Published May 5, 2017

Soybean Response to Inoculation with *Bradyrhizobium japonicum* in the United States and Argentina

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ABSTRACT

Although the relevance of biological N nutrition of soybean [*Glycine max* (L.) Merr.] is recognized worldwide, inoculation with *Bradyrhizobium japonicum* shows variable results and the benefit needs to be validated under current crop production practices. We conducted statistical analysis of soybean field trial data to provide insight into factors affecting the efficacy of soybean inoculation under contrasting crop production conditions. Most experimental sites, 187 trials in the United States and 152 trials in Argentina, were in soils with soybean history and naturalized *B. japonicum* strains. Yield increases were greater in Argentina (190 kg ha^{-1} equivalent to 6.39%) than in the United States (60 kg ha^{-1} equivalent to 1.67%). Tillage methods did not affect inoculant performance. In the United States, inoculation was more effective in soils with higher pH (>6.8) while in Argentina the greatest inoculation effect on crop production occurred in soils with a lower pH (<5.5). In the United States, where most of the trials were in rotation with corn (*Zea mays* L), the greatest positive effect of inoculation was observed in late planted soybean crops and independent of soil organic matter (SOM). In Argentina, the inoculant had its greatest effect in soils with no soybean history, a relatively high SOM, higher levels of soil extractable P and S, and in areas with greater precipitation during early reproductive growing stages. In both regions, the yield increases due to *B. japonicum* inoculation support the regular use of this practice to help provide adequate conditions for soybean production.

Core Ideas

- Soybean seed inoculation with *Bradyrhizobium japonicum* enhances grain production.
- Greater inoculation response happens in Argentinean sites than in the United States.
- Several soil properties and crop management practices are related with the responses to inoculation.

Soybean was first cultivated in China and is now the most important annual grain legume in the world, with the United States, Brazil, and Argentina the leading producers (Vieira et al., 2010). Soybean is a host for the N_2–fixing bacteria, *B. japonicum* and *B. elkanii*, and they can obtain up to 50% or more of their N needs through biological nitrogen fixation (BNF) when *B. japonicum* and *B. elkanii* are present in the soil (Elmore, 1984; Collino et al. 2015). As the crop is not native to North and South America, the soils in these areas do not have a native rhizobia population, unless there has been prolonged use of inoculated seed (Zerpa et al., 2013). In South America, soybean may be infected by other native species of rhizobia but their effectiveness in fixing atmospheric N_2 is variable (Althabegoiti et al., 2008). Inoculation of soybean seed at planting with *B. japonicum* came into common practice in the United States in the early part of the 20th century (Pueppke, 2005) and in Argentina in the mid-1970s (Hungria et al., 2005). Soybean inoculation at planting is more common in South America than in the United States. In Argentina, approximately 80% of soybean is inoculated each year (Perticari, 2015), while in the United States, only 15% of farmers use inoculants (Graham et al., 2004). Factors, among others, that may have contributed to a decreased utilization of inoculation practices in the United States are the use of plant varieties limited in their ability to fix N_2 in symbiosis, and edaphic constraints that include soil acidification, drought, and shortage of specific nutrients (Graham et al., 2004) as well as the limited yield increase of seed inoculation in soils with soybean history (De Bruin et al., 2010). Studies conducted in 2000 estimated that the average N_2 fixation in dryland soybean in the United States was 100 kg N ha^{-1} for aboveground biomass and as much as 142 kg N ha^{-1} if root biomass was included (Pueppke, 2005). The portion of plant N derived via BNF varies based on the calculation method used, agronomic practices, and soil and environmental conditions.

Published in Agron. J. 109:1031–1038 (2017)
doi:10.2134/agronj2016.04.0214
Received 15 Apr. 2016
Accepted 20 Jan. 2017
Available freely online through the author-supported open access option

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Abbreviations: BNF, biological nitrogen fixation; IT, inoculation treatment; LCO, lipo-chitooligosaccharide; PLS, partial least squares; SOM, soil organic matter; VIP, variable importance in the projection.

As an example, Schipanski et al. (2010) found that the percentage of plant N attributed to BNF ranged from 36 to 82% and the total N_2 fixed ranged from 40 to 224 kg N ha^{-1}. In the United States, since 1985, the portion of N derived from BNF in soybean has declined from 65% to only 54% (Graham et al., 2004). In Argentina, BNF accounted for 45 to 58% of total plant N (Di Ciocco et al. 2008; Collino et al. 2015).

