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Original article

Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity

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ABSTRACT

The beneficial effects of inoculating with *Azospirillum brasilense* on crop productivity have been widely described, but extensive use in typical agricultural field environments is scarcely documented. The objective of this study was to quantify the productivity of wheat (*Triticum aestivum* L.) whose seed was inoculated with a liquid formulation containing *Azospirillum brasilense* INTA Az-39 strain under typical dryland farming conditions. The study was performed in the 2002–2006 growing seasons, evaluating inoculated and non-inoculated seed at 297 experimental locations in the Pampas region of Argentina. The inoculated crops exhibited more vigorous vegetative growth, with both greater shoot and root dry matter accumulation (12.9 and 22.0%, respectively). The inoculation increased the number of harvested grains by 6.1%, and grain yield by 260 kg ha⁻¹ (8.0%). Positive responses were determined in about 70% of the sites, depending mostly on the attainable yield and independently of fertilization and other crop and soil management practices. In general, more response to inoculation was observed in the absence of major crop growth limitations, suggesting the complementary contribution of the *Azospirillum brasilense* treatment to more efficiently developing higher yielding wheat.

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1. Introduction

Plant growth-promoting rhizobacterias (PGPRs) are microorganisms living in the rhizosphere of cultivated crops with known plant growth promotion effects. Their introduction into cropping systems could contribute to better crop productivity. The effects of PGPRs on plant growth and productivity are either direct (e.g. biological N fixation, S oxidation or P solubilization, increasing nutrient availability) or indirect “catalytic” actions [11].

Azospirillum sp. are non-specific PGPRs providing varied contributions to the enhancement of growth and productivity in many agricultural crop species [22,31]. Initially, these bacteria were known for their availability to provide associative N fixation [13]. Today, several other multiple complementary mechanisms have been described for these organisms, resulting in better nutrient and water use in inoculated crops. For example, their ability to improve root growth, both water and nutrient uptake, and to also trigger root and shoot growth promotion in inoculated plants has been reported [9,10,14].

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The increased wheat (*Triticum aestivum* L.) grain yield and grain protein content was attributed to an increase in root uptake of inorganic N [29]. Creus et al. [6] concluded that inoculated wheat plants under water stress during anthesis exhibited better growth due to an “elastic adjustment” that enhanced grain yield and quality. Similar results were described for corn (*Zea mays* L.) plants under water stress during flowering [5]. This complex of multiple, interactive, mechanisms affecting crop growth and productivity is also known as the “additive hypothesis”, which states that while a single mechanism can account for some of the observed growth promotion, the complete magnitude of growth promotion is only realized by the additive effect of several mechanisms operating during the growing season [3].

Information about the beneficial effects of *Azospirillum* sp. inoculation on the growth of cultivated plants is not new and was originally developed under greenhouse and laboratory conditions. The results of individual field studies showing the effects of *Azospirillum* sp. inoculation of agricultural crops are now available, but knowledge of those impacts within the context of more extensive inoculation in large on-farm trials is still quite limited. Limited on-farm work is often justified by the reportedly low consistency of field results conducted in a context of more realistic crop management production conditions [9].

The variability in the results could be due to the interaction of the inoculation practice with environmental conditions (e.g. soil type, water balance, etc.) and/or crop management practices (e.g. fertilization, chemical disease control, genotypes, etc.), among others [15]. For example, Sala et al. [27] found that wheat's grain yield response to inoculation with *Azospirillum* sp. and other diazotrophic bacteria varied among Brazilian locations. They attributed this variation to complex interactions between the crop, the bacteria and the environment. In Entic Haplustolls from the Pampas region of Argentina, Rodríguez Cáceres et al. [24] concluded that the variation in wheat grain yield response to *Azospirillum brasilense* inoculation was mainly due to differences in soil fertility and water availability. Also in Argentina, differences in the responses of corn to PGPR inoculation were attributed to interactions between crop genotypes and the N fixation potential of the evaluated *Azospirillum* sp. strains [17]. Puente et al. [23] observed greater tiller numbers, root dry matter and number of spikelets per plant when wheat seeds where inoculated with several *Azospirillum brasilense* strains. However, only wheat crops inoculated with the INTA Az-8 or INTA Az-39 strains exhibited a significant increase in grain yield. The INTA Az-39 strain was isolated from washed wheat roots originating in Marcos Juárez, Córdoba Province, Argentina. Inoculation with this strain resulted in a significant increase in wheat grain yield when evaluated in the semiarid region of Argentina [25].

