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3 **Nitrogen fertilization and inoculation of seeds**
4 **with *Rhizobium tropici* on the agronomic**
5 **performance of common beans**

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11 **ABSTRACT**
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Aims: This study aimed to evaluate the influence of nitrogen fertilization and inoculation of seeds with *Rhizobium tropici* on the agronomic performance of common bean (*Phaseolus vulgaris* L.).

Study design: The experimental design consisted of a randomized block in a 4x2 factorial layout, with 4 replicates, the first factor referring to inoculant doses (0, 50, 100 and 200 mL for each 25 kg of seed), while the second refers to nitrogen fertilization (0 and 40 kg ha of N).

Place and Duration of Study: The study was conducted to the field in a no-till system area, in the southwestern region of Paraná, Brazil. The soil is classified as a Purple Latosol, with a clayey texture.

Methodology: The adopted spacing was 0.45 m between rows, and the seeding density used was 12 seeds per furrow meter. The inoculants NITRO 1000 *Rhizobium tropici* SEMIA 4077 and SEMIA 4088 were applied, varied according to the treatments. The cultivar used was IPR – Tangará.

Results: As for grain yield, it was observed that nitrogen fertilization did not contribute to the increase in grain yield, and higher productivity was obtained in the absence of N. This was probably due to the high content of organic matter present in the soil.

Conclusion: Nitrogen fertilization at sowing and seed inoculation with *Rhizobium tropici* did not influence the plant population and the 1000-grain mass. In the absence of nitrogen fertilization at sowing, pods with a longer length and higher grain yield were obtained. Inoculation of the seeds with *Rhizobium tropici* exerts a positive influence on plant height, number of nodes of the main stem, number of pods per plant, and number of beans per pod, the dose recommended by the manufacturer (100 mL) being efficient, with the possibility of applying a dose of 50 mL, to satisfactory results.

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14 *Keywords: Phaseolus vulgaris L., Biological Nitrogen Fixation, Inoculation, IPR-Tangará*

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18 **1. INTRODUCTION**

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20 The common bean (*Phaseolus vulgaris* L.) is grown in more than 100 countries, although
21 over 60% of world production is taking place in only five countries, Brazil is the largest
22 producer and consumer in the world. The area cultivated with beans in Brazil as of the
23 2017/18 harvest was estimated at 3.2 million hectares, 0.5% higher than in the 2016/17

24 harvest. The mean productivity is projected at 1,043 kg ha⁻¹, 2.4% less than in the last
25 harvest. Considering the sown area and expected productivity, bean production in the
26 2017/18 harvest is expected to reach 3.35 million metric tons, 1.9% less than in the previous
27 harvest [1].

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29 Several factors interfere in the yield of common bean, including nitrogen fertilization
30 management. Inadequate nitrogen fertilization is a factor that often determines the failure of
31 common bean crops. While a few producers apply excessive doses of N, others apply
32 insufficient amounts of this element, limiting crop yield even if other factors of production are
33 optimized [2]. Furthermore, it is necessary to correctly manage the N, as it is a nutrient that
34 can easily be lost through the leaching, volatilization or denitrification processes [3].

35

36 N is the nutrient required in the greatest quantity by beans [4] and although there is a
37 recommendation for the use of nitrogen fertilizer for common bean crops, research results
38 suggest that this crop can benefit from biological nitrogen fixation (BNF), it may contribute to
39 increased crop yield and reduce or replace the use of nitrogen fertilizers [5], thereby
40 decreasing production costs and the environmental impact of the use of chemical fertilizers.
41 It should be noted that the high dependence on fertilizers by crops, for example, causes an
42 increase in the energy costs for conversion of atmospheric nitrogen, in addition to the
43 extraction process of the other elements, such as phosphorus and potassium [6]. Moreover,
44 excess nutrients applied in conventional agriculture may cause environmental problems.
45 Environmental costs of all nitrogen losses in Europe have been estimated at €70-320 billion
46 per year, which outweighs the direct economic benefits of nitrogen in agriculture [7].