The question, of why in some countries, such as the United States, the yield response to soybean inoculation is small and difficult to quantify, while in countries like Brazil and Argentina larger yield increases are common, is intriguing. Determining whether there is a scientific basis for regular inoculation in the United States and in Argentina, and which environmental conditions enhance the success, will help agronomist make better recommendations to producers on the benefits of inoculants. Soybean cultivars developed in the United States show appreciable variation in ability to respond to inoculation with *B. japonicum*. The inoculant strains used with these cultivars induce only 5 to 15% of the nodules formed, and even superior N_2–fixing strains are sometimes unable to establish in the soil (Graham and Temple, 1984). After several years of inoculation, *B. japonicum* becomes established in the soil and any new organisms introduced with the seeds have low infection rate. This "competition" problem has been one of the major constraints to the introduction of new and more efficient strains (Vieira et al., 2010). Competitive exclusion is site specific and determined by a combination of factors related to the environment, the host plant, and the size and composition of the population of indigenous rhizobia (Vieira et al., 2010).

Quantifying real benefits of inoculation can be difficult as yields continue to improve in agro-ecosystems that may, have yields near to their capacity (Karamanos et al., 2014). Detecting small but consistent yield improvements, against a background of biological variation, can present problems in field experiment designs using few replicates and limited numbers of sites (Edmeades, 2002; Vieira et al., 2010), especially when the weights recorded by most harvest equipment used in field trials are not precise and therefore also contribute to the variability of the results. Often, results from similar experiments will disagree as even well-planned studies are limited in statistical power (Olkin and Shaw, 1995). More sophisticated modeling techniques using a large number of trials conducted over several years may be a better way to investigate inoculant response and the factors affecting inoculation efficacy.

A fundamental shift has taken place in agricultural research and world food production. In the past, the principal goal was to increase the yield potential of food crops and to maximize productivity. Today, the drive for productivity is increasingly combined with a desire for sustainability (Peoples et al., 1995; Vieira et al., 2010). The effective management of N is an essential element of agricultural sustainability (Zahran, 2010; Vieira et al., 2010). In the United States most corn–soybean producers have not used N fertilizers, but those who are aiming for higher yields have been relying more on synthetic fertilizers which is not a sustainable solution due to the large energy required to synthesize N fertilizers (Pueppke, 2005). Significant N demand for soybean, coupled with poor N_2 fixation and inadequate amounts of fertilizer N, can lead to depletion of soil N fertility (Althabegoiti et al., 2008; Ferreira et al., 2000; Vieira et al., 2010). Therefore, interest in the use of inoculants in soybean is increasing along with the awareness of the environmental benefits of BNF. Researchers, extension personnel, and policymakers worldwide should think of BNF as a key process, with long-term benefits, essential for sustainable agriculture, improving productivity, ensuring food security, and maintaining environmental quality (Olivares et al., 2013; Hungria et al., 2005).

The soybean grain response to inoculation with *B. japonicum* varies greatly among seasons and production regions. Hungria et al. (2005) reported that the yield increases (4.5%) recorded in Brazil on soils with high number of indigenous rhizobia were much higher than observed in the United States, and that the differences warrant further research. Yield response to inoculation is unpredictable and, while there are reports about environments that are more likely to respond to inoculation, few are scientifically supported. Understanding the factors that contribute to this variability is an essential step to developing sustainable farming systems that truly contribute to increased world food production. Most literature reports describe trials conducted in limited geographic areas covering only a few years of results, which make it difficult to make any conclusions on the benefits of the *B. japonicum* inoculation. Relatively small, but consistent, differences in grain yield can still provide an economic benefit to farmers. A more comprehensive approach is required by combining results of multiple studies to increase the power of the analysis (Fisher, 2015).

The extensive data set used in this study was collected to determine the effect of a commercial soybean inoculant with *B. japonicum* (Optimize produced by Novozymes BioAg), in two contrasting soybean growing regions, the United States and Argentina. This inoculant also contains a signal molecule, lipochitooligosaccharide (LCO) that increases the consistency and effectiveness of the rhizobium–soybean symbiosis. The LCO is a molecule, produced by rhizobia and received by the plant that This molecule initiates nodulation, stimulates the root system and enhances nodule development leading to enhanced N_2 fixation (Zhang and Smith, 1997; Smith and Habib, 2009; Vieira et al., 2010; Smith et al., 2015).

The purpose of this study was to establish the contribution of soybean seed inoculation with *B. japonicum*, under current crop production practices in contrasting regions, to improving yield. In addition, the paper describes the use of a multiple site and season analysis, to determine the principle variables among environmental and management factors that explain the variability in the responses to the practice.