The multitude of possible interacting factors that might impact the effect of *Azospirillum* sp. inoculation on crop productivity explains the low consistency of results observed in field studies. Thus, crop response to *Azospirillum* sp. inoculation should be evaluated under multiple environmental and crop management conditions for a more comprehensive understanding of the benefits to this practice. The objective of this study was to quantify the productivity of dryland

farmed wheat that was seed inoculated with a liquid formulation containing *Azospirillum brasilense*, strain INTA Az-39, across the Pampas region of Argentina.

2. Materials and methods

This study was performed over five consecutive wheat growing seasons between 2002 and 2006, at a total of 297 on-farm experimental sites utilizing typical soil and crop management practices and located across the Pampas region of Argentina (Fig. 1).

2.1. Environmental characterization of the Pampas region

The Pampas region is a vast plain of approximately 52 Mha, located in the central part of Argentina, and having warm temperate weather with adequate to less than adequate rainfall for normal crop production [18]. Rainfall amounts exhibit high inter-annual variability, with most rainfall occurring between October and April (spring through fall seasons), and the long-term annual average ranges from 500 mm in the southwest to 1000 mm in the northeast of the region. The soils, developed in loess deposits, exhibit texture variation according to their distance from the Andes Mountains, and are sandier to the southwest. The most frequently cropped soils of the Pampas are the Mollisols (USDA Soil Taxonomy) with udic and thermic moisture and temperature regimes prevailing, respectively [7]. In this region, soil water storage capacity is an important factor differentiating soils according to overlying crop productivity. Potential available water in the top 100 cm of the soil profile varies between 83 and 172 mm for Entic Haplustolls and Vertic Argiudolls, respectively. Shallow soils are a serious limitation to crop production in the southern and western Pampas, where the topsoil depth, due to the presence of a petrocalcic horizon, is less than 50 cm over approximately 50% of the area.

Of the 297 studied sites, most of them (89.6%) were located on soil with a udic moisture regime, the remainder on ustic soils (Table 1). In general, the study was performed on deep soils. Only 13.5% of the sites were dominated by shallow soils, mostly due to the presence of a petrocalcic layer within the surface 50 cm of the soil profile.

2.2. General crop and soil management practices

At each on-farm location, the wheat crop and soil were managed according to the best locally recommended practices for achieving high wheat yields. At 82% of the experimental sites, soybean (*Glycine max* (L.) Merrill) was the previous crop. Corn, sunflower (*Helianthus annuus* L.) and pastures composed of mixtures of fescue (*Festuca arundinacea* L.) and alfalfa (*Medicago sativa* L.) were the previous crops at the rest of the locations. In each of the studied seasons, the wheat seeding date ranged from the middle of May to the middle of August with a mean seeding date of July 1st, and following a fallow period of at least 30 days. Long and intermediate-long maturity cultivars were sown early (before late June) and short-season cultivars were usually sown at later dates.