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48 The practice of seed inoculation with rhizobia represents a low-cost alternative to increase
49 the yield of common bean, in addition to avoiding the contamination of water resources by
50 nitrogen fertilizer and reducing greenhouse gas emissions. It is proven to be a cheap
51 technology, simple to use, and with a good economic return. Being a legume, the common
52 bean presents conditions to benefit from symbiotic association with *Rhizobium*, which
53 contributes specifically to N savings. Nevertheless, according to Rabelo et al. [8], there is still
54 a need for improvements to develop an inoculation that fully replaces nitrogen fertilization in
55 common beans, as is the case with soybeans. FBN contributes positively to the development
56 of this culture, but not to a sufficient degree to dispense N applications.

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58 This study aimed to evaluate the influence of nitrogen fertilization and inoculation of seeds
59 with *Rhizobium tropici* on the agronomic performance of common beans.

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61 2. MATERIAL AND METHODS

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63 The experiment was conducted in an area under a no-till system, located on the São Pedro
64 da Bandeira line, in the municipality of Dois Vizinhos, Paraná, Brazil. The municipality is
65 located in the Southwest region of the State, between the geographic coordinates 25°44' S
66 and 53°03' W, with an altitude of 509 m.

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68 The climate of the region according to the Köppen classification subtropical hot humid (Cfa),
69 with a mean annual rainfall of 2,044 mm, well distributed throughout the year. The mean
70 daytime temperature is 19.6°C and the mean nighttime temperature is 15.2°C [9]. The mean
71 rainfall and temperature in the months of the experiment are shown in Table 1.

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75 **Table 1. Rainfall (mm), maximum temperature (MAX), minimum temperature (MIN), and**
76 **mean (MDA) in °C, in the months of execution of the experiment.**

Month	Precipitation (mm)	Temperature		
		MAX	MIN	MDA
November	112	31.4	21.4	26.4
December	266	32.03	24.09	28
January	200	32.96	22.23	27.5

Data obtained at the property where the experiment was conducted.

The soil is classified as a Purple Latosol, with a clayey texture [10]. The area is characterized by presenting a slightly uneven topography, with a level curve and no erosion. Before the sowing of beans, the area had been sown with an oat crop. Before the bean sowing, a soil sample was collected at a 0-20 cm depth. The results obtained were: pH CaCl₂ 6,7, P 40.05 mg dm⁻³, organic matter 38.28 g dm⁻³, Ca, Mg, K, Al and H+Al, respectively, 8.18, 3.33, 0.78, 0.0, 2.37 cmolc dm⁻³ and V 83.83%.

A Semeato Personale seed drill was used for sowing the beans. The adopted spacing was 0.45 m between rows, and the seeding density used was 12 seeds per furrow meter. The seeds were treated with thiamethoxan 350 g L⁻¹ at the dose 0.7 L of the commercial product for 100 kg of seed. Following that, the inoculants NITRO 1000 *Rhizobium tropici* SEMIA 4077 and SEMIA 4088 were applied. According to the manufacturer, the product concentration is 3.0 x 10⁹ viable cells per ml g⁻¹. The inoculant dose, as well as the nitrogen fertilization, varied according to the treatments. For phosphate and potassium fertilization, 40 kg ha⁻¹ was used. The fertilizer was applied to the side and below the seed.

The cultivar used was IPR – Tangará. This cultivar belongs to the Carioca group and presents an indeterminate growth habit, erect plants with long vines, a mean cycle of 87 days from emergence to harvest, and mean the productive potential of 3,326 kg ha⁻¹. It has resistance to common mosaic, *Curtobacterium* wilt, *Fusarium* wilt and rust, mild resistance to powdery mildew and angular leaf spot, and susceptibility to anthracnose and common bacterial blight. It presents intermediate tolerance to high temperatures and droughts occurring during the reproductive phase. The seeds have a light beige coat with light brown stripes.

