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Can inoculation with diazotrophic bacteria decrease the productivity loss of defoliated *Vigna unguiculata* (L.) Walp?[1]

A inoculação com bactérias diazotróficas pode diminuir a perda de produtividade de *Vigna unguiculata* (L.) Walp desfolhada?

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HIGHLIGHTS:
Cowpea nodulation is affected by artificial defoliation during the vegetative stage of the plant.
Cowpea plants inoculated with Bradyrhizobium elkanni are more tolerant to defoliation.
Cowpea plants inoculated with Bradyrhizobium elkanni display higher growth and increments in productivity and contents of N and P.

ABSTRACT: The objective of this study was to evaluate the effect of artificial defoliation on vegetative and productive stages of cowpea inoculated with diazotrophic bacteria, in two experiments. The first experiment was performed in a greenhouse with 5 × 5 factorial (five defoliation percentages and five N sources), with four repetitions. N sources consisted of bacterial inoculation with strains INPA 03-11B, UFLA 03-84, UFRB FA34C2-2, and two control treatments: i - with N fertilization and ii - blank control, without N fertilization and without inoculation. The second experiment was performed in the field, in a 3 × 5 factorial scheme, with three repetitions. Treatments consisted of three N sources: with N fertilization, with bacterial strain INPA 03-11B that was selected in the first experiment, and five percentages of artificial defoliation. Defoliation percentages for both experiments were 0, 25, 50, 75, and 100%. Artificial defoliation during the vegetative stage caused reduction in the dry mass of bacterial nodules. Inoculation increased plant tolerance to defoliation and enhanced grain nutrient concentration (N and P). Inoculation with the strain INPA 03-11B allowed cowpea plants to tolerate 50% defoliation in the vegetative stage. The mean productivity of cowpea was reduced under > 50% defoliation during the productive stage; therefore, control of defoliating pests until the productive stage is not necessary under field conditions.

Key words: *Bradyrhizobium elkanni*, *Bradyrhizobium viridifuturi*, agroecological management, nutrition

RESUMO: Objetivou-se no presente estudo avaliar o efeito da desfolha artificial no crescimento vegetativo e produtivo do feijão caupi sob inoculação com bactérias diazotróficas, em dois experimentos. O primeiro experimento foi realizado em casa de vegetação com fatorial 5 × 5 (cinco percentagens de desfolha e cinco fontes de nitrogênio), com quatro repetições. As fontes de nitrogênio consistiram na inoculação bacteriana das cepas INPA 03-11B, UFLA 03-84, UFRB FA34C2-2 e dois tratamentos controle: i - com adubação nitrogenada e ii - sem adubação nitrogenada e sem inoculação. O segundo experimento, foi realizado em campo no esquema fatorial 3 × 5, com três repetições. Os tratamentos consistiram de três fontes de nitrogênio: com adubação nitrogenada, sem adubação nitrogenada e uso da cepa bacteriana INPA 03-11B selecionada no primeiro experimento, e cinco percentagens de desfolhamento artificial, cujos valores, nos dois experimentos, foram: 0, 25, 50, 75 e 100%. O desfolhamento artificial do feijão caupi durante o estágio vegetativo causou redução na massa seca dos nódulos bacterianos. A inoculação aumentou a tolerância das plantas ao desfolhamento e aumentou o teor de nutrientes dos grãos (N e P). A inoculação com a cepa INPA 03-11B permitiu que as plantas de caupi tolerassem 50% de desfolhamento no estádio vegetativo. A produtividade média do feijão caupi é reduzida a partir de 50% de desfolhamento artificial durante a fase produtiva e, portanto, dispensa o controle de pragas desfolhantes até esse nível, em condições de campo.

Palavras-chave: *Bradyrhizobium elkanni*, *Bradyrhizobium viridifuturi*, manejo agroecológico, nutrição

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Introduction

During its cycle, cowpea is challenged by defoliating herbivorous insects. Such insects reduce the leaf area and photosynthetic capacity of the plant, promoting significant production losses (Ibrahim et al., 2010; Santos et al., 2017; Smiderle et al., 2017; Addai & Ghanney, 2018; Lima-Primo et al., 2019).

Cowpea may tolerate some defoliation levels, depending on the season and cultivar. Data show that defoliation levels up to 50%, during the pre-blossoming stage, do not reduce grain production; however, severe defoliation at any stage before maturation drastically reduces production (Ezedinma, 1973). When the cultivar BR 17-Gurguéia is submitted to defoliation levels of 60 and 47% during the vegetative stage, a reduction of grain yield is observed, while during the reproductive stage, 47% defoliation already affects yield (Moura et al., 2014). Defoliation levels > 33% during the blossoming stage reduce the number of pods and seeds per plant and their weight, and the number of pods per plant. Moreover, defoliation of 67% reduces the number of pods per plant during blossoming stage, and during the pod stage, it reduces the number of seeds per pod (Smiderle et al., 2017).