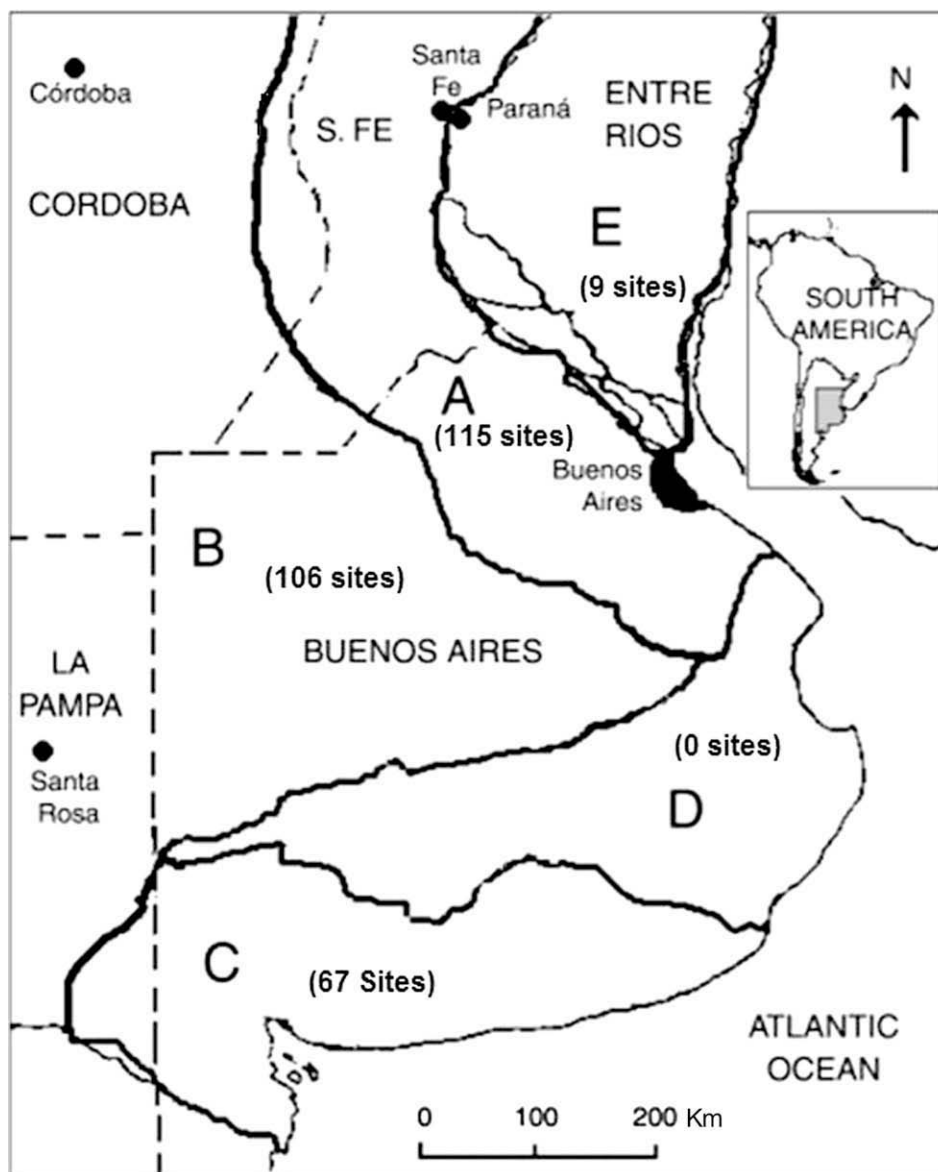


Fig. 1 – Location of the Pampas region (Argentina) showing boundaries for the subregions (solid lines): (A) rolling Pampas; (B) Central or Inland Pampas; (C) Southern Pampas; (D) Flooding Pampas; (E) Mesopotamian Pampas (Adapted from [7]). The number in parentheses shows the quantity of sites evaluated in each subregion. Inset shows location of the area within South America. Provinces lying partly within the area of interest are named and their boundaries are shown (dashed line).

At all sites, the sown wheat varieties were regionally adapted and recommended for high yielding environmental and crop management conditions. The mean seeding rate was 125 kg seed ha⁻¹, within a range of 80–187 kg ha⁻¹. At almost all of the sites, seed was treated with fungicide, usually containing carbendazim and thiram, tebuconazole or triticanazole as active ingredients. Previous field trials in the Pampas region found no significant effects of these active fungicidal ingredients on the mean response to *Azospirillum brasilense* inoculation with the studied formulation [12].

The predominant soil management was continuous no-tillage with chemical weed control for fallow preparation and maintenance, used at 79% of the sites. Phosphorus (P), nitrogen (N), or NP fertilization was done at almost 85% of

the sites. Nitrogen fertilization was mostly performed by broadcasting urea (46:0:0) during early wheat growth stages, before tillering. In soils low in plant available P, triple superphosphate (0:46:0), monoammonium phosphate (11:52:0) or diammonium phosphate (18:46:0) fertilizers were placed with the seed at planting. The N and P fertilization rates varied with field soil test results and local recommendations. When necessary, foliar and reproductive diseases were controlled with fungicides.