The experimental design consisted of a randomized block, with a 4x2 factorial layout, with four replications. The first factor refers to four levels of seed treatment with different doses of inoculant (0 mL, 50 mL, 100 mL, and 200 mL for every 25 kg of seed). The recommended dose according to the manufacturer is 100 mL for 25 kg of seed. The second factor refers to two levels of nitrogen fertilization (0 and 40 kg ha⁻¹ of N at sowing). The treatments were as follows: T₁ = 0 kg ha⁻¹ N and 0 mL inoculant; T₂ = 0 kg ha⁻¹ N and 50 mL inoculant; T₃ = 0 kg ha⁻¹ N and 100 mL inoculant; T₄ = 0 kg ha⁻¹ N and 200 mL inoculant; T₅ = 40 kg ha⁻¹ N and 0 mL inoculant; T₆ = 40 kg ha⁻¹ N and 50 mL inoculant; T₇ = 40 kg ha⁻¹ N and 100 mL inoculant; and T₈ = 40 kg ha⁻¹ N and 200 mL inoculant.

Each experimental plot had 5 m of length composed of 5 lines and spacing of 0.45 m, totalling an area of 11.25 m². The three central lines were considered as the usable area of the plot, excluding 0.5 m from each end of the line, totalling 4.5 m².

During the development of the crop, two manual weeding treatments were performed, at 10 and 25 days after sowing. Phytotoxic treatments (125 mL ha⁻¹) were also prepared aiming at the control of pests such as whitefly, white mite, leafhoppers, Cucurbit beetle and thrips with thiamethoxam (125 mL ha⁻¹) and diseases such as angular leaf spot, anthracnose and rust with fentin hydroxide (700 mL ha⁻¹), these comprising pests and diseases of major importance, which may cause greater damage to the common bean in the southwestern region of Paraná.

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126 The evaluations were carried out at the end of the crop cycle. The following variables were
127 evaluated: plant population, plant height, number of main stem nodes, number of pods per
128 plant, number of grains per pod, mean pod length, 1000-grain mass (13% moisture), and
129 productivity.

130

131 To determine the plant population, the number of plants was counted in 2 m of the usable
132 plot. Plant height was determined by averaging 20 plants harvested from each usable plot,
133 measured from the plant neck to the end of the main stem. The number of nodes of the main
134 stem, the number of pods per plant, the number of beans per pod and the mean pod length
135 were determined by averaging 20 plants randomly collected from each usable plot. The
136 1000-grain mass was determined by averaging 2 samples of each usable plot, each sample
137 having 250 grains, then weighed in a semi-analytical balance and then calculated the
138 moisture content corrected to 13%, estimating the 1000-grain mass. Grain productivity was
139 determined throughout the plot area, correcting for moisture content of 13% and,
140 subsequently, estimated productivity in kg ha⁻¹.

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142 3. RESULTS AND DISCUSSION

143

144 There was no significant effect of the interaction between the inoculant doses and nitrogen
145 fertilization for the variables of plant population, mean pod length, one 1000-grain mass, and
146 productivity. Thus, the results are presented independently. For these variables, there was
147 also no significant effect of inoculant doses. A significant difference was observed for
148 nitrogen fertilization only for the variables of mean pod length and grain yield (Table 2).

149

150 Nitrogen application at sowing did not affect the plant population (Table 2). This can be
151 explained by the fact that the seed contains the nutrients necessary for the establishment of
152 the seedling, such as in the Fabaceae (beans, soybean), in which the endosperm is partially
153 or fully absorbed during the development of the seed in favour of the cotyledons, which
154 assumes the function of tissue [11]. Additionally, the temperature, which had an average
155 value of 26.4 °C (Table 1) may have contributed to the homogeneity of the booth, it is noted
156 that the optimal temperature for bean germination is around 28 °C, and rainfall occurred
157 shortly after sowing (Table 1) [12].

158

159 **Table 2. Population of plants per hectare, mean pod length (cm), 1000-grain mass (g)**
160 **and yield (kg ha⁻¹) of common beans as a function of nitrogen fertilization.**

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	Plant population (ha)	Mean pod length (cm)	1000-grain mass (g)	Yield (kg ha ⁻¹)
Without N	239.777 a	9.12 a	284.31 a	1,716.6 a
With N	237.333 a	8.88 b	287.37 a	1,686.6 b
CV	3.7	2.31	2.33	1.05

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163 Mean values followed by the same lowercase letter in the columns do not differ significantly from each other by the
164 F test (P<0.05).

