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Handbook for Azospirillum

Technical Issues and Protocols

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Chapter 24
Field Evaluation of Extensive Crops Inoculated with *Azospirillum* sp.

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Abstract The available information about the changes in growth and production of extensive crops due to the inoculation with formulations containing *Azospirillum* sp. is abundant. The crop responses to the presence of *Azospirillum* sp. are generally related with changes in their growth during early stages of development. It leads to variable contributions to the final grain production depending on the differences in the occurrence of abiotic stresses (i.e., water and nutrient uptake). We present, based on recently available articles, the design of field trials and its analysis for describing the value of the inoculation practice in terms of grain production as a tool to support crop management decisions. The ecophysiological responses require the interpretation of the results based on the frequency of their occurrence as well as the discrimination among hierarchical productivity factors (i.e., spatial and temporal variability). Because of the variability in the crop responses to the inoculation with *Azospirillum* sp., a large number of observations are needed to describe significant differences between treatments. In the case of rainfed wheat (*Triticum aestivum* L.) crops from

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the semiarid pampas (Argentina), the range can vary between 60 in seasons with normal rainfall patterns and almost 200 cases in dry seasons. The analysis of distribution response values is a more realistic approach to measure the crop performance to inoculation under the interaction of complex random factors and provides also a probabilistic answer to stakeholders. For example, based on the distribution of maize (*Zea mays* L.) responses to *Azospirillum* sp. from 316 cases located in the pampas region of Argentina it was observed that in more than 81.1 % of the cases the incremental revenue of the practice exceeded its cost and it could be included as a crop management practice under the current production conditions.

24.1 Introduction

The diverse single modes of actions to enhance plant growth and crop production in the presence of *Azospirillum* sp. has been widely studied also under diverse experiences setups. In general, the modes of action that partially explain crop growth and yield responses to the inoculation with *Azospirillum* sp. are both direct and derivate effects enhancing the nutritional status of the plants and mitigating other abiotic stresses. Abundant studies show that *Azospirillum* sp. has the capability, within other attributes, for fixing nitrogen from the air, delivering hormones and enzymes to the rhizosphere that can enhance root growth and promote also the growth of other microbes (Bayan and Levanony 1990; Okon and Labandera-Gonzalez 1994; Dobbelare et al. 2003). Most of the studies that describe several of its modes of action have been performed within pots under controlled conditions in greenhouses (Van Dommelen et al. 2009; Rodrigues et al. 2008) with limitations for the extrapolation to regular crop conditions.

Field trials analyzing the contribution of the inoculation with *Azospirillum* sp. on extensive crops under regular crop production conditions are less abundant. Furthermore, the interpretations of their results are variable and not always conclusive. This could be partially explained because of the complexity in the design of the field studies in response to the diverse mode of interaction of *Azospirillum* sp. and plants under multiplicity in abiotic stress conditions to be mitigated in the presence of the microorganism. The objective of this contribution is to briefly describe several field and data analysis procedures for the evaluation of crop production responses to the inoculation with *Azospirillum* sp.

24.2 Design of Field Experiments

We reviewed 47 articles worldwide published during the last decade showing grain crop production in response to the application of diverse *Azospirillum* sp. inoculants in 12 countries, collecting 347 cases. Most of the studies were performed in cereals' crops (86.7 %), mainly rainfed maize (*Zea mays* L.) and they were located in Latin America, mostly Brazil, and Asia. Approximately, among all the reported crops,

Table 24.2 Maize distribution parameters of control and *Azospirillum brasilense* inoculated maize crops in a database from 316 field experiments performed in Argentina

	Percentile							Mean	SD
	0	5	25	50	75	95	100		
Treatment	Grain yield (kg ha^{-1})								
Control	2,020	4,563	7,169	8,792	10,545	12,855	15,924	8,794	2,512
Inoculated	2,394	5,092	7,583	9,196	11,015	13,095	18,654	9,237	2,562
	Relative yield increase to inoculation (%)								
	–43	–8	1	4	9	24	66	6	11

SD Standard deviation

were needed to describe differences between the means while in the driest and low yielding season more than 200 samples should be required. This large number of observations needed for describing significant differences between the treatments could be diminished limiting the location of samples to spatially homogeneous sites.