MATERIALS AND METHODS

Trial Description and Experiment Design

The field trials were conducted at 187 sites in the United States (location × year combinations) covering a wide geographic region from 2009 to 2013 (Table 1). Most of the trials were established using a standard corn–soybean rotation and most sites (62%) had corn as the previous crop. Site locations were sampled for soil fertility and nutrients, excluding N, were applied as needed to eliminate nutrient deficiency as a factor. Site managers were instructed to manage the trials based on local University/Extension recommendations for high-yield soybean production. The mean characteristics of the soils in all the trial sites are summarized in Table 1. Managers were asked to monitor the trials for insect and disease

Table 1. Location and soil general description for Bradyrhizobium japonicum inoculation trials in the United States (USA) and in Argentina. Soil organic matter (SOM) and pH measured in the 0- to 20-cm layer.

Country	Years	Locations (States or provinces)	Soil textural class or USDA soil classification	pH	SOM
					g kg^{-1}
USA	2009–2012	Arkansas, Iowa, Illinois, Indiana, Kansas, Minnesota, Mississippi, Missouri, North Dakota, Nebraska, Ohio, South Dakota, Wisconsin	loam, silt, clay loam, sandy clay, sandy loam, silt loam, silty clay loam	5.5–7.9	14.0–60.0
Argentina	2009–2013	Buenos Aires, Chaco, Córdoba, Corrientes, Entre Ríos, La Pampa, Rio Negro, Santa Fe, Tucumán	Typic Argiduolls, Typic Ariudolls, Typic Hapludolls, Entic Hapudolls, Vertic Hapludolls	5.1–6.8	16.0–52.0

thresholds throughout the growing season and treat if necessary. Weeds were controlled with commercially available herbicides common for the growing area and cultivation methods.

Soybean crops were planted at a mean seeding rate of 370,500 seeds ha^{-1} using standard research field equipment suited for small plot research and best production practices (i.e., planting dates during May and June, mostly glyphosate [*N*-(phosphonomethyl)glycine]- tolerant varieties, without N fertilization). In all the locations, the seeds were inoculated immediately before planting by mixing the recommended amount of seed thoroughly with the required amount of inoculant (Optimize, Novozymes BioAg, Milwaukee, WI). In 79% of the experimental sites from the United States, the seeds in both inoculated and un-inoculated control treatments were previously coated with commercial seed treatments (e.g.,. Acceleron [Monsanto, St. Louis, MO], ApronMaxx [Syngenta Crop Protection, Greensboro, NC], CruiserMaxx Syngenta Crop Protection, Greensboro, NC], or Trilex [Bayer Crop Science, Research Triangle Park, NC])

Each trial consisted of an untreated control and the Optimize treatment in a randomized complete blocks (RCB) design with four to six replicates in plots of at least five rows wide and 10 m long.

A similar set of trials were conducted over a wide geographic area in Argentina at 152 sites from 2009 to 2013 (Table 1), 60% of the sites with corn as previous crop. The mean characteristics of the soils in all the trial sites are summarized in Table 1. Also, the crops and soils were managed following the best local recommended practices for achieving high- yielding soybean crops. Soybean crops were planted at a mean seeding rate of 350,000 seeds ha^{-1} and mostly under no-tillage practices with chemical control of weeds and using standard field equipment. Best production practices (i.e., planting dates during late October and November, mostly glyphosate-tolerant varieties, P fertilization in 40% of the sites and none with N fertilization, foliar fungicides) were applied when needed. The seeds were inoculated immediately before planting by mixing a known amount of seed thoroughly with the required dose of inoculant (Optimize, Novozymes BioAg, Pilar, BA, Argentina). In almost all the sites, the seeds were previously treated with fungicide using rhizobia-compatible formulations like Protreat (Novozymes, Pilar, Buenos Aires, Argentina) containing carbendazim (methyl 1*H*-benzimidazol-2-ylcarbamate) and thiram (dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithioate).

As in the United States field trials, trials in Argentina had a RCB design with each treatment in two to five replicates in plots of at least five rows wide and 10 m in length.

Soil and Crop Measurements

The soil type, based on soil textural classes and USDA soil taxonomy classification, was determined for each of the experimental sites. Also, composited soil samples from the 0- to 20-cm layer were taken at planting and the soil pH, SOM, and extractable P (Bray Kurtz 1 method) and S levels were determined based on routine soil testing procedures. The soil textural class from the upper soil layers was manually estimated. At physiological maturity of the crops, duplicate 1 m^2 sampling units were randomly chosen within a uniform 10 m^2 area in each treatment for measuring grain yield using small plot combines or hand harvest. Grain weight was adjusted to 14% of moisture content. Rainfall data was recorded from public weather stations nearby each of the experimental sites.