2.3. Experimental design and crop evaluation

In each field, the experiment was a completely randomized design with two replicates of plots with a size greater than

Table 1 – Distribution of the 297 experimental sites among soil types (USDA Soil Taxonomy) and subregions in the Pampas

Soil type	Pampas subregion (number of sites)				Total
	Rolling	Central or inland	Southern	Mesopotamic	
Typic Argiudolls	86	28	33		147
Vertic Argiudolls	5			9	14
Typic Argiustolls		1	8		9
Petrocalcic Calciustolls			6		6
Entic Hapludolls		39			39
Petrocalcic Hapludolls			20		20
Thaptoargic Hapludolls	1	13			14
Typic Hapludolls	23	9			32
Typic Haplustolls		16			16

0.5 ha. The two treatments consisted of wheat seed not inoculated and inoculated with *Azospirillum brasilense*. The inoculant was an aqueous formulation containing the INTA Az-39 strain, applied at a rate of 10 mL kg⁻¹ of seed approximately 6 h before seeding. The inoculant (Nitragin Bonus®) was prepared by Merck Crop BioScience Argentina SA (Pilar, Buenos Aires, Argentina), stored at room temperature for approximately 5 months before application to seed and had a mean of 1 × 10⁹ colony forming units mL⁻¹ at the time of application.

Duplicate 1-m² sampling units were randomly chosen within a uniform 100-m² area within each treatment plot and used for performing the following crop measurements: plant stand 45 days after seeding, dry root and shoot accumulation at tillering (Z23 to Z25 [33]) and grain yield at physiological maturity (Z92 [33]). Grain weight was adjusted to a moisture content of 0.14 g g⁻¹. The weight of individual seeds was determined from the weight of three samples of 300 grains taken from each treatment plot. The seed number per spike was determined from the average grains per spike for 10 consecutive spikes.

Means for inoculated and non-inoculated crops were separated by the LSD (T) test. Environmental and management effects on treatment (not inoculated and inoculated) grain yields were evaluated by comparing regression lines [30] between both treatments, according to Jennrich [19] using Statistix [1].

3. Results

Wheat grain yield varied between 850 and 8050 kg ha⁻¹ and, averaged over the 297 experimental sites, indicated that seed inoculation with this liquid formulation containing *Azospirillum brasilense* increased that yield by 260 kg ha⁻¹ (Table 2). Inoculation also increased shoot and root dry matter accumulation during wheat's vegetative growth, and grain number and kernel weight at harvest (Table 2). The available information was not sufficient to find a significant change in plant establishment due to inoculation. Wheat grain yield rose with increasing grain number or kernel weight, independent of the treatments (Table 3). In general, greater grain yields were observed at locations exhibiting more shoot and root dry matter accumulation, as well as greater numbers of spikes and grains. Differences in kernel weight and plant establishment contributed modestly to observed grain yield variability.

Averaged over each of the predominant Pampas soil types, the mean grain yield response to seed inoculation varied between 0 and 334 kg ha⁻¹ (Table 4). In general, the grain yield response to inoculation was greater at sites under a udic (subhumid) moisture regime, or with deep soil profiles, than under ustic (semiarid) environments or with shallow soils, respectively. Among the different soil types studied, there was no significant grain production response to inoculation only on those soils subject to both limitations (i.e. Petrocalcic

Table 2 – Mean effect of seed inoculation with a liquid formulation of *Azospirillum brasilense* on wheat growth and grain yield at 297 experimental sites in the Pampas region of Argentina