Biological N fixation (BNF) encompasses a series of processes through chemical and molecular mechanisms. BNF initiates with diazotrophic bacterial colonization of plant roots and latter establishment of symbiotic relations, which ultimately results in the fixation of atmospheric N_2 into the plant root. However, under defoliation conditions, the availability of carbon in the plant may be a limiting factor for this symbiotic relationship (Silva et al., 2010).

Well-nourished plants are more resistant to defoliation because they compensate leaf loss by producing leaves regularly (Cordeiro et al., 2004); moreover, inoculation with diazotrophic bacteria may improve N availability to the plant. Thus, it might be hypothesized that plants inoculated with diazotrophic bacteria are more tolerant to artificial defoliation than plants fertilized with N. The objective of the present study was to evaluate the effect of artificial defoliation on the vegetative and productive stages of cowpea inoculated with diazotrophic bacteria.

Material and Methods

Two experiments were performed. The firsts experiment was performed in a greenhouse at the Federal University of Recôncavo da Bahia (UFRB), Cruz das Almas, BA, Brazil (12° 40' 19" S, 39° 06' 22" W, and altitude of 224 m), with minimum and maximum air temperatures of 28.9 and 29.8°C, respectively, and a mean air temperature of 29.35 °C during the experimental period. Seeds of *Vigna unguiculata* L., cultivar EPACE 10, were used in the experiment.

A completely randomized design with 5 × 5 factorial scheme (five defoliation percentages × five N sources), with four repetitions, was used. Defoliation percentages were 0, 25, 50, 75, and 100% to mimic the damage caused by defoliating insects (Moura et al., 2014).

The N sources were: two bacterial strains recommended as inoculants for cowpea by MAPA (2011), INPA 03-11B–SEMIA 6462 (*Bradyrhizobium elkanii*) (Lacerda et al., 2004; Soares et al., 2006; Guimarães et al., 2015) and UFLA 03-84–SEMIA 6461 (*Bradyrhizobium viridifuturi* symbiovar tropici) (Soares et al., 2006; Almeida et al., 2010; Costa et al., 2019); one strain under test, UFRB FA34C2-2 (Sousa, 2017); and two controls, N fertilization (105.3 mg N per bag, using urea as a source of 45% N) and a blank control, without N fertilization or inoculation.

The soil used as substrate was Oxisol, medium-textured, collected at UFRB from 0–0.20-m depth and conditioned in plastic bags (0.12 × 0.20 m and 1.2 dm^{-3} capacity). The soil chemical and physical characteristics were: pH (H_2O): 6.0; OM: 12.8 g dm^{-3}, P (Mehlich 1):10.5 mg dm^{-3}, K^+: 67.7 mg dm^{-3}, Ca^{2+}: 0.7 $cmol_c$ dm^{-3}, Mg^{2+}: 0.3 $cmol_c$ dm^{-3}, (H + Al): 2.0 $cmol_c$ dm^{-3}, SB: 1.2 $cmol_c$ dm^{-3}, CEC effective: 1.4 $cmol_c$ dm^{-3}, CEC potential: 3.2 $cmol_c$ dm^{-3}, V: 37.5%, Zn^{2+}: 5.05 mg dm^{-3}, Fe^{2+}: 49.05 mg dm^{-3}, Mn^{2+}: 9.1 mg dm^{-3}, Cu^{2+}: 1.6 mg dm^{-3}, and B:0.28 mg dm^{-3}, Sand: 760; Silt: 60; Clay: 180 g kg^{-1}.

To obtain the inoculum, bacterial strains were cultivated in 79 semisolid medium (Fred & Wakman, 1928) and incubated in a germination chamber for 4 days at 25 °C until they reached the log phase of growth.

The seeds surface was disinfected as described by Costa et al. (2016). For each replicate, 1 mL of inoculant was added to the seed. For control treatments, 1 mL of 79 semisolid culture medium, without inoculum, was used.

Artificial defoliation was performed 25 days after planting, during the plant vegetative stage (stage V9). Plants were collected 54 days after planting, at phenological stage R2, when the following variables were evaluated: chlorophyll a (CLA), chlorophyll b (CLB), and total chlorophyll content (CLT), which were quantified as described by Li et al. (2014), the number of nodules (NN) and dry mass of nodules (DMN), shoots (SDM), and roots (RDM) were also evaluated, as well as the efficiency of the strains compared with plants fertilized with mineral N (REN) according to the formula: REN = (SDM inoculated plants) × 100/(SDM plants fertilized with mineral N) (Bergersen et al., 1971). The most efficient strain promoting increase in cowpea biomass in this experiment was selected to evaluate its agricultural efficiency under field conditions. The field experiment was performed under dry-cultivation conditions at UFRB, a cycle of 87 days in an Oxisol. Rain precipitation during the experimental period was 310 mm. The minimum and maximum air temperatures were 21.6 and 22.7 °C, with a mean air temperature of 22.15 °C.

The experiment was set under a randomized block design and a 3 × 5 factorial scheme with three repetitions. Treatments consisted of three N sources: i- with mineral N; ii- without mineral N; and iii- inoculation of strain INPA 03-11B (BR 3301), under five defoliation percentages: 0, 25, 50, 75, and 100%. Plots consisted of five lines of 3 × 5 m, with three central lines for grain harvest and two border lines, separated by 0.20 m between plants. Plots included 15 plants per line and 75 plants per parcel.