Statistical Analysis

Yield data from the United States and Argentina were separately analyzed with the GLIMMIX procedure of SAS (Littell et al., 2006; SAS Institute, 2013). The analysis considered the effects of replicates and site (year × location combination) as random, and the effect of the inoculation treatment (IT) as fixed. A Gaussian error distribution was used for all analyses (SAS Institute, 2013). Exploratory analyses revealed that residual variances were heterogeneous among sites. The corrected Akaike's information (AICc) model fit criterion confirmed that the preceding model parameterization was better than a model that did not consider residual variance heterogeneity. Variance heterogeneity was modeled using a random statement with the effect set to *_residual_* and the *group* option set to site. Some of the analyses required that starting covariance parameter estimates be estimated with PROC HPMIXED and then passed to GLIMMIX using procedures like those described in SAS documentation (SAS Institute, 2013).

The site × inoculation treatment interaction was further investigated independently for the United States and Argentinean data. An extension of the mixed model described in the preceding paragraph (sensitivity analysis) was used to assess the site × IT interaction (Littell et al., 2002). This analysis used site means for yield, and other agronomic and environmental data from each site, as covariables to explain variability of inoculation responses across sites. Each covariable was considered in a separate analysis, and for each analysis a covariable × treatment effect was included in the model statement. A statistically significant ($P \le 0.05$) covariable × IT interaction indicated that changing the level of covariable affected the difference between the control and IT. Treatment means were estimated at the minimum and maximum levels for each covariable. For those covariables that were class factors (e.g., tillage system and previous crop), covariable, IT, and covariable × treatment effects were included in the model statement, and treatment means were estimated for each level of the covariable.

The relative importance of covariables reflecting site characteristics was assessed using partial least squares (PLS), also known as projection to latent structures PLS method. The data for the PLS analysis consisted of a matrix with each site as a row, and site means for crop yield and other site and environment indicators (covariables) as columns. The PLS analysis was performed using the PROC PLS procedure of SAS (Tobias 1995; SAS Institute, 2013). All covariables were included as predictor variables for crop yield in the PLS model (Tobias, 1995). Predictors that best explained crop yield were selected based on the criterion of variable importance in the projection (VIP) greater than 0.8 (Wold, 1994). Additionally, PLS regression coefficients based a raw (i.e., not standardized data) were outputted with VIP to help assess relative importance and to assist in interpretation.

RESULTS

Grain Yield Increases

The mean soybean grain yields were 3.70 Mg ha^{-1} in the U.S. field trials and 3.16 Mg ha^{-1} in the sites located in Argentina. Inoculation with *B. japonicum* increased ($P < 0.001$) soybean yield in both the United States and Argentina (Table 2). The absolute and percent yield increase due to inoculation was greater in Argentina. Both in the United States and in Argentina, the yield increase due to inoculation was different in sites with low and high yields (Table 3). In the United States, both the absolute yield increase (0.13 Mg ha^{-1}) while in Argentina the greatest absolute yield difference (0.17 Mg ha^{-1}) occurred in the higher-yielding sites. However, when we look at the percent increase of the inoculated crops compared to the control at each yield level, the highest increase relative to the control was obtained in the lower yielding sites (14.0% in United States and 9.5% in Argentina) compared the higher yielding sites (0.6% in United States and 3.5% in Argentina). This difference suggests that the BNF contribution explains more of the attainable yield in low yielding environments probably with greater soil N availability limitations than in sites with lesser growth restrictions for the crops and consequently fewer plant N limitations.

The relative importance of covariables assessed using PLS analysis showed contrasting results for the United States and Argentina. For the United States, delayed planting was the variable most affecting yield (regression coefficient = −0.3 and VIP = 1.64). In the other hand, this analysis showed, for the trials in Argentina, that soil related variables provided contrasting production results. The PLS regression coefficient and VIP results indicated that S (regression coefficient = −0.3 and VIP = 1.64), SOM (regression coefficient = 0.34 and VIP = 1.46), and pH (regression coefficient = 0.26 and VIP = 1.4) were important predictors of yield.

Factors Affecting the Efficacy of the Soybean Response to Inoculation

Tillage Practices

There was a distinct difference in tillage practices in the United States and Argentina. In the United States, only 9% of the studied sites used minimum till or no till methods and those sites were in Iowa (2011), Illinois (2010), and Ohio (2009). In Argentina, most of the sites used minimum or no till practices. Conventional tillage practices were only used in few locations in the province of Buenos Aires of Argentina. Tillage had no effect on inoculant performance in the United States or Argentina (Table 3).