164

165 For mean pod length, it was observed that there were no significant differences in the
166 different doses of the inoculant (Table 2). This can be explained by the fact that this
167 component presents high genetic heritability, being intrinsically linked to the characteristic
168 of the cultivar [13]. Notwithstanding, a significant difference was observed for nitrogen
169 fertilization, in which the best result was observed in the absence of N, with 9.12 cm (Table
170 2). This was probably due to the high content of organic matter present in the soil.

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172 The 1000-grain mass and grain yield were also not influenced by nitrogen fertilization at
173 sowing (Table 2), as well as by seed inoculation. As for grain yield, it was observed that
174 nitrogen fertilization did not contribute to the increase in grain yield, and higher productivity
175 (1,716.6 kg ha⁻¹) was obtained in the absence of N (Table 2). This was probably due to the
176 high content of organic matter present in the soil.

177
178 It was also possible to observe that the different inoculant doses did not present significant
179 differences. The high production levels obtained, even in the absence of inoculation,
180 demonstrate the great capacity of competition and effectiveness in symbiotic N fixation by
181 native strains. The yields obtained in the absence of inoculation were similar to high doses in
182 some treatments. The non-response to seed inoculation about productivity can be explained
183 by the presence of native *Rhizobium* strain in the soil, which is usually more aggressive than
184 the introduced strain. The presence of native strains in the soil makes it difficult to perform
185 the introduced strain, as they compete for the sites of nodular infection, their presence in the
186 nodulation in plants that did not receive the inoculant being evident, even in a smaller
187 number of nodules, which may be equivalent to other treatments thanks to their greater
188 efficiency [14].

189
190 Ferreira et al. [14] studying *Rhizobium* strains, concluded that the inoculation of efficient
191 strains in common bean-nodulating cultivar, or their cultivation in soils with an efficient native
192 population, may allow the non-use of nitrogen in the bean crop without affecting productivity.
193 Rabelo et al. [8] mention that FBN contributes positively to the development of this crop, but
194 not enough to dispense N applications.

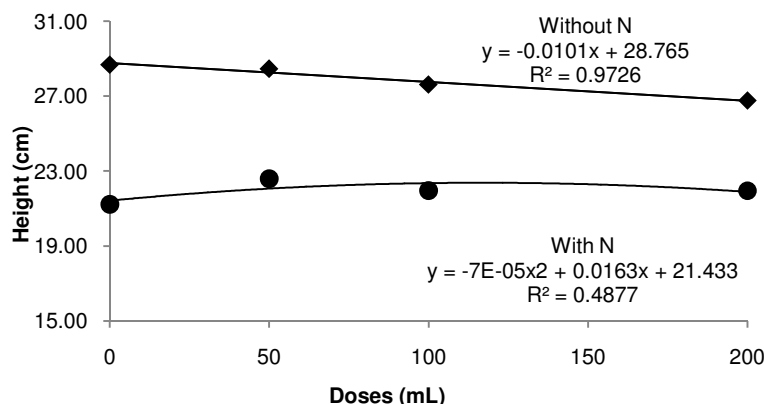
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196 Peres et al. [15], in three years of cultivation and using several cultivars, verified that the
197 inoculation with rhizobia contributed to the increase in grain yield. These data show that the
198 biological nitrogen fixation may supplement the nitrogen fertilization in the bean crop,
199 allowing a reduction in nitrogen fertilizer rates, without losses in grain yield. Nevertheless,
200 Matoso and Kusdra [16] did not find positive results regarding the inoculation of common
201 bean with *R. tropici*.

202
203 There was a significant effect of the interaction between inoculant doses and nitrogen
204 fertilization for the variables of plant height, number of main stem nodes, number of pods per
205 plant, and number of grains per pod.

206
207 Following the interaction between nitrogen fertilization and seed inoculation, similar
208 behaviour can be observed for plant height, number of nodes of the main stem per plant,
209 number of pods per plant, and number of grains per pod. Nitrogen fertilization obtained the
210 lowest mean values in all studied variables, even in inoculated treatments. In the absence of
211 nitrogen fertilization, inoculation provided a considerable increase in some agronomic
212 variables of importance, such as the number of pods per plant and number of grains per
213 pod.