All treatments received phosphate and potassium fertilization with 90 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O, using triple superphosphate and potassium chloride as sources, respectively. The control treatment with N, in addition to

P and K, was fertilized with 70 kg ha^{-1} N (urea: 45% of N), administered as 35 kg ha^{-1} N during seeding and 35 kg ha^{-1} N 35 days after emergence. Both experiments received these fertilizer doses.

The seed surface was disinfected as previously described. The inoculum was supplied with sterilized peat in a proportion of 3:1 (peat: 79 semisolid culture medium). Seeds were inoculated with 500 g of inoculant per 50 kg of seeds (10^8 cells mL^{-1}). Seeding was performed immediately after inoculation; the distance used was 1 m between lines and 0.2 m between plants, with four seeds per hole.

Forty days after seeding, ten plants from each plot were harvested in the border area to evaluate NN, DMN, SDM, and REN, to determine the symbiotic efficiency between N-fixing bacteria and cowpea in the field. Artificial defoliation was completed 45 days after seeding, during the initial period of blossoming (stage R2) (Campos et al., 2000). The experiment was extended until harvest (87 days after seeding).

The second, third, and fourth lines from each plot were harvested. Pods, dried in the field, were measured and manually threshed to evaluate: number of pods per plant (NPPL), number of grains per pod (NGP), number of grains per plant (NGPL), mean length of pods (MLP), dry mass of hundred grains (W100), production per plant (PPL), mean productivity per hectare (PHA), and grain content of N (GN) and P (GP).

Data were submitted to analysis of variance using the R software (R Core Team, 2018). Tukey test at p ≤ 0.05 was performed to compare strains (greenhouse experiment) and Scott-Knott (field experiment) and regression analysis for defoliation percentage.

Results and Discussion

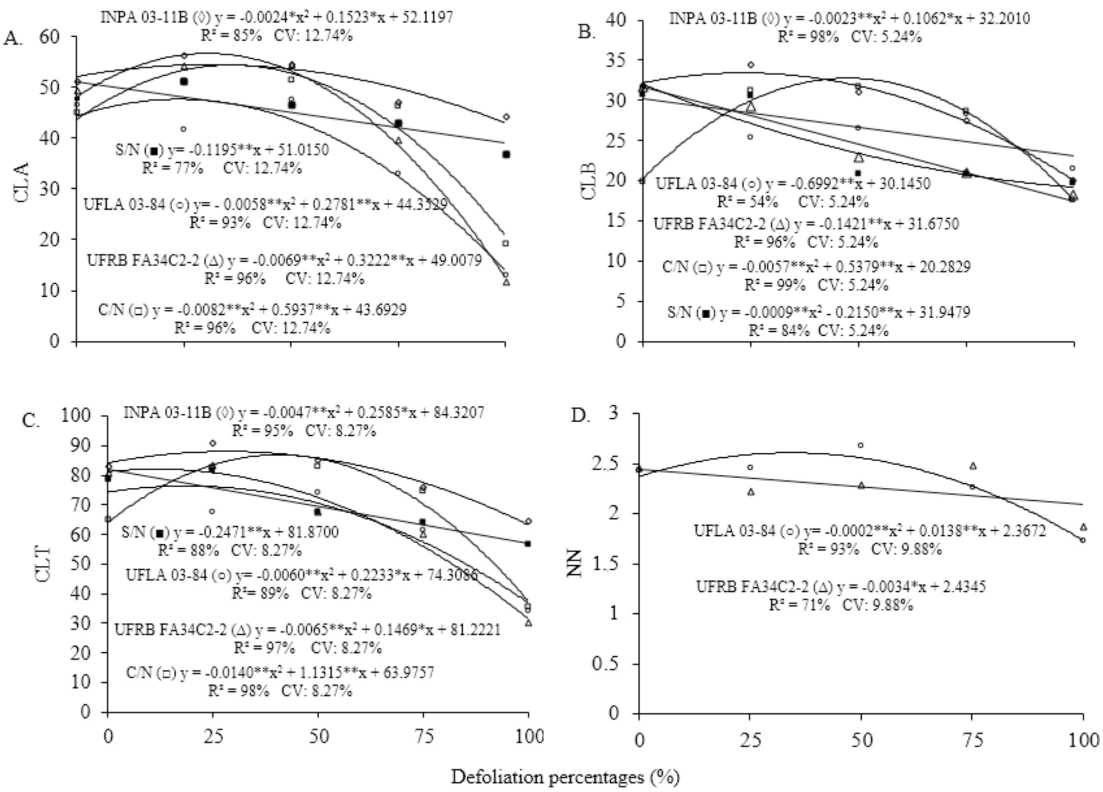
In greenhouse the treatments influenced all production components of cowpea.

A quadratic effect was verified for CLA, CLB and CLT (Figures 1A, B and C).