Field Cropping History and Preceding Crop

Corn was the most common rotation crop in the U.S. trials accounting for 62% followed by soybean (23%) and other crops such as cabbage (*Brassica oleracea*), strawberries (*Fragaria* × *ananassa* Duch.), cotton (*Gossypium hirsutum* L.), kidney bean (*Phaseolus vulgaris* L.), sorghum [*Sorghum bicolor* (L.) Moench] and winter wheat (*Triticum aestivum* L.) which all accounted for 15% of sites. Inoculation had a positive effect on grain yields when soybean or corn was the previous crop. No inoculation benefit was detected in sites with other preceding crops (Table 3). In the studied locations in Argentina, corn was also the most common crop to precede soybean (59% of the sites) with soybean second (17% of the sites). Other crops such as rye (*Secale cereale* L.), sorghum, winter wheat, fescue (*Festuca arundinacea* Schreb.) and alfalfa (*Medicago sativa* L.) based pastures were listed as previous crops for 24% of sites. The preceding crop, however, did not influence the ability of the inoculant to increase soybean yield in Argentina (*P* value for previous crop by inoculation interaction = 0.479). In Argentina, the vast majority (88%) of sites previously had been sown to soybean, but the inoculant effect was more effective in fields which had no history of soybean in the crop sequences (Table 3). The discrimination between sites rotated with soybean or from virgin lands for soybean production was not feasible in the U.S. trials because almost all the sites had a history of soybean production.

Seeding Date

The wide range on seeding dates for U.S. trials from early in April until late planting during June allowed us to look at early and late seeding effects on the responses to inoculation. The inoculant had a greater impact on soybean yield, both in absolute terms and percent increase, when planting occurred later in June compared with early dates (Table 3). In Argentina, this analysis was not feasible because of its limited variation because all trials were seeded in early planting periods from late October until mid-November.

Table 2. Soybean mean grain yield increases in the United States (USA) and Argentina due to inoculation with *Bradyrhizobium japonicum*. *n* = number of experimental sites, UCL = upper confidence level, LCL = lower confidence level.

Country (*n*)	Mean yield			Differences			
	Inoculated	Control	Difference	UCL$_{(95\%)}$	LCL$_{(95\%)}$	Relative increment	*P* value
	Mg ha^{-1}						
USA (187)	3.70	3.64	0.06	0.10	0.02	1.67%	0.007
Argentina (152)	3.16	2.97	0.19	0.25	0.13	6.39%	<0.001

Soil Properties and Rainfalls

The soil pH ranged from slightly acidic to alkaline in the U.S. trails and to neutral values in the sites in Argentina (Table 1). In the United States, pH had no interaction with the performance of the inoculation practice (Table 3). In Argentina, however, the inoculation practice was more effective in terms of yield response in soils with lower pH (Table 3).

Soil organic matter contents showed similar ranges of variation among field trials in both countries (Table 1). This soil property did not affect the performance of the inoculant in the United States. However, in Argentina, SOM affected both the yield of the crops and their responses to inoculation. While the response to inoculation was not significant at two contrasting levels of SOM the absolute (Mg ha^{-1}) differences in yield between inoculated and control crops were greater ($P < 0.001$) in the sites with greater levels of SOM (Table 3).

Only in the sites from Argentina there was available data for soil extractable P and for soil extractable S contents at 82 and at 31 sites, respectively. Monthly rainfall data during the growth of the crops (October–April), recorded in 63 of the Argentinean sites, was also available. It was observed that the inoculation practice had a larger effect on soybean yield in sites with lower January precipitation (Table 3). Although a significant soil P ′ inoculation interaction was observed, the response to the inoculation treatment was not significant for either level of soil P. Soil S and rainfalls for other months in the growing season did not impact yield responses obtained by inoculation (Table 3).

DISCUSSION

Soybean grain yield increases ($P < 0.05$) due to inoculation of soybean with *B. japonicum* occurred in both countries but were greater in Argentina (Table 2). An average of 1.67% yield increase occurred over the 4 yr of trials in the United States compared to 6.39% increase in the 5 yr of trials in Argentina. The higher response to inoculation in Argentina may be due to lower levels of indigenous rhizobia in Argentinian soils in which the dry winter reduces the indigenous rhizobia populations, leading to a greater opportunity for the response to introduced elite strains. This is, however, unlikely to be the sole reason for the difference as the data from Argentina is like that from Brazil on sites where the indigenous populations of *Bradyrhizobium* spp. were greater than 10^6 cells kg^{-1} (Hungria et al., 2005). The difference in the

Table 3. Soybean grain yield increases in the United States (USA) and in Argentina due to inoculation with *Bradyrhizobium japonicum* under different conditions. UCL = upper confidence level, LCL = lower confidence level. DAA1 = days after 1 April·, SOM = soil organic matter.