24.4 Applying Experimental Data to Evaluate Crop Management Decisions

Experimental data are particularly valuable if they are used to support management decisions. In the previous sections of this chapter, it was shown that *Azospirillum* sp. inoculation is expected to increase crop yields. However, response to *Azospirillum* sp. cannot be predicted with certainty since it is a random variable partially because of its diverse modes of actions mitigation also multiple abiotic stresses during crop growth. In fact, negative, neutral, and positive responses to inoculations with *Azospirillum* sp. were found in any crop species (Araujo et al. 2013; Hungria et al. 2013; Piccinin et al. 2011; Turan and Fikrettin 2013). So, how can the experimental information be used to take decisions? To choose some experiments according to predefined criteria (i.e., soil type or growing season) could be a way and has been discussed in the previous section. But, incorporating particular criteria to select information could be misleading, particularly if it is done by inexperienced persons. In this section, we are analyzing an alternative way to look and explore experimental data to evaluate the convenience of crop inoculations. For this purpose, we used results from 316 field experiments where the grain yields of maize crops under control without inoculation and inoculated with *Azospirillum brasilense* strain INTAAz39 treatments were measured in Argentina. The data were obtained in various locations of the pampas region under regular production practices (i.e., NP fertilization, rotated land, mostly uner zero tillage practices) and during 12 consecutive growing seasons (M. Díaz-Zorita, unpublished data). Because, an ANOVA analysis of the treatment response showed no significant ($P=0.05$) differences among them, the whole database (316 data set) was used.

Crop management decisions are usually evaluated on the basis of the difference between incremental revenue and incremental costs. If the incremental revenue is higher than the incremental cost, there appears to be an advantage in the technology. In this case, incremental revenue is provided by yield response (yield increment provided by the technology) multiplied by net price (gross price discounted all marketing expenses). As both yield response and price are random variables, incremental income turns out to be a random variable itself. As such, it cannot be described by a static value. Instead, it needs to be described by a distribution function. Using empirical data, the probability distribution of maize yield in the control and inoculated treatments may be described by various percentile values in the range from 0 to 100 (Table 24.2). Moreover, maize yields may be compared statistically in various percentile ranges (Table 24.3). In the database used, the treatments were compared in a wide range of conditions, from crops yielding as low as 2,020 kg ha^{-1} to as high as 18,654 kg ha^{-1} (Table 24.2). Although percentile values differed along the whole distribution, only mean yields of the treatments distribution in the range from 5 to 95 % were significant (Table 24.3). The probability to find differences in both extremes of the data distribution was low; however, in the central 90 % of the distribution, in crops yielding between 6,000 and 12,000 kg ha^{-1}, crops inoculated with *Azospirillum brasilense* had a consistent higher probability of greater yields than control crops (Table 24.3).

Potent tools may be used to extend and analyze the data described. One of this, Monte Carlo simulation, is a technique that allows estimating the probability distribution of an outcome that depends on random variables. Even though it was originally developed with other purposes (i.e., operations research field), it has been widely applied to crop decision analysis under uncertainty (Clow and Flaskerud 2001; Ferreyra et al. 2001; Berger and Pena 2013). In order to use Monte Carlo simulation, probability distributions need to be defined for any random variable involved. From the experimental data of maize yield responses to *Azospirillum brasilense* inoculation in Argentina, a general probability distribution was built showing a range from –43 to 66 % and less than 20 % change of negative responses (Table 24.2). But, as crop response data are not normally distributed, other probability distributions that are

Table 24.3 Mean values of percentile ranges in the empirical distribution of maize grain yields from control and *Azospirillum brasilense* inoculated crops in a database from 316 field experiments performed in Argentina

	Range of the empirical distribution				
	0–5	5–25	25–75	75–95	95–100
Treatment	Grain yield (kg ha^{-1})				
Control	3,691	6,118	8,755	11,541	13,838
Inoculated	3,912	6,566	9,216	11,948	14,448
P	ns	<0.001	<0.001	<0.001	ns
n	16	63	157	64	16
SE	209	92	78	82	316