Strain INPA 03-11B promoted the highest CLA and CLB among all treatments, with means of 54.54 and 33.43 at 31.73 and 23.09% estimated defoliation levels, respectively. CLA from inoculated plants was similar to that of plants fertilized with mineral N at 36.20% estimated defoliation (Figure 1A). Plants fertilized with N showed a maximum CLB of 32.97 when submitted to 47.18% estimated defoliation (Figure 1B).

A decreasing linear effect was verified for NN in treatments inoculated with the strain UFRB FA34C2-2 and blank control. Plants inoculated with the strain UFLA 03-84 showed a higher NN with a maximum of 2.61 nodules plant^{-1} than that of plants from other treatments at 34.5% defoliation; at 100% defoliation, a reduction of 28.93% in NN was verified when compared with no-defoliation plants (Figure 1D).

Evaluating N sources and defoliation levels in greenhouse conditions, it was verified that defoliated cowpea plants inoculated with the strain INPA 03-11B showed higher mean values for all production components compared with plants from other treatments (Figure 2). Thus, this strain was selected for the field experiment, as cowpea showed tolerance to < 50% defoliation in the vegetative stage, as previously observed by Ibrahim et al. (2010). This may be attributed to the competitive ability of the inoculum (Costa et al., 2016) and its symbiotic efficiency, already described for cowpea in the literature



*; ** - Significant at p ≤ 0.05 and p ≤ 0.01, respectively, according to the F test
INPA 03-11B (◊) - bacterial inoculation with strain INPA 03-11B; S/N (▪) – blank control, without N fertilization and without inoculation; UFLA 03-84 (○) - bacterial inoculation with strain UFLA 03-84; UFRB FA34C2-2 (Δ) - bacterial inoculation with strain UFRB FA34C2-2; and C/N (□) - with N fertilization

Figure 1. Content of chlorophyll a, CLA (A); chlorophyll b, CLB (B); total chlorophyll, CLT (C), and number of nodules, NN (D) in cowpea plants [*Vigna unguiculata* (L.) Walp], as function of the artificial defoliation percentage at each N source

(Lacerda et al., 2004; Costa et al., 2013; 2016). Moreover, the diazotrophic bacteria might have produced hormones (auxin, cytokinin, gibberellin, and gibberellic acid) that are responsible for physiological processes that stimulate plant growth (Silva et al., 2016).

While evaluating N sources and artificial defoliation under field conditions, the efficiency of strain INPA 03-11B was verified (Table 1). Production components of cowpea inoculated with INPA 03-11B showed similar behavior to those verified in the greenhouse experiment; precisely, this treatment showed higher mean values when compared with the control treatments under defoliation conditions.

A decreasing linear model fit was observed in treatments inoculated with the strains INPA 03-11B and UFRB FA34C2-2 and blank control, regarding DMN. Plants inoculated with the strain UFLA 03-84 showed maximum DMN of 0.92 mg plant^{-1} when submitted to 37.5% estimated defoliation (Figure 2A).

The treatment inoculated with the strain INPA 03-11B produced higher SDM, with a maximum of 7.67 g plant^{-1} at 20.0% estimated defoliation. In this treatment and in the control with N fertilization, the mean values for SDM with 50% defoliation were similar to those without defoliation. In plants fertilized with mineral N, the maximum SDM was 5.60 g plant^{-1} at 27.86% estimated defoliation (Figure 2B).

Quadratic decreasing adjustment was observed for RDM in plants inoculated with the strain INPA 03-11B in response to the increase in defoliation (Figure 2C). Inoculation with UFRB FA34C2-2, at 13.75% estimated defoliation, showed a maximum RDM of 0.99 g plant^{-1}. In plants fertilized with mineral N, subjected to 36% estimated defoliation, a maximum RDM of 0.80 g plant^{-1} was observed. The lowest RDM was verified in the blank control treatment, without N fertilization and inoculation (Figure 2C).

The strain INPA 03-11B, followed by the strain UFRB FA34C2-2, showed better REN efficiency, with higher mean values than that of the control with mineral N (Figure 2D).