		Mean, Mg ha^{-1}			Differences			
Country	Comparison	Inoculant	Control	Difference	UCL $_{(95\%)}$	LCL $_{(95\%)}$	*P* value	*P* value interaction
USA	Low yield, 1 Mg ha^{-1}	1.07	0.93	0.13	0.19	0.08	<0.001	<0.001
	High yield, 5 Mg ha^{-1}	5.01	4.99	0.03	0.06	0.00	0.042	
Argentina	Low yield, 1 Mg ha^{-1}	1.05	0.95	0.09	0.13	0.05	<0.001	<0.001
	High yield, 5 Mg ha^{-1}	5.08	4.92	0.17	0.21	0.12	<0.001	
USA	Early seeded (40 DAA1)	4.02	3.97	0.05	0.09	0.00	0.075	<0.001
	Late seeded (80 DAA1)	2.74	2.53	0.21	0.30	0.11	<0.001	
USA	Low soil pH (5.5)	3.49	3.45	0.04	0.18	–0.10	0.574	0.139
	High soil pH (6.8)	3.77	3.69	0.08	0.12	0.04	<0.001	
Argentina	Low soil pH (5.5)	3.70	3.54	0.16	0.32	0.00	0.050	0.002
	High soil pH (6.8)	2.78	2.69	0.09	0.22	–0.04	0.149	
USA	Tillage: no	3.34	3.32	0.02	0.12	–0.08	0.661	0.283
	Tillage: yes	3.80	3.72	0.08	0.12	0.04	<0.001	
Argentina	Tillage: no	3.20	3.04	0.15	0.25	0.05	0.008	0.4562
	Tillage: yes	2.68	2.45	0.23	0.42	0.04	0.018	
USA	Low SOM, 16.0 g kg^{-1}	3.81	3.72	0.10	0.15	0.04	<0.001	0.520
	High SOM, 52.0 g kg^{-1}	3.70	3.68	0.02	0.13	–0.08	0.670	
Argentina	Low SOM, 16.0 g kg^{-1}	2.77	2.65	0.11	0.25	–0.02	0.102	<0.001
	High SOM, 52.0 g kg^{-1}	5.06	4.80	0.26	0.76	–0.25	0.303	
USA	Soybean previous crop	3.51	3.41	0.09	0.18	0.00	0.040	0.011
	Corn previous crop	3.88	3.81	0.07	0.12	0.02	0.003	
	Another previous crop	3.50	3.60	–0.10	0.01	–0.21	0.069	
Argentina	Soybean previous crop	3.01	3.06	–0.05	–1.51	–1.47	0.748	0.479
	Corn previous crop	3.10	2.90	0.20	–0.25	–0.44	0.148	
	Another previous crop	2.97	2.84	0.13	–0.03	–0.16	0.100	
Argentina	Soybean in rotation: no	3.66	2.83	0.83	1.14	0.51	<0.001	<0.001
	Soybean in rotation: yes	3.12	3.01	0.11	0.19	0.03	0.007	
Argentina	Low soil P, 10 mg kg^{-1}	2.34	2.21	0.13	0.32	–0.05	0.158	<0.001
	High soil P, 40 mg kg^{-1}	4.52	4.42	0.10	0.43	–0.23	0.544	
Argentina	Low soil S, 2 mg kg^{-1}	4.46	4.26	0.20	0.35	0.05	0.009	<0.001
	High soil S, 16 mg kg^{-1}	1.81	1.45	0.37	0.59	0.15	0.001	
Argentina	Low January rainfalls, 10 mm	2.75	2.56	0.18	0.34	0.03	0.027	<0.001
	High January rainfalls, 250 mm),	4.89	4.79	0.10	0.33	–0.13	0.363	

response to inoculation between Argentina and U.S. field trials could also be explained by the potential need of N for crop production. In general, soils in the United States are richer in available N due to both high SOM contents and N fertilization doses applied in the corn rotation than Argentine soils. In this study, the soybean yields overall were lower in Argentina, which might be due to more frequent stressful conditions under negative water balances and high temperatures during the growth of the crops. Also, it could be because of the more frequent limitations in P nutrition related to the infrequent P fertilization practices for soybean production in Argentina. In other words, the N requirement fulfilled by BNF represents almost 2% of the attainable grain yield under U.S. cropping conditions and approximately 6% under Argentine conditions. There may be greater responses in Argentina because the soils are of lighter texture with lower SOM contents and N-binding capacity that limits yields. Low N availability in soils and greater plant N requirement leads to an increased requirement for added N inputs, and consequently greater responses to inoculation. This also can be seen in the data from the U.S. trials where the relative benefits from BNF are greater when the overall yields are lower (Table 3).

