates

This indicates the more easy N availability in the MIN treatment. The intensive nutrient uptake, however, was in-efficient for yield formation of the plants. Earlier investigations with winter rye in this experiment revealed the same situation (Raupp, 2001). Obviously the high nitrogen availability from mineral fertilizer at early growth stages is the reason why cereals create a large capacity of grains that cannot be developed properly in later growth stages because of lack of nutrients and possibly also of water.

**Table 4:** Concentration of nitrogen, phosphorus and potassium (in % dry matter) in straw of wheat plants fertilized with farmyard manure (CMBD) or mineral fertilizer (MIN) at 2 different rates

|           |      | CMBD   | MIN    | av.    |
|-----------|------|--------|--------|--------|
| N<br>% dw | Low  | 0.40 a | 0.56 b | 0.48   |
|           | High | 0.53 b | 0.91 c | 0.72   |
|           | av.  | 0.47   | 0.73   |        |
| P<br>% dw | Low  | 0.10   | 0.11   | 0.11 a |
|           | High | 0.12   | 0.14   | 0.13 b |
|           | av.  | 0.11   | 0.12   |        |
| K<br>% dw | Low  | 0.66   | 0.94   | 0.80 a |
|           | High | 1.11   | 1.38   | 1.25 b |
|           | av.  | 0.89   | 1.16   |        |

**Table 5:** The AM fungal colonized root length in percent of the total root length estimated in two different soil depths.

| Soil depth | CMBD   |        | MIN    |        |
|------------|--------|--------|--------|--------|
|            | Low    | High   | Low    | High   |
| 0 – 5 cm   | 54.2   | 37.3   | 58.7   | 31.5   |
| 5 – 10 cm  | 64.7   | 40.2   | 67.2   | 32.5   |
|            | 59.4 c | 38.8 b | 63.0 c | 32.0 a |

*Effect of fertilization on AM fungal root colonization and spore production*

The AM fungal colonized root length was generally decreased in response to increasing amounts of fertilizer application. The type of fertilizer applied had only a small effect on the extent of AM fungal root colonization. Table 5 indicates that the P nutritional status of the ‘High’ fertilization treatments was higher compared with the ‘Low’ fertilization treatments, irrespective of the type of fertilizer applied. The results of this study thus support earlier findings, indicating that the plant P status is a major determinant of the external AM fungal root colonization.

Spore numbers estimated for this field site (Table 6) were in a relatively high range compared with those found in other field samples, which were usually below 100 per g dry soil (Dandan & Zhiwei, 2007; Mathimaran *et al.*, 2005; Oehl *et al.*, 2004). Since the field site was cropped with mycotrophic plants before the wheat plants were grown, it is possible that not all spores found in the soil samples were produced by the wheat plant mycorrhizae, but also by those of the previous crops. However, there was a significant increase in the number of spores when organic instead of mineral fertilizers were applied. The precise reasons for this effect need to be investigated in more detail. A stimulation of AM sporulation by organic substances like humic acids or chitin has been described previously (Gryndler *et al.*, 2003, 2004), and it is possible that such effects played a role in this study as well. Since spores are the major reproductive organs of AM fungi, our results suggest that (i) AM fungal fitness in terms of reproductive success was higher in organically compared with minerally fertilized soil, and that (ii) organic soil fertilization may increase the AM infective potential over that of minerally fertilized soils.

Spore numbers were higher in the upper 5 cm of the soil compared with soil in 5 to 10 cm depth. This supports earlier findings, where a decreasing number of spores was found with increasing soil depth (Oehl *et al.*, 2004). Increased sporulation at the soil surface may facilitate AM fungal spore dispersal by soil wind and water erosion.

**Table 6:** The number of AM fungal spores per g dry soil<sup>-1</sup>

| Soil depth | CMBD  |      | MIN    |      | Means |
|------------|-------|------|--------|------|-------|
|            | Low   | High | Low    | High |       |
| 0 – 5 cm   | 203   | 223  | 133    | 157  | 179 y |
|            | 213 c |      | 145 ab |      |       |
| 5 – 10 cm  | 154   | 166  | 121    | 137  | 145 x |
|            | 160 b |      | 129 a  |      |       |

Summary and conclusions

Our results suggest that at high fertilization levels, organic fertilizers are superior compared with mineral fertilizers to secure yields under conditions of limited water supply. At low fertilization levels there was no difference in yield between minerally and organically fertilized plots. However, higher numbers of AM spores were found in organically fertilized soil, indicating that fitness and reproduction of beneficial soil microbes was promoted by organic soil fertilization on a dry, sandy soil.

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