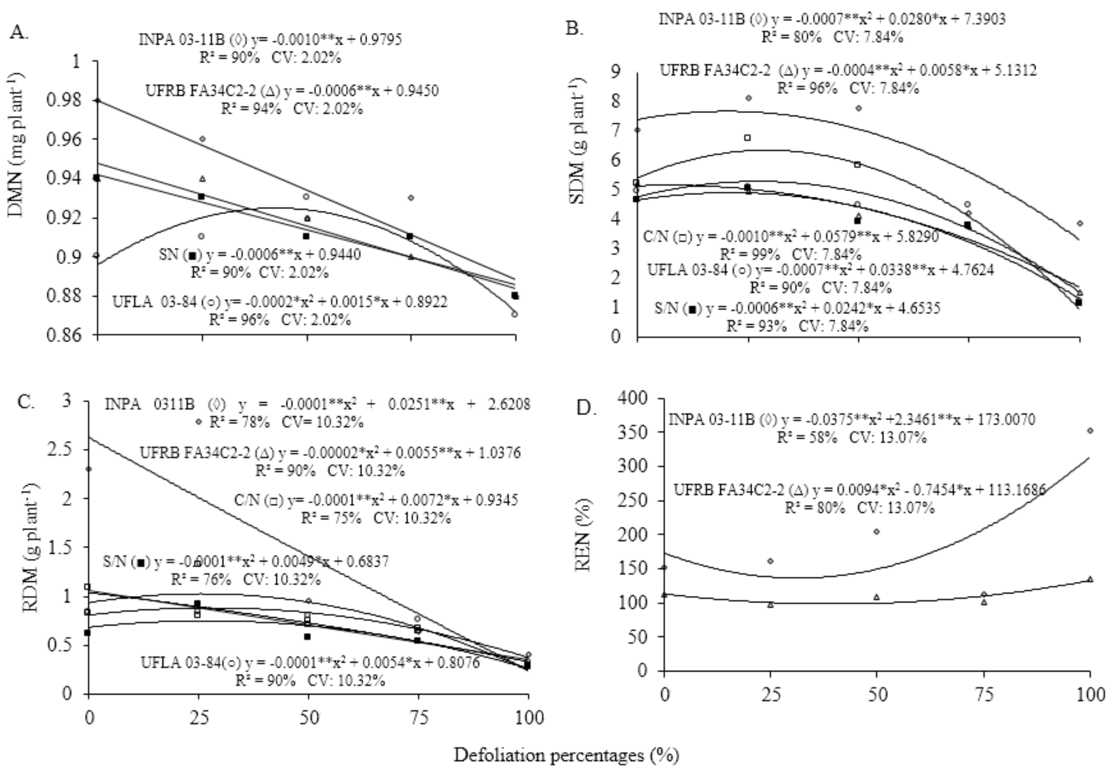
Plants inoculated with the strain INPA 03-11B in the field showed higher NN, DMN, SDM, and REN, indicating the ability of cowpea to nodulate under symbiosis with this strain and the mentioned soil (Table 1). Plants in the blank control, without N fertilization without inoculation, showed higher NN and DMN than those of the control with mineral N. Inoculation with strain INPA 03-11B was more efficient than fertilization with mineral N (Table 1).

The lowest values for production components, observed in drastic defoliation (> 50%), may be related to higher consumption of reserves stored in roots (Figures 2A and C)

Table 1. Mean number of nodules (NN), dry mass of nodules (DMN), dry mass of shoots (SDM), efficiency of the strain when compared to plants fertilized with mineral N, (REN) in cowpea plants [*Vigna unguiculata* (L.) Walp] inoculated with *Bradyrhizobium elkanii*

Sources of N	NN (number plant^{-1})	DMN (mg plant^{-1})	SDM (g plant^{-1})	REN (%)
INPA 03-11B	7.26 a	69.33 a	21.79 a	122.70 a
Without mineral N	5.96 b	44.82 b	17.78 c	89.00 c
With mineral N	0.71 c	4.13 c	19.86 b	100.00 b
CV (%)	7.28	7.19	2.19	4.69

* - Means followed by the same letter in the column are not statistically different according to Tukey's test, p ≤ 0.05



*; ** - Significant at p ≤ 0.05 and p ≤ 0.01, respectively, according to the F test

INPA 03-11B (◊) - bacterial inoculation with strain INPA 03-11B; S/N (▪) – blank control, without N fertilization and without inoculation; UFLA 03-84 (○) - bacterial inoculation with strain UFLA 03-84; UFRB FA34C2-2 (Δ) - bacterial inoculation with strain UFRB FA34C2-2; and C/N (□) - with N fertilization

Figure 2. Dry mass of nodules, DMN (A); shoots, SDM (B); roots, RDM (C); and efficiency of strains when compared to mineral N-fertilized cowpea plants [*Vigna unguiculata* (L.) Walp], REN (D), as function of artificial defoliation percentage at each N source

to reestablish leaf area and due to reduction of photosynthetic process in the plant (Figures 1A, B, and C) caused by defoliation. Such facts interfere with the availability of chemical energy (ATP) from photosynthesis, which is produced only in cells containing chlorophyll in the presence of light (Ibrahim et al., 2010; Addai & Ghanney, 2018; Lima-Primo et al., 2019). Reduction of leaf area interferes with photosynthesis and, consequently, with hormonal equilibrium, index of chlorophylls, stomatic resistance, and ATP production (Ibrahim et al., 2010; Santos et al., 2017; Addai & Ghanney, 2018), resulting in a production decline (Figures 3 and 4).

Individual effects were verified for inoculation ($p \leq 0.05$) and defoliation ($p \leq 0.01$) for MLP and NPPL. Plants inoculated with strain INPA 03-11B showed higher MLP and NPPL (Table 2). At 23.08% estimated defoliation, a maximum MLP of 15.73 cm plant^{-1} was observed (Figure 3A).

It was verified that plants (MLP) tolerated 50% of defoliation (Figure 3A), as the mean values were similar to the treatment without and < 50% defoliation.

There was an individual effect for inoculation ($p \leq 0.05$) (Table 2) and defoliation levels ($p \leq 0.01$) (Figure 3B) for NPPL.