Variable	Treatment		Response (%)	Statistics from LSD (T)
	Control	Inoculated		
Plants m ⁻²	290 (7.9)	301 (9.8)	4.2 (3.0)	NS
Shoot dry matter (kg ha ⁻¹)	5181 (411.5)	5658 (455.3)	12.9 (2.5)	**
Root dry matter (kg ha ⁻¹)	3168 (330.9)	3519 (305.0)	22.0 (6.3)	*
Spikes m ⁻²	434 (7.1)	447 (7.3)	3.6 (0.7)	**
Grains m ⁻²	10450 (188.3)	10972 (189.5)	6.1 (0.8)	**
Grains spike ⁻¹	22.9 (0.4)	23.5 (0.4)	2.0 (1.1)	**
Kernel weight (mg grain ⁻¹)	35.8 (0.2)	36.4 (0.2)	2.8 (0.3)	**
Grain yield (kg ha ⁻¹)	3900 (73.3)	4160 (75.2)	8.0 (0.8)	**

Differences between inoculation treatments: NS, $p > 0.10$; * $p < 0.05$; ** $p < 0.01$. Standard errors of the means are given in parentheses.

Table 3 – Single factor regression models of grain yield (y, kg ha⁻¹) predicted by grain yield components

Treatments	Regression model	r ²
Both treatments	$y = 299.48 \times KW - 6897.1$	0.5542
Control	$y = 300.36 \times KW - 6962.6$	0.5574
Inoculated	$y = 296.59 \times KW - 6757.8$	0.5439
Both treatments	$y = 0.4124 \times GN - 491.13$	0.9424
Control	$y = 0.4098 \times GN - 482.94$	0.9481
Inoculated	$y = 0.4139 \times GN - 488.23$	0.9362

KW, kernel weight (mg grain⁻¹); GN, grain number (grains m⁻²).

Calciustolls, which are both ustic in moisture regime and shallow) (Table 4).

The application of different crop and soil management practices modified mean wheat grain yield with a minor impact on crop response to *Azospirillum brasilense* inoculation (Table 5). Similar mean grain yields and inoculation responses were observed with different wheat tillage systems (Table 5). In Argentina, among other attributes, sown varieties are classified according to their germplasm origin. Cultivars derived from European genotypes have exhibited greater yields than other genotypes of Argentine or Mexican-CIMMYT origins [26]. Our results are in agreement. Cultivars derived from European germplasm exhibited greater mean grain yield, but were not more, or less, responsive to inoculation than other genotypes (Table 5). The seeding rate positively affected wheat grain yield but the influence was weak (yield (kg ha⁻¹) = 1788.7 + 18.46 × seeding rate (kg ha⁻¹), r² = 0.122, p < 0.01). Seeding rate did not affect the yield response to inoculation (p < 0.18). The relationship between seeding date and grain yield was weak and of little agronomic importance (yield (kg ha⁻¹) = 2926.8 + 6.35 × days after 1 January, r² = 0.009, p < 0.08).

Wheat sown after a previous pasture crop yielded less and did not exhibit a significant response to the inoculation, while those sown after row crops, generally responded positively, 230–375 kg ha⁻¹, to inoculation (Table 5). Fertilization with N, P or both macronutrients enhanced wheat yield relative to the unfertilized crop (Table 5). Inoculation significantly and positively affected yield, regardless of fertilization practice, with mean yield responses of 259 and 260 kg ha⁻¹ for unfertilized and fertilized wheat, respectively (Table 5).

Within four filed brackets, on sites with attainable yield potentials greater than 1499 kg ha⁻¹, the proportion of trials exhibiting a significant grain yield response to inoculation (p < 0.10) varied between 49 and 75%, with a mean yield response of 289 kg ha⁻¹, which was 7.5% greater than that of the untreated control (Table 6). There was not enough information to discern a significant difference between the inoculation treatments when the attainable yield potential was lower than 1499 kg ha⁻¹ (Table 6).

