Peculiar effect of *Azospirillum* inoculation on growth and nitrogen balance of winter wheat (*Triticum aestivum*)

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Summary. Wheat plants (*Triticum aestivum*) grown in pots and in the field under the Mediterranean climate of the south of France were inoculated with a strain of *Azospirillum brasilense*. Comparisons with non-inoculated plants grown under the same conditions showed significant responses to inoculation with an increase in the number of fertile tillers, shoot and root dry weight, and root to shoot biomass ratio. The roots of inoculated plants attracted relatively more assimilates than those of the control plants until a late stage of growth (heading stage) but the rhizosphere respiration expressed per unit of root growth was not increased by inoculation. Nitrogen yield, both total and in grains, was also enhanced; however, N percentages of all aerial parts of the plants grown in pots were always statistically lower after inoculation than in the control. At maturity, the N % in seeds was 1.81 and 2.45, respectively. The possible mechanisms of this effect of inoculation under the experimental conditions of this study are discussed.

Key words: *Azospirillum brasilense* — *Triticum aestivum* — Inoculation — N and dry matter yield — N percentages in plant parts — Associative N₂ fixation

There have been many reports on the effect of *Azospirillum* inoculation on growth, dry matter and N yield of wheat, both in greenhouses and in the field (Reyners and Vlassak 1982; Kapulnik et al. 1983; Mertens and Hess 1984). Which process may contribute to yield increases of inoculated plants remains to be assessed and depends on the various effects of *Azospirillum* in association with host plants (Okon and Kapulnik 1986).

Two major mechanisms of the association have been reported: Firstly biological nitrogen fixation (Okon 1984) and secondly growth promoting substances (Tien et al. 1979; Vlassak and Reyners 1981) have been considered to contribute to the enhancement of yields. However, the amount of nitrogen fixed in such systems measured with current methods is too small to explain the positive responses so far recorded (Okon 1984). Production of growth substances by either bacteria or inoculated plants is under investigation.

In view of the results quoted in the literature and of the discrepancies concerning the effects of inoculation in wheat, a careful analysis of the main modifications in the carbon and nitrogen dynamics of plants in association with *Azospirillum* was planned. It involved measurements of the main growth components (dry matter, carbon and nitrogen content, root to shoot ratio) as well as the carbon allocation to various plant parts. Exposure of the plants to ¹⁴CO₂ was designed to estimate the carbon consumption by the root systems of inoculated as compared with noninoculated plants.

This paper reports and discusses the main changes in the carbon and nitrogen balance of winter wheat after inoculation.

Materials and methods

Pot and field experiments were conducted on winter wheat (*Triticum aestivum* L. cv. Fidel) inoculated or not with a strain of *Azospirillum brasilense* under the Mediterranean climate of the south of France (Montpellier).
Table 1. Effect of inoculation with *Azospirillum brasilense* (Sp Br 14) on growth and development of winter wheat (*Triticum aestivum* cv. Fidel) grown in pots (expressed per plant at maturity ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of fertile tillers</th>
<th>Height of plants (cm)</th>
<th>Shoot dry weight (mg)</th>
<th>Root dry weight (mg)</th>
<th>Grain dry weight (mg)</th>
<th>Root/shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6 ± 0.3</td>
<td>53 ± 6</td>
<td>5037 ± 724</td>
<td>1480 ± 952</td>
<td>1161 ± 464</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Sp Br 14</td>
<td>3.0 ± 0.2</td>
<td>65 ± 2</td>
<td>7145 ± 673</td>
<td>3114 ± 585</td>
<td>1939 ± 366</td>
<td>0.43 ± 0.06</td>
</tr>
</tbody>
</table>

* a 7 × 2 plants; b 16 × 2 plants

Table 2. Effect of inoculation with *Azospirillum brasilense* (Sp Br 14) on nitrogen yield of winter wheat (*Triticum aestivum* cv. Fidel) grown in pots (expressed in milligrams N per plant at maturity ± SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaves (mg)</th>
<th>Stems (mg)</th>
<th>Grain (mg)</th>
<th>Kernel without grain (mg)</th>
<th>Roots (mg)</th>
<th>Total (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.6 ± 9.0</td>
<td>6.0 ± 3.0</td>
<td>34.3 ± 6.5</td>
<td>5.6 ± 2.8</td>
<td>9.6 ± 6.0</td>
<td>76.0 ± 18.0</td>
</tr>
<tr>
<td>Sp Br 14</td>
<td>7.5 ± 3.0</td>
<td>5.1 ± 2.5</td>
<td>48.5 ± 14.0</td>
<td>7.9 ± 3.1</td>
<td>22.3 ± 7.2</td>
<td>91.3 ± 20.0</td>
</tr>
</tbody>
</table>