Table 2. Means of pod length (MLP) and number of pods per plant (NPPL) in cowpea [*Vigna unguiculata* (L.) Walp] inoculated with *Bradyrhizobium elkanii*

Sources of N	MLP (cm)	NPPL (pod plant^{-1})
INPA 03-11B	16.54 a	17.94 a
Without mineral N	14.10 b	16.51 b
With mineral N	13.36 b	16.42 b
CV (%)	5.86	8.90

* - Means followed by the same letter in the column are not statistically different according to Tukey's test, $p \leq 0.05$

Plants inoculated with INPA 03-11B showed higher NPPL than those of other treatments, with a mean value of 17.94 pods plant^{-1}. At 16.65% estimated defoliation, a maximum NPPL of 21.18 pod plant^{-1} was verified. Cowpea preserved normal production until 25% defoliation.

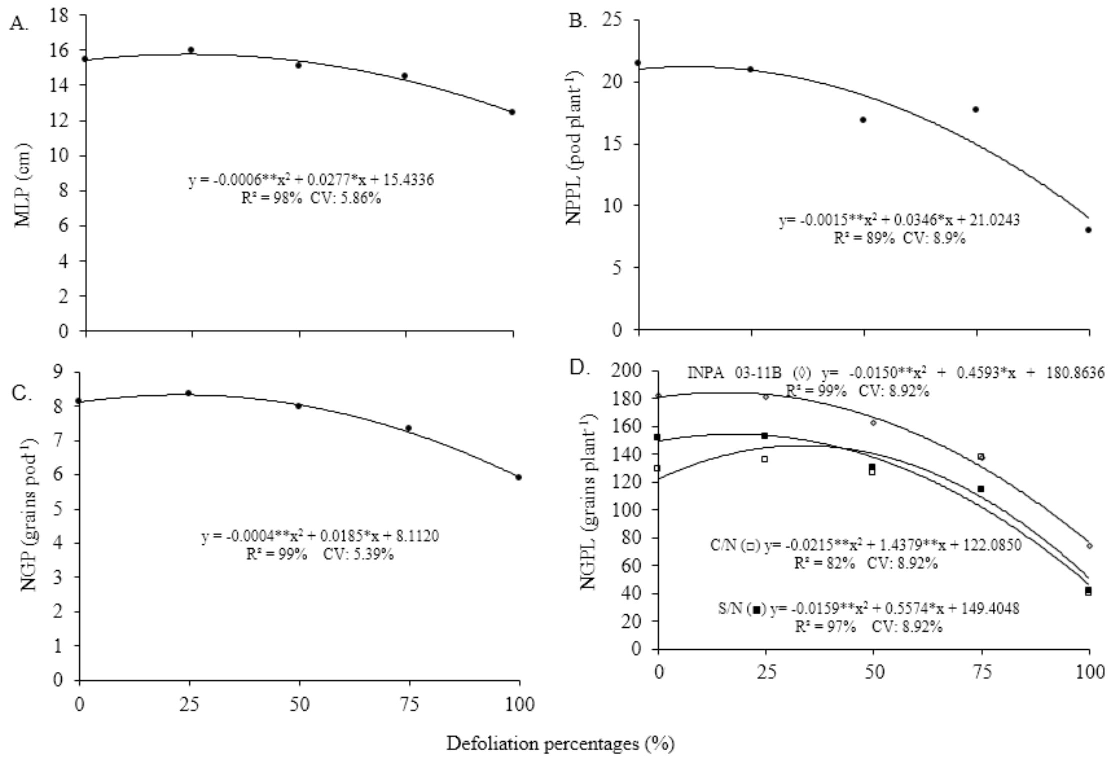
Regarding NGP, there was an individual effect of defoliation levels ($p \leq 0.05$) (Figure 3C). The maximum NGP was 8.33 grains pod $^{-1}$, obtained at 23.13% defoliation level. Cowpea plants showed tolerance to up to 50% defoliation regarding NGP.

Concerning NGPL, there was an interaction between N source and defoliation levels, with the highest mean value observed for INPA 03-11B at 15.31% defoliation level, and a maximum of 184.38 grains plant^{-1} (Figure 3D). At 100% defoliation, a reduction of 56.71% was observed when compared to the 0% defoliation level.

In the blank control treatment, the estimated defoliation level of 17.53% resulted in a maximum NGPL of 154.28 grains plant^{-1}, with a reduction of 72.42% when compared with the 100% defoliation level.

Plants inoculated with INPA 03-11B, at 43.10% estimated defoliation, produced W100 of 14.51 g plant^{-1} (Figure 4A). In the blank control treatment, the estimated defoliation level of 45.22% resulted in a maximum W100 of 13.45 g plant^{-1}. In plants fertilized with N, at 43.17% estimated defoliation, the maximum W100 was 13.50 g plant^{-1}. Thus, cowpea preserved W100 averages until 75% defoliation when under different N sources (Figure 4A).