Seasonal wheat grain yield increased yearly (Fig. 2), exhibiting a trend similar to that observed over the Pampas region since the beginning of the 1990s. The trend is a response to the broad adoption of yield-improving production practices (no-tillage, crop protection and fertilization). Comparing the seasonal yield regression lines for uninoculated and inoculated wheat crops, there was no significant difference in the slopes (p < 0.40). Between the period from 2003 to 2006, the mean difference between the two treatments was significantly different, and the inoculated wheat exhibited a greater mean yield in each of these years (Fig. 2). In the 2002 growing season, there was insufficient information to determine a significant difference due to inoculation, and this was the only season where greater rainfall was evident at the beginning of the season (Fig. 3). The opposite behavior was observed in the 2006 growing season, when the greatest response to inoculation (405 kg ha⁻¹, p < 0.01) was observed, which was

Table 4 – Effect of soil type (USDA soil taxonomy) and seed inoculation with a liquid formulation containing *Azospirillum brasilense* on wheat grain yields in 297 experimental sites in the Pampas region of Argentina

	Grain yield (kg ha ⁻¹)		Statistics from LSD (T)	Inoculation response (%)
	Control	Inoculated		
Soil type				
Typic Argiudolls	3848 (98)	4118 (99)	**	8.7 (1.2)
Vertic Argiudolls	3308 (183)	3569 (209)	**	7.9 (2.4)
Typic Argiustolls	3322 (342)	3569 (378)	**	8.6 (3.8)
Petrocalcic Calciustolls	1582 (101)	1580 (133)	NS	0.5 (4.2)
Entic Hapludolls	4278 (192)	4524 (185)	**	7.6 (2.0)
Petrocalcic Hapludolls	4671 (260)	5005 (245)	**	8.1 (1.7)
Thaptoargic Hapludolls	2868 (352)	3060 (362)	*	8.8 (5.0)
Typic Hapludolls	4505 (203)	4768 (209)	**	6.3 (1.7)
Typic Haplustolls	3890 (316)	4167 (361)	**	8.0 (3.4)
Moisture regime				
Udic	3972 (76)	4238 (76)	**	8.2 (0.8)
Ustic	3278 (245)	3493 (277)	**	6.5 (2.2)
Soil profile				
Deep	3950 (74)	4214 (76)	**	8.1 (0.8)
Shallow	3577 (257)	3811 (270)	**	7.1 (2.1)

Differences between inoculation treatments: NS, p > 0.10; *p < 0.05; **p < 0.01. Standard errors of the means are given in parentheses.

Table 5 – Wheat grain yield response to crop and soil management practices and seed inoculation with a liquid formulation containing *Azospirillum brasilense* across 297 experimental sites in the Pampas region of Argentina

	Yield response (kg ha ⁻¹)		Statistics from LSD (T)	Inoculation response (%)
	Control	Inoculated		
Tillage system				
No-Tillage	3822 (86)	4053 (89)	**	7.4 (0.9)
With-tillage	3977 (197)	4273 (212)	**	7.6 (1.2)
Germplasm				
European	4619 (172)	4935 (184)	**	7.6 (1.9)
Argentine/Mexican	3791 (86)	4013 (87)	**	7.1 (0.9)
Previous crop				
Pasture	3066 (487)	3137 (508)	NS	1.8 (3.6)
Soybean	3848 (96)	4078 (96)	**	7.8 (0.9)
Sunflower	3762 (248)	4099 (248)	**	10.0 (2.3)
Corn	4089 (272)	4463 (288)	**	9.8 (2.3)
Fertilization practice				
No N or P fertilizer added	3125 (260)	3383 (284)	**	9.4 (4.4)
P added	3377 (246)	3544 (258)	*	5.0 (1.5)
N added	3438 (185)	3669 (175)	**	9.6 (2.4)
N and P added	4160 (84)	4439 (86)	**	7.8 (0.8)

Differences between inoculation treatments: NS, $p > 0.10$; * $p < 0.05$; ** $p < 0.01$. Standard errors of the means are given in parentheses.

the season exhibiting the greatest dryness during wheat's vegetative growth (Fig. 3).

4. Discussion

The seed application of this liquid formulation containing *Azospirillum brasilense* raised wheat grain yield by an average of 260 kg ha⁻¹, equivalent to 8.0% of the mean wheat yield attained under the dry land farming conditions found in the Pampas region of Argentina. A positive response to seed inoculation was observed at about 70% of the 297 studied sites. Okon and Labandera-Gonzalez [22], from greenhouse and field experiments around the world, also reported a 5–30% increase in grain yield in 70% of the inoculation trials evaluated.