P statistical significance, *n* number of sites considered in each range, *SE* standard errors of the means, *ns* nonsignificant differences between means

frequently employed, betapert and cumulative, were also tested. The betapert distribution is simple to use in a variety of situations, both when counting with observed data and when working with limited information or also just with only expert opinion. The cumulative distribution can be chosen when large amount of data is available because it implies no data manipulation. These distributions need the subjective estimation of two values to be used as parameters for the range. The values defined as minimum and maximum have zero probability of being sampled in a Monte Carlo simulation process. Therefore, the lowest and the highest values of a sample of real data cannot be used as estimators, which for the exercise performed here were set at –45 and 75 %. In this example, the maize grain yield response to the inoculation with *Azospirillum brasilense* in 316 sites from Argentina was better reproduced when using a cumulative distribution (Table 24.2). The normal and general distribution replicated better the observed data than the betapert distribution but both overestimate the probability of null responses.

To analyze and to make decisions with the results, the maize price has to be described and it is also a random variable. In this analysis, the maize price distribution was defined using a betapert function and the parameters were estimated on the basis of historical prices with inflation adjustment and expert opinion. This is a common procedure when using Monte Carlo simulation in daily decision making. Because of structural changes in commodities markets long time series may not be relevant for producer's decision making in Argentina and we used the last 7 years of maize prices in Argentina at harvest. The resulting betapert distribution for gross price was: 118.7 (minimum), 170.0 (most probable), and 200.0 (maximum) US$ ton^{-1}. For the analysis, the marketing expenses were set at current values with fixed expenses (freight and drying) of 38 US$ ton^{-1} and 3 % of commissions and taxes. Then, the net price was calculated as 97 % of the gross price minus the fixed expenses per produced ton. Using agricultural simulation models and expert opinion, a betapert distribution was defined for maize "base yield" without *Azospirillum brasilense* inoculation. The parameters for this distribution were 5,009 (minimum), 10,630 (most probable), and 12,623 (maximum) kg ha^{-1}, with an expected grain yield of 10,025 kg ha^{-1}. The revenue was calculated as the product between the base yield, the percent of response and the net price. With the distribution shown in the Fig. 24.1, the probability of incremental revenue exceeding the cost of inoculation with *Azospirillum brasilense* can be calculated. If this probability is high enough, there may be no doubt that *Azospirillum brasilense* inoculation will be included as a crop management practice. In this case, based on the distribution of maize responses to *Azospirillum* sp. from 316 cases located in the pampas region of Argentina, it was observed that in more than 81 % of the cases the incremental revenue of the practice exceeded its cost, and it could be included as a crop management practice under the current production conditions.

The approach presented is quite different from the way that usual experimental results are used to help stakeholders. A deterministic result, usually the mean response value, was here replaced by a distribution of values and a probabilistic answer to the stakeholders. This may be considered a more realistic approach to measure the response of the maize crops to inoculation with *Azospirillum brasilense* in Argentina.

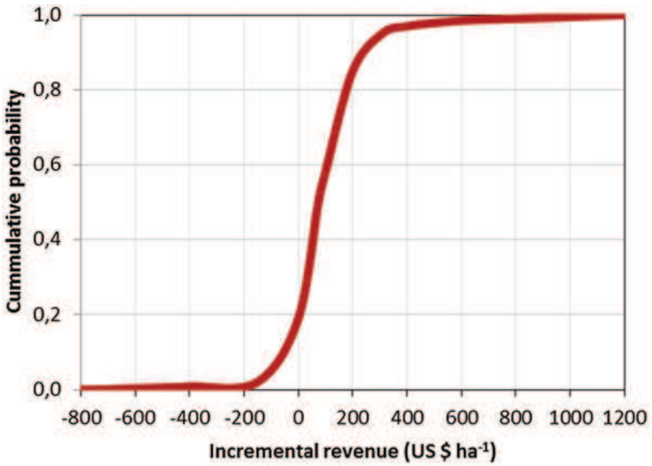

Fig. 24.1 Incremental revenue distribution for *Azospirillum brasilense* inoculation in maize crops from the North of Buenos Aires Province, Argentina