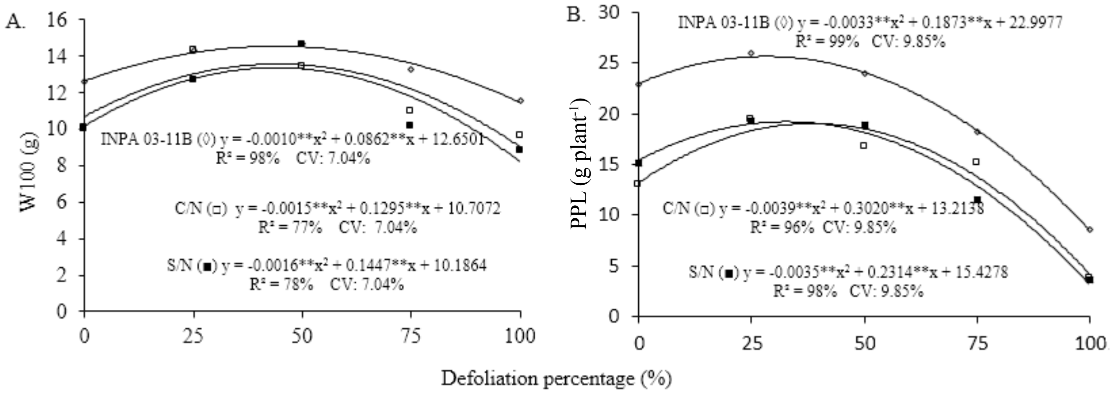
Plants inoculated with strain INPA 03-11B submitted to 28.38% estimated defoliation showed maximum PPL of 25.66 g plant^{-1}, whereas plants cultivated without inoculation



*; ** - Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to F test

INPA 03-11B (◊) - Bacterial inoculation with strain INPA 03-11B; S/N (▪) – Blank control, without N fertilization and without inoculation; and C/N (□) - With N fertilization

Figure 3. Mean length of pods, MLP (A); number of pods per plant, NPPL (B); number of grains per pod, NGP (C) and number of grains per plant, NGPL (D) in cowpea under artificial defoliation percentage



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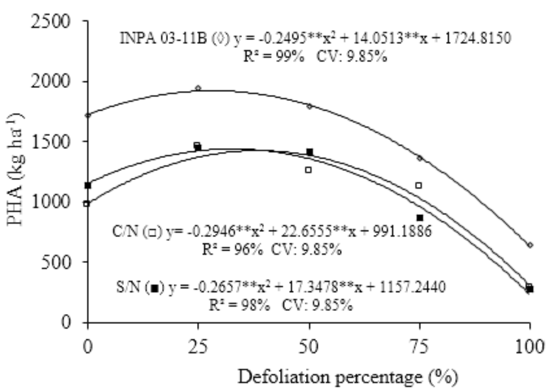
INPA 03-11B (◊) - Bacterial inoculation with strain INPA 03-11B; S/N (▪) – Blank control, without N fertilization and without inoculation; and C/N (□) - With N fertilization

Figure 4. Dry mass of one hundred grains, W100 (A) and production per plant, PPL (B) in cowpea as function of artificial defoliation percentage at each N source

and without N fertilization at 33.06% estimated defoliation, showed a maximum PPL of 19.25 g plant^{-1}. Plants fertilized with N showed a maximum PPL of 19.06 g plant^{-1} at 38.72% estimated defoliation. Cowpea preserved PPL averages until 50% defoliation when cultivated under different N sources (Figure 4B).

An interaction between inoculation and defoliation levels was verified for PHA. Plants inoculated with the strain INPA 03-11B showed higher PHA, with a maximum mean of 1922.7 kg ha^{-1} at 28.16% estimated defoliation (Figure 5). When fertilized with mineral N, plants showed a maximum PHA of 1,426.8 kg ha^{-1} at 38.45% estimated defoliation (Figure 5). Without both mineral fertilizer (N) and inoculation, plants showed maximum PHA of 1,440.4 kg ha^{-1} at 32.65% estimated defoliation. Above 50% defoliation, a significant decrease in production was observed in cowpea plants (Figure 5).

A reduction in leaf number and area, when well distributed, may lead cowpea plants to be more productive (Ezedinma, 1973; Mondal et al., 2011). This may be related to the reduction of the transpiration rate, reducing energy consumption, and enhancing production. Moreover, CO_2 and water supply limits photosynthesis, and water is lost through stomata (Silva et al., 2010). With defoliation under 50% (Figure 2B), the reduction of foliar mass may diminish water loss and contribute to the supply of CO_2 for photosynthesis, promoting high PHA



INPA 03-11B (◊) - Bacterial inoculation with strain INPA 03-11B; S/N (▪) – Blank control, without N fertilization and without inoculation; and C/N (□) - With N fertilization

Figure 5. Average productivity, (PHA) in cowpea [*Vigna unguiculata* (L.) Walp] as function of artificial defoliation percentage at each N source