Table 6 – Wheat grain yield response to seed inoculation with a liquid formulation containing *Azospirillum brasilense* across 297 experimental sites in the Pampas region of Argentina, grouped according to the attainable yield potential

Attainable yield	Yield (kg ha ⁻¹)		Statistics from LSD (T)	% Sites with response ($p \leq 0.10$)
	Control	Inoculated		
<1499	1124 (68)	1164 (71)	NS	0
1500–2999	2146 (61)	2394 (58)	**	53
3000–4499	3633 (45)	3834 (44)	**	49
4500–5999	4824 (53)	5140 (45)	**	58
>6000	6044 (165)	6435 (172)	*	75

Differences between inoculation treatments: NS, $p > 0.10$; * $p < 0.05$; ** $p < 0.01$. Standard errors of the means are given in parentheses.

In our study, the increment in shoot and root dry matter with inoculation was greater than that for grain yield (Table 2) and agrees with the observations of Dobbelaere et al. [9], who also found that the positive effects of inoculation on early growth were not always translated into increased yield. Most of the difference in grain yield was related to an increase in harvest grain number with inoculation. In agreement with these results, Kapulnik et al. [20], and Dobbelaere et al. [9] observed a significant increase in the shoot dry weight of *Azospirillum* sp. inoculated wheat, due to an increased number of tillers. They also observed more root dry weight with inoculation, but no significant yield increase was observed at harvest due to growth limitations during the seed filling period. Caballero-Mellado et al. [4], reporting on seven experimental sites in

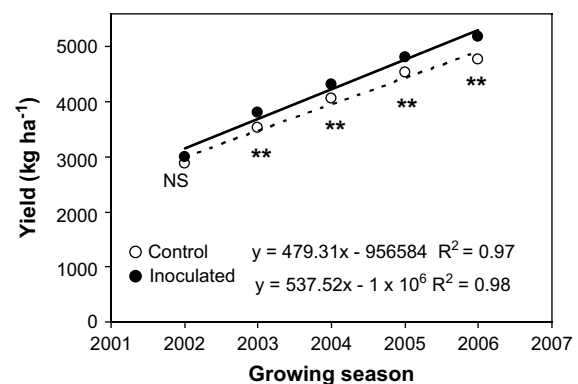


Fig. 2 – Seasonal wheat grain yield response to seed inoculation with *Azospirillum brasilense* in the Pampas region of Argentina. Average of 34, 101, 84, 45 and 33 experimental sites for the 2002, 2003, 2004, 2005 and 2006 growing seasons, respectively. Difference between inoculation treatments: NS, $p > 0.10$; ** $p < 0.01$.

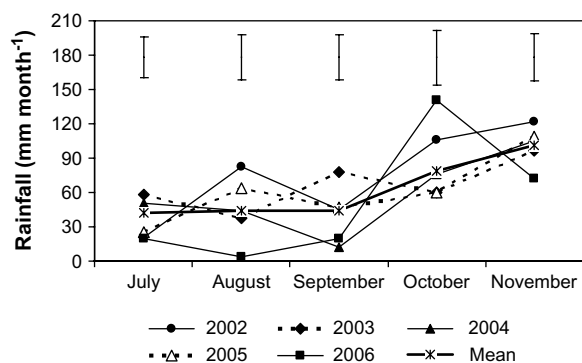


Fig. 3 – Monthly rainfall during the 2002–2006 wheat growing seasons. Average of 297 experimental sites in the Pampas region (Argentina).

Mexico, found a mean wheat grain yield increase of 22% with *Azospirillum brasilense* inoculation of crops fertilized with less than 90 kg N ha⁻¹. In North America, wheat seed inoculation with *Azospirillum brasilense* was evaluated between 2004 and 2007 at 36 winter wheat and 19 spring wheat locations spread across several wheat production regions, under dryland conditions and with otherwise typical on-farm management. Location-average grain yield varied between 2730 and 5040 kg ha⁻¹, with a 6.3 and 8.0% mean grain yield increase over the untreated control for the winter and spring wheat crops, respectively (Dr R.S. Smith, EMD Crop BioScience Inc., personal communication).