In our study, tillage did not affect the performance of inoculant on soybean grain yield. However, this result may be biased as minimum or no till was the standard in Argentina while conventional tillage was the most common practice in the trials performed in the United States. The difference in tillage practices in studies between the two countries might lead to the conclusion that the larger response to inoculation in Argentina compared to the United States was, at least in part, due to differences in tillage. Generally, under no-tillage practice the extractable NO_3–N levels are smaller than under tillage (Hungria et al., 2005). Thus, it could be assumed that the need for biological N nutrition should be greater in soils under no tillage practices, which supports the greater response to inoculation described in Argentina compared with similar studies performed in the United States. However, a previous Argentinean study of soybean BNF concluded that tillage did not have a significant effect on the amount of fixed N (Di Ciocco et al., 2008). Brazilian soils that had not received any inoculant for the last 15 yr in areas under no-till cultivation practices had higher numbers of *Bradyrhizobium* spp. than conventionally tilled soils. And, in addition, isolates with higher rates of BNF were isolated from no-till sites (Ferreira et al., 2000). The increased build-up of effective *Bradyrhizobium* spp. in soils could increase the competition between natural and induced populations reducing the effectiveness of the inoculation practice. Tillage could, however, have an important effect on inoculation effectiveness at certain geographic locations. In the northern part of the United States, lower early season soil temperatures and high soil moisture contents under reduced tillage can delay nodulation and contribute to lower exudation of flavanoids leading to reduced nodulation (Hungria et al., 2005; Zhang et al., 1996). Flavanoids are normally responsible for signaling the production of LCO by the rhizobia. In our studies, the effect of reduced exudation of flavanoids could have been overcome by the addition of the LCO in the product formulation (Smith et al., 2015). No tillage practices, compared with tillage operations, modifies not only mineral N availability but also other soil physicochemical characteristics (Díaz-Zorita et al., 2002) that directly interact with plant growth. The effect of tillage needs more investigation in more controlled site selection within each region (Zhang et al., 1996).

The findings in this study support the influence of soil factors on the effectiveness of inoculation. Soil acidity, texture, moisture, low soil P, or high soil N levels can all affect symbiosis between host and rhizobia, due to effects on rhizobium survival in soil, the host, or the process of nodulation itself (Graham et al., 2004; Sadowsky, 2005; Schipanski et al., 2010). Soil N is often thought to be the dominant controlling factor on the efficacy of N_2 fixation, but this can be secondary to environmental and site characteristics (Schipanski et al., 2010). The differential response to soil pH in the United States and Argentina was unexpected. Understanding pH effects is however a complex process as acidity also influences both the growth of the legume plant and the infection process which may be due, to a disruption of signal exchange between macro- and micro-symbionts as well as a repression of nodulation genes and excretion of nodulation factors in the rhizobia (Sadowsky, 2005). The results of the sensitivity analysis of pH in the U.S. trials agree with findings that the rhizobia do not function in acidic soils with pH values below 5.0 and between pH 5.0 and 6.0 the BNF process may also be limited by Mo deficiency (Elmore, 1984). In the pampas region of Argentina, if soil pH is greater than critical levels for normal soybean production, lower pH values relates to more available or extractable nutrients (i.e., P) and, when combined with higher SOM and fine-textured soils, to a greater soil water holding capacity and increased crop productivity (Díaz-Zorita and Buschiazzo, 2006). Collino et al. (2015) also showed that in Argentina the BNF increases when the soil pH decreases and it was positively related with increasing soil P levels. Under the current production conditions in Argentina, soil pH is not a limiting factor for soybean and the production of other row crops, no liming is recommended and no significant responses to lime application are frequently observed.

The relationship between SOM and the response to inoculation was also surprising as our results showed no effects of SOM contents in U.S. trials while in the sites performed in Argentina the percent yield increase due to inoculation was greater in those with higher SOM contents (Table 3). It is thought that the statistical power to detect differences between the control and inoculated treatments was diminished at Argentina sites with greater SOM and with greater standard error of difference. Most of the agricultural land in Argentina lays mainly in semiarid and subhumid regions where increasing the SOM levels supported by greater water holding capacity and availability of nutrients provide better conditions for crop production (Díaz-Zorita et al., 1999). Thus, greater SOM contents could suggest greater attainable yields or higher plant N requirement, supporting the crop growth as well as better growth conditions for supporting the nodulation and BNF process. Waterer and Vessey (1993) proposed that as SOM levels increase in legume-based systems over time, the mineralization of N from larger SOM pools may suppress BNF. This is a complex subject as the N_2 fixation process involves a symbiosis between a plant and a microbe; and it can be affected by any factor that affects the growth of the rhizobia or the host plant (Sadowsky, 2005). In Argentina, the higher response to inoculation occurred when rainfall was higher at the beginning of the growing season and at higher soil P and S levels. This agrees with other studies on the effects of soil conditions on N_2 fixation. Soil water directly affects the growth of rhizobia, and indirectly affects the symbiosis by altering plant growth root architecture and exudations (Sadowsky, 2005). January rainfalls in Argentina occur during flowering and the early seed-filling period. Consequently, having adequate rainfall during January supports more grain yield

Table 4. Economic benefit of using *Bradyrhizobium japonicum* inoculation for soybean production in the United States. *n* = number of experimental sites.