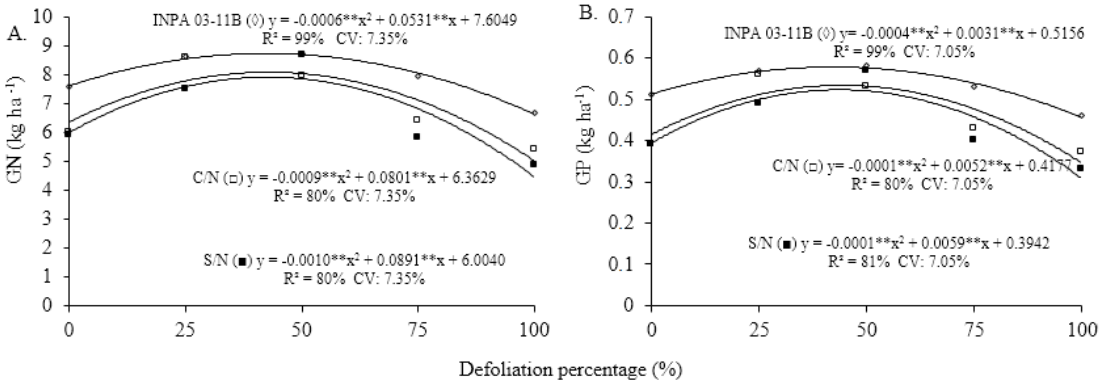
(Figure 5). This scenario may benefit agroecological production systems, where pesticides are avoided and inoculated plants may still produce as much as non-defoliated plants even under herbivory. In this context, it is acknowledged that, besides the already known benefits of diazotrophic bacteria, such as BNF, promotion of plant growth, and tolerance to salinity (Costa et al., 2013; 2016; Silva et al., 2016; Rocha et al., 2017), there is evidence of beneficial effects concerning tolerance against defoliation in cowpea.

During blossoming, plants demand more photo assimilates; critical levels of defoliation interfere in the translocation of such compounds to the grains, reducing plant productivity (Figures 4A and B). The energy consumed in the vegetative recovery jeopardizes productivity, mainly when damage occurs during blossoming and grain-filling periods (Silva et al., 2007). In cowpea cultivar BR 17-Gurguéia, the control level for defoliating herbivorous insects was determined at 47% defoliation, in the reproductive stage (Moura et al., 2014) and without inoculation.

Artificial defoliation reduces production components, reflecting in productivity loss. However, production was not affected in the reproductive stage under 50% defoliation, as mean values for MLP, NGP, NGPL, W100, PPL, and PHA were still within the ranges of non-defoliated treatments. Addai & Ghanney (2018), while evaluating different cowpea varieties, verified that plants subjected to 50% defoliation exhibited responses of production components analogous to those from plants without defoliation (0%).

As defoliation was performed during blossoming (stage R2), new leaves were produced, which may have extended the activity of nitrogenase by strain INPA 03-11B, when higher accumulation of N was observed in the grains (Figure 5). Thus, as verified in the greenhouse experiment, plants inoculated with INPA 03-11B in the field showed higher production than those in the other treatments. The advent of new leaves after defoliation may recover or extend, or both, the activity of this enzyme in soybeans and beans (*Phaseolus vulgaris*), as observed by Hungria & Neves (1986), and increase the production of mungobeans (*Vigna radiata*) (Mondal et al., 2011).

A quadratic effect was verified for N sources related to defoliation levels for N (GN) and P (GP) contents in the grains (Figures 6 A and B). Plants inoculated with the strain



*; ** - Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to F test
INPA 03-11B (◊) - Bacterial inoculation with strain INPA 03-11B; S/N (▪) – Blank control, without N fertilization and inoculation; and C/N (□) - With N fertilization

Figure 6. Accumulation of N (GN) (A) and phosphorus (GP) in the grains (B) in cowpea [*Vigna unguiculata* (L.) Walp] as function of artificial defoliation percentage at each N source

INPA 03-11B excelled when compared with the control treatments, showing maximum GN of 8.78 kg ha^{-1} and GP of 0.58 kg ha^{-1} at 44.25 and 38.75% estimated defoliation levels, respectively.

Inoculation increased the tolerance of cowpea to defoliation and improved phosphate and N nutrition of grains (Figures 6A and B). Nitrogen is a key nutrient for cell structure and metabolism, thus increased N absorption compensates defoliation losses. In field experiments performed in different Brazilian regions, the effect of strain INPA 03-11B on N (Almeida et al., 2010; Ferreira et al., 2013) and P nutrition (Ferreira et al., 2013) in leaves and grains was similar to that of plants fertilized with mineral N, confirming the beneficial effect of P and N nutrition promoted by this strain. Thus, diazotrophic bacteria inoculation may benefit the agroecological management of pests and diseases in cowpea, as it preserves the tolerance of this crop, as reported by Ezedinma (1973), Moura et al. (2014), and Addai & Ghanney (2018), and increases production when compared with N fertilization.

The inoculation of the strain INPA 03-11B promoted high increments in cowpea productivity, representing a feasible alternative to N fertilization and agroecological production systems.

Conclusions

1. The artificial defoliation of cowpea in the vegetative stage (V9) decreased dry mass of nodules.

2. Inoculation with the strain INPA 03-11B allowed cowpea to tolerate 50% defoliation in the vegetative stage.

3. The productivity of cowpea was reduced by > 50% of artificial defoliation in the reproductive stage (R2).

4. Plants inoculated with diazotrophic bacteria were more tolerant to artificial defoliation than plants that received N fertilization.

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