The generally greater grain yield observed with N and P fertilization confirms that the management of both nutrients is crucial to high yielding Argentine wheat production systems [28]. The response to inoculation was more pronounced at the non-fertilized locations, but was also significant for both N and P fertilized situations (Table 5). That the inoculation response was generally independent of fertilization practices is in agreement with the results of several studies suggesting that *Azospirillum* sp. can make a moderate N contribution to the crop [32]. For example, seed inoculation with *Azospirillum brasilense* resulted in greater N, P, and K concentrations in winter wheat flag leaves and grain, as well as increased grain yield, across a range of N fertilization rates [8]. Similar effects were observed in Mexico, where significant increments in grain yield and N, P, and K uptake were found in inoculated wheat crops, independent of the N fertilization rate, which ranged from 0 to 120 kg N ha⁻¹ [4]. Saubidet et al. [29] concluded that the increment in yield and grain protein found with inoculation of wheat was in response to increased inorganic N uptake by roots. Dr R.S. Smith (EMD Crop BioScience Inc., personal communication), from four experimental sites located in Wisconsin (USA), adequately fertilized with N, observed greener (chlorophyll measurements) flag leaves in inoculated crops. This suggested more efficient N uptake during the vegetative growth stage, contributing to the setting of a greater kernel number in the inoculated crop. In agreement with these observations, Bashan et al. [2] observed a positive increment in photosynthetic pigment concentrations in wheat seedlings inoculated with *Azospirillum brasilense*.

Because wheat grain yield results from the product of grain number per unit area and the single grain weight, differences in these yield components can help us to understand how the treatments induced changes in wheat yield. In this study, inoculation with *Azospirillum* promoted greater grain numbers per unit area more than a greater single grain weight, suggesting that the treatment effectively improved photosynthate availability, largely prior to anthesis [16]. Increased root growth in the inoculated plants improved access to soil water and nutrients, improving growth conditions during early vegetative stages, and resulting in more shoot dry matter accumulation. Furthermore, vigorous shoot growth can lead to more efficient radiation use, supporting greater grain numbers under the moderate stressful production conditions normally observed in the Pampas [28].

No significant response to inoculation was found under strongly limiting growth conditions like soils with shallow profiles in semiarid regions (Table 4) or when crops were low yielding (Table 6). On the other hand, a greater positive contribution from the inoculation treatment was observed with moderate water shortage, such as occurred in the 2006 growing season (Figs. 2 and 3). And, because positive and consistent grain yield benefits to inoculation were observed under a wide range of production conditions (Tables 4 and 5), we conclude that the complex contribution of *Azospirillum* complements adequate resource availability rather than substituting for those resources.

Azospirillum's role as a PGPR, its contribution as a yield-promoting biofertilizer for field crops, appears to be one of modifying soil-plant processes so that N and other nutrients are more completely retained in the plant-soil system [21]. Inoculation with *Azospirillum* has the potential for economic and environmental benefit by causing a more efficient use of the resources required for crop production. Our results quantify the contribution of this practice under typical dryland farming conditions and shows that to obtain maximum benefit the use of this inoculant should be complemented with use of the best locally adapted production practices. In the presence of severe crop growth limitations the benefit of inoculation with *Azospirillum brasilense* was not significant, whereas consistent and significant yield benefits were described under adequate crop and soil management systems.

5. Conclusions

Under dryland farming conditions in the Pampas region of Argentina, wheat that was not inoculated with *Azospirillum brasilense* had as a result a mean yield limitation of 260 kg ha⁻¹. At about 70% of the studied sites, application to the seed of a liquid formulation containing these bacteria provided positive grain yield responses and better crop early growth. The initial root growth response benefit potentially contributed to more efficient use of water and available nutrient resources and, in combination with enhanced shoot growth, resulted in a greater grain number per unit area. However, with low yielding environments (i.e. shallow soils, crops seeded without fallow, etc.) there was no significant contribution from the inoculation of seed with this

formulation, either to crop growth or grain yield. These general observations were largely independent of regular production practices (i.e. cultivar choice, fertilization and tillage practices, etc.), suggesting that the contribution of seed inoculation with *Azospirillum brasilense* to a higher yielding, more efficient wheat crop was complementary to existing soil resources.

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