

## Nitrogen sources and defoliation levels in cowpea bean

Fontes de nitrogênio e níveis de desfolha em feijão-caupi

Fuentes de nitrógeno y niveles de defoliación en el caupi

Received: 07/12/2024 | Revised: 07/30/2024 | Accepted: 08/02/2024 | Published: 08/07/2024

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### Abstract

This study was aimed at evaluating the effect of artificial defoliation and different nitrogen sources on production traits of cowpea using mixed generalized linear models. The experiment was developed in a 3x5x2 factorial arrangement, being: three Nitrogen sources; five levels of artificial defoliation; and two defoliation seasons, with four repetitions. The plots were distributed in the Randomized Block Design, developed in a greenhouse and in the field. The following characteristics were evaluated: number of pods produced per plant; number of grains produced per pod; number of grains produced per plant; average length of pods; average weight of 100 grains; and estimated production. The GLIMMIX procedure was used because it allows the probability distribution of the response variable to take the appropriate distribution model. Production per Hectare (PHA) of plants subjected to leaf loss area can be increased up to 50% defoliation level, the variety Tumucumaque overcompensates in productivity, with maximum yields at 27.03% of defoliation and significant decrease above 75%, regardless of phenological stage. Even with losses above these levels, this cultivar had high indexes of productivity, revealing its high resistance to leaf loss injury. The use of chemical nitrogen fertilization and inoculation of cowpea seeds, variety Tumucumaque with *Bradyrhizobium* is unnecessary in the edaphoclimatic conditions of Bom Jesus – PI; The use of nitrogen fertilization with ammonium sulfate and inoculation of cowpea seeds with *Bradyrhizobium* does not influence the resistance of the Tumucumaque variety to defoliation levels; Defoliation levels above 75% impair the performance of production traits of cowpea, Tumucumaque.

**Keywords:** BNF; *Bradyrhizobium*; *Vigna unguiculata*; Generalized linear models.

### Resumo

Esse experimento teve como objetivo avaliar o efeito da desfolha artificial e diferentes fontes de nitrogênio em características produtivas de feijão-caupi usando mistura de modelos lineares generalizados. Experimento desenvolvido em esquema fatorial de 3x5x2, sendo: três fontes de Nitrogênio; cinco níveis de desfolha artificial; e duas épocas de desfolha, com quatro repetições. O experimento foi distribuído em Delineamento de Blocos Aleatórios, desenvolvido em uma casa de vegetação e em campo. As seguintes características foram avaliadas: número de vagens produzidas por planta; número de grãos produzidos por vagem; número de grãos produzidos por planta; peso médio de 100 grãos; e estimativa de produção. O procedimento GLIMMIX foi utilizado pois permite que a distribuição de probabilidade das variáveis obtidas se encaixe no modelo apropriado de distribuição. Produção por Hectare (PHA) de plantas submetidas à perda de área foliar pode ser aumentada a níveis de desfolha acima de 50%, a variedade Tumucumaque compensa em produtividade, com máximos rendimentos a 27.03%, e queda significativa acima de 75%, independente do estado fenológico. Mesmo com perda acima desses níveis, essa cultivar possui grande índices de produtividade, revelando uma alta resistência a desfolha. O uso de adubação química nitrogenada e inoculação de sementes de variedades de feijão-caupi Tumucumaque com *Bradyrhizobium* é desnecessário nas condições edafoclimáticas de Bom Jesus – PI; O uso de adubação nitrogenada com sulfato de amônia e inoculação de sementes de feijão-caupi com *Bradyrhizobium* não

influencia na resistência de níveis de desfolha em Tumucumaque. Níveis de desfolha acima de 75% prejudicam a produtividade de Tumucumaque.

**Palavras-chave:** BNF; *Bradyrhizobium*; *Vigna unguiculata*; Modelo linear generalizado.

### Resumen

El experimento pretendía evaluar el efecto de la defoliación artificial y de diferentes fuentes de nitrógeno sobre las características productivas del caupí utilizando una mezcla de modelos lineales generalizados. Se realizó en un diseño factorial 3x5x2: tres fuentes de nitrógeno; cinco niveles de defoliación artificial; y dos tiempos de defoliación, con cuatro repeticiones. Se planteó en un Diseño de Bloques Aleatorizados, desarrollado en invernadero y en campo. Se evaluaron las siguientes características: número de vainas producidas por planta; número de granos producidos por vaina; número de granos producidos por planta; peso medio de 100 granos; y producción estimada. Utilizó el procedimiento GLIMMIX porque permite ajustar la distribución de probabilidad de las variables obtenidas al modelo de distribución apropiado. La producción por hectárea (PHA) de las plantas sometidas a pérdida de área foliar puede incrementarse a niveles de defoliación superiores al 50%, la variedad Tumucumaque compensa en productividad, con rendimientos máximos al 27,03%, y una caída significativa por encima del 75%, independientemente del estado fenológico. Incluso con pérdidas por encima de estos niveles, este cultivar presenta altos índices de productividad, revelando una elevada resistencia a la defoliación. Fertilización nitrogenada química e inoculación de las semillas de las variedades de caupí Tumucumaque con *Bradyrhizobium* es innecesario en las condiciones edafoclimáticas de Bom Jesus - PI; Fertilización nitrogenada con sulfato de amonio e inoculación de las semillas de caupí con *Bradyrhizobium* no influye en la resistencia de los niveles de defoliación en Tumucumaque. Niveles de defoliación superiores al 75% son perjudiciales para la productividad.

**Palabras clave:** BNF; *Bradyrhizobium*; *Vigna unguiculata*; Modelo lineal generalizado.

## 1. Introduction

Nitrogen (N) is an essential nutrient for all living beings and crucial for several agricultural crops. It is classified as a highly mobile macronutrient in plants and both lack and excess affect metabolic processes in plants (Marschner, 2012).

The nitrogen requirements of cowpea plants vary according to their developmental stage, with higher leaf concentrations being found in the vegetative stage compared to the pod-filling stage (Taffouo et al., 2014). Its availability in the soil is among the constraints in bean development and production, when advanced technologies are not used (Monteiro et al., 2012). Therefore, information on limiting factors is essential for the expansion of this crop in northeastern Brazil (Filho et al., 2013).

Fixation by industrial processes and soil bacteria (BNF) are the main sources of N for legume crops (Marschner, 2012 – cap 16). In arid and semi-arid regions, BNF frequently supply more than half of the N used by plants (Freitas, et al., 2009; Andrews et al., 2011; Souza et al., 2012). In cowpea plants (*Vigna unguiculata* (L.) Walp), nitrogen-fixing bacteria are known to be efficient and can supply most of the N necessary for the development and production of this crop, depending on the *Rhizobium* strain available (Brito et al., 2011; Vieira et al., 2010).

The use of fertilizer formulas with low N concentration can have economic advantages in cowpea