* a 3 × 2 plants; b 5 × 2 plants

**Growth conditions.** For pot experiments, seeds were sown in autumn (15 November) in soil, in PVC containers 50 cm deep and 11 cm in diameter holding approximately 6 kg soil. The soil was a sandy loam soil (pH 6.5; N content, 0.10%; C content, 1.2%) from Belgium, where positive responses of winter wheat to inoculation had already been observed (Reynders and Vlassak 1982). A dose of nitrogen equivalent to 80 kg/ha (calculated on a per area basis) was added in the form of ammonium nitrate. Half was applied at sowing, the other half during the tillering stage (1 April). The containers housing 2 plants each were split into 2 sets (15 in one and 20 in the other) and buried in the field at two different locations to prevent contamination when inoculation was done on the second set. In both cases, growth was conducted under natural conditions and irrigation was applied when necessary throughout the growing period.

Two field plots 5 × 5 m, adjacent to the two sets of pots, were sown with the same cultivar of wheat. The soil was a red Mediterranean soil (pH 7.6; N content 0.12%; C content 1.4%). Nitrogen fertilization and irrigation were carried out according to the procedure used in the pot experiments. These two plots, inoculated and noninoculated, were used as a reference for comparison with the plants grown in containers.

**Inoculation.** *Azospirillum brasilense* strain SpBr14 was obtained from J. Döbereiner, EMBRAPA, Brazil. This tropical strain was isolated from the rhizosphere of wheat. Stock cultures kept on nitrogen-free agar medium were subcultured and grown on nitrogen-free liquid medium for inoculation. Fifty milliliters containing 10⁶ to 10⁷ cells/ml were added to each of the 20 containers. Inoculation was repeated 3 times at 1-week intervals during the tillering stage (1 to 20 April). In the field, the bacterial solution sprayed around the plants was made in order to reach 5 × 10¹⁰ cells/m². The inoculation was repeated twice at a 1-week interval near the end of April.

**Labelling with ¹⁴CO₂.** From tillering to the late heading stage (April to June), containers holding inoculated and noninoculated plants were selected for exposure of the shoot system to ¹⁴CO₂ according to a procedure already described (Warembourg et al. 1982). Exposure (1 day) was done under a plastic canopy in which temperature and CO₂ concentration were maintained according to outside conditions. Harvesting of the plants took place after 1 week when primary translocation of ¹⁴C to various plant parts had occurred. This was estimated from root respiration measurements undertaken on inoculated and noninoculated plants for which a tight separation between aerial and soil atmospheres had been inserted on top of the containers. Measurements were made by flushing each container with CO₂-free air and collecting ¹⁴CO₂ into NaOH solutions (Warembourg 1983). After 1 week, more than 95% of the labelled C respired by the roots had evolved, indicating that ¹⁴C remaining in plant parts represented growth increment after ¹⁴CO₂ application.

Plants were carefully separated into roots, stems, leaves, and kernels. Subsamples were used for C, ¹⁴C and N analysis.

**Other harvests and analyses.** At maturity, all plants of the remaining containers were harvested and separated into roots, stems, leaves and kernels for biomass, C and N determinations. In the field, sampling was performed on 6 subplots of 1 m² for each treatment. Number of fertile tillers and biomass of aerial plant parts were determined for each sampling area. Subsamples of plant materials were used for N analysis.

**Results**

**Pot experiments**

**Plant growth.** In the pot experiments, growth characteristics were compared between inoculated plants and control plants at maturity (Table 1). There was a significant effect of inoculation on the plant development with an increase in the number of fertile tillers, in plant height and therefore in shoot and root biomass. Grain yield was also enhanced. However, this general effect on plant dry weight for both aerial and below-ground parts was more pronounced in the roots as indicated by the root to shoot ratio, which increased...