214
215 The height of the plants showed a significant interaction ($P < 0.05$). It was noted that, in the
216 absence of N, the values were higher, and that these values decreased when increasing the
217 inoculant dose (Figure 1), thereby reinforcing the idea of the action of the native strains in
218 the area. The adaptation of rhizobia to the soil depends on the biotic and abiotic conditions
219 of the environment and the wild or cultivated legume species, both in size and variability. As
220 the cultivated area has a high content of organic matter and has been working under a no-till
221 system for a long time, the biotic, physical and chemical conditions of the soil are in
222 favourable conditions for the development of the native strains.

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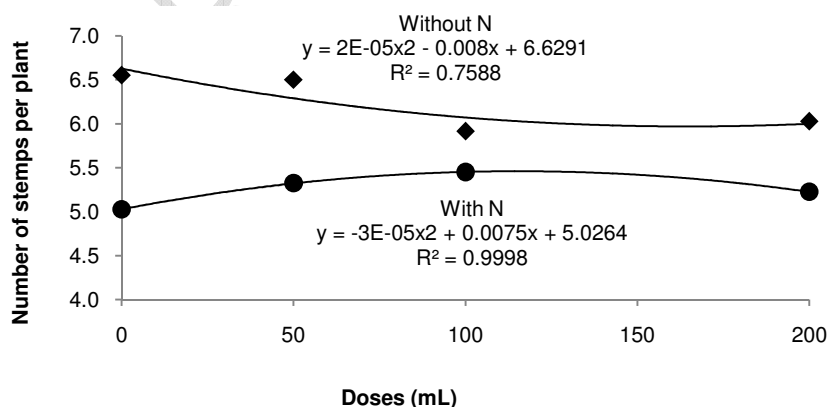
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Fig 1. Plant height of cv. IPR Tangará bean as a function of inoculant doses and nitrogen fertilization.

228 When the same inoculant doses associated with nitrogen fertilization (40 kg ha^{-1} of N) were
229 studied, the best inoculation response was obtained, and the best result was observed when
230 applied at half the recommended inoculant dose (50 mL). Although the mean values were
231 lower than in treatments without N use (Figure 1). N fertilizer may cause a reduction in
232 symbiotic efficiency but, when applied in small amounts in the bean crop, allows an increase
233 in nodule growth and higher BNF, and very low levels of nitrate in the soil may be limiting to
234 symbiotic activity. According to Novo et al. [17], root nodulation supplies the needs of the
235 plants, avoiding the nitrogen fertilization, as it inhibits the formation of the nodules, affecting
236 BNF the biomass production.

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As for the number of nodes of the main stem, results similar to those already studied were
observed, the best results being observed in the absence of nitrogen fertilization. In the
latter, the lower dose of inoculant stands out over the others (Figure 2), followed by half of
the recommended dose (50 mL), as previously discussed on the aggressiveness of the
native strains in the area, with the best performance of these strains in the absence of
nitrogen fertilization at sowing.



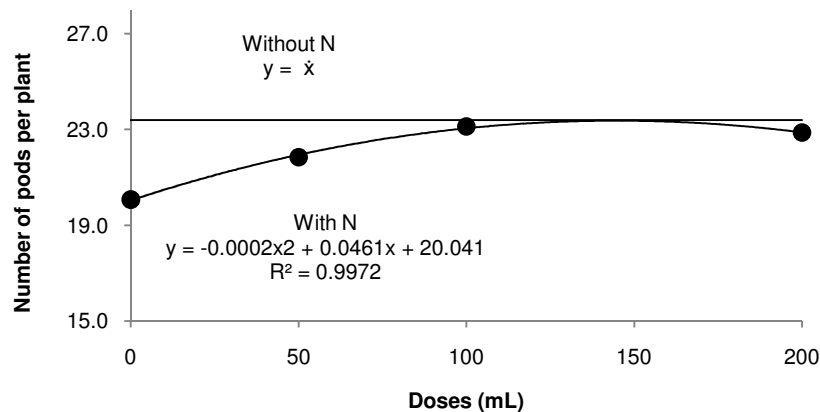
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Fig 2. The number of stems per plant of the cv. IPR Tangará as a function of inoculant doses and nitrogen fertilization.