Year (*n*)	Mean Inoculant	Mean Control	Mean Difference	Increase	*P* value	Grain mean price	Gross return
	Mg ha^{-1}			%		US$ Mg^{-1}	US$ ha^{-1}
2009 (39)	3.67	3.69	–0.02	0.66	0.675	369	–7.39
2010 (34)	3.94	3.84	0.10	4.30	0.006	366	36.63
2011 (54)	3.58	3.48	0.1	3.90	<0.001	460	46.00
2012 (58)	3.43	3.38	0.05	1.40	0.021	512	25.63

and consequently more N demand and provides a better growth to support the BNF process (Collino et al., 2015). If this process is based on introduced strains, we can also support the results of greater responses to inoculation practices under good production conditions.

In both regions, the use of rhizobia inoculants provides consistent crop yield enhancement. Although the yield contribution is smaller in the United States than in Argentina, inoculation with rhizobia in both regions provides consistent benefits that justify the cost of the practice. For example, the 0.06 Mg ha^{-1} increase in the U.S. yield averaged over four consecutive growing seasons, and based on yearly averaged U.S. soybean prices published by the National Agricultural Statistics Service, would give an average gross return of approximately US$25 ha^{-1} (Table 4). Based on these results and considering an average inoculant cost of $12 ha^{-1} the net return due to the inoculation practices is approximately $13 ha^{-1} and realize an economic advantage in 3 out of 4 yr. This agrees with Beuerlein (2015) who pointed out that because inoculants are low cost inputs for crop production, a response of 33.5 kg ha^{-1} can give an economic benefit for farmers. These yield increases should also be viewed as conservative estimates because small plot data can under estimate the yield increases that can be obtained with inoculants evaluated under large plots (Leggett et al., 2015). For example, in 37 inoculation trials conducted in 10 states over 4 yr in the United States, soybean grain yield increases in large demonstration plots showed a mean increase of a 187.6 kg ha^{-1} equivalent to 5.2% of relative contribution over the control without inoculation (Leggett, unpublished data, 2012). Furthermore, inoculation may increase seed N levels and N levels in plant residues (Vessey, 2004). And, also the BNF has the potential to reduce the excess application of synthetic N fertilizers and their risky impact on the environment (Olivares et al., 2013).

CONCLUSIONS

Small plot trials conducted in a narrow geographic region are commonly used to test the effectiveness of agricultural inputs. These inputs may have small, but important, benefits which may not be detected in trials conducted at a few sites in a limited region. This leads many researchers to conclude that there is no benefit to be gained by tested inputs. An example of such a benefit might be a yield increase due to rhizobia seed treatment in soybean grown on soils without history of rhizobia-inoculated crops.

The data from this analysis, with a large number of studies from a wide geographical and seasonal range, showed significant increase in soybean grain yield due to inoculation with *B. japonicum*.

The multivariable analysis of the data showed no correlation between soil tillage methods and grain yield increase due to *B. japonicum* inoculation. Similarly, we found no clear correlation between other soil properties such as pH or SOM contents and the extent of yield increase. In the United States, the inoculation practice increased yields more in alkaline soils while in Argentina the biggest yield increases were measured in soils with pH values lower than 5.5. The field history had positive effects on the performance of the inoculant practice in the United States, where fields previously under soybean or corn produced the largest yield increases due to inoculation. As expected, in Argentina, fields with no history of soybean had the greatest yield response to rhizobia inoculation.

While the relative importance of the variables on soybean production and the responses to inoculation was assessed, the available information and the scope of the study did not support the development of a full model involving the interactions. The results reported in this study can contribute to identify individual factors that should be included in full models involving environmental and crop management variables.

The response to inoculation varied across locations and years with the greatest benefit in sites with lower than normal yields. The average yield increase obtained in field trials were 1.67% in the sites located in the United States and 6.39% in those from Argentina equivalent to 60 and 190 kg ha^{-1}, respectively. With the current soybean prices, this response would translate to an increase in profit, which confirms the conclusion that in most years, inoculation is a good practice as it is cheap input that provides economic benefits in the long run. These results support, under the current soybean production practices in the United States and in Argentina, that regular soybean seed inoculation providing *B. japonicum* strains is profitable.

ACKNOWLEDGMENTS

The authors declare that, although several of the authors are from the Research and Development and Technical Development teams of Novozymes BioAg and Monsanto BioAg, there is neither censorship of results nor conflict of interest regarding the results presented and the conducted research.

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