By adding 40 kg ha^{-1} of nitrogen at sowing, a 15.8% reduction was obtained in the number of
nodes, achieving the best results associated with the recommended inoculant dose (100 mL)
(Figure 2).

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Regarding the number of pods per plant, significant differences were observed between treatments, with better results obtained in the absence of nitrogen fertilization (Figure 3). In this sense, Souza et al. [18] verified that seed inoculation only increased the number of pods per plant in the absence of N application. Nevertheless, in this variable, it is possible to observe that nitrogen fertilization with the aid of the recommended inoculant dose (100 mL) was able to reach values similar to the recommended inoculant dose without N in fertilization (Figure 3). Nevertheless, the cheapest form of fertilization, without N, with the influence of the inoculation, comprising half of the dose (50 mL), exceeded all other treatments.



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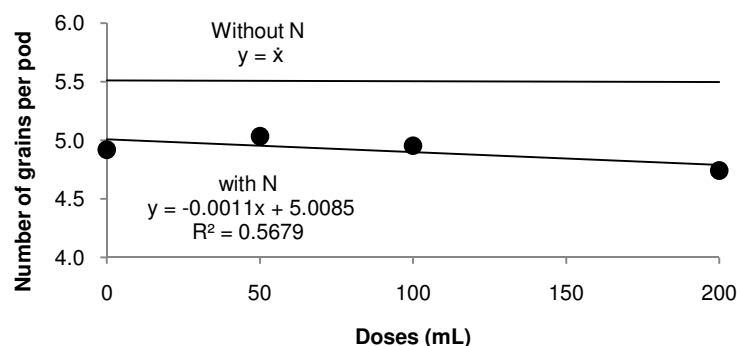
Fig 3. The number of pods per cv. IPR Tangará as a function of inoculant doses and nitrogen fertilization.

Soratto et al. [19] and Alvarez et al. [20] observed positive results for the number of pods per plant when the N doses were increased. Araújo et al. [21] observed no effect of inoculation on the number of pods per plant. The number of pods per plant depends on the cultivar being used.

The number of grains per pod, despite being a characteristic of high genetic heritability [13], and thus related to the cultivar used, showed an interaction between the treatments with the two forms of fertilization and the different doses of the inoculant. In the treatment without nitrogen fertilization, the results were higher (Figure 4), following those found by Soratto et al. [22] which also did not have significant effects on the number of grains per pod with the use of different levels of N.

With the interaction between inoculant doses, it can be observed that half of the recommended dose (50 mL) was superior compared to the others, reaching 5.7 grains per pod (Figure 4). Similar results were found by Araújo et al. [21] and Romanini Júnior et al. [23], who noted that the inoculation with rhizobia contributed to the increase in productivity. Binotti [24] did not find significant differences for the number of grains per pod, using *R. tropici* in the inoculation of the bean seeds.

In the treatment that received nitrogen fertilization, the results were lower, but half of the inoculant dose again presented superior results compared to others in this treatment, reaching 5.1 grains per pod. A different result was found in a study carried out by Farinelli et al. [25], in the state of São Paulo, Brazil, evaluating the application of N doses in the bean crop, in a no-till system and in conventional planting, finding maximum yield with the application of 185 kg ha⁻¹ of N.



292 Fig 4. The number of grains per pod of cv. IPR Tangará bean as a function of
 293 inoculant doses and nitrogen fertilization.
 294

295 4. CONCLUSION

296 Nitrogen fertilization at sowing and seed inoculation with *Rhizobium tropici* did not influence
 297 the plant population and the 1000-grain mass.
 298

299 In the absence of nitrogen fertilization at sowing, pods with a longer length and higher grain
 300 yield were obtained
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302 Inoculation of the seeds with *Rhizobium tropici* exerts a positive influence on plant height,
 303 number of nodes of the main stem, number of pods per plant, and number of beans per pod,
 304 the dose recommended by the manufacturer (100 mL) being efficient, with the possibility of
 305 applying a dose of 50 mL, to satisfactory results.
 306

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311 CONSENT (WHERE EVER APPLICABLE)

312 Not applicable.
 313

314 ETHICAL APPROVAL (WHERE EVER APPLICABLE)

315 Not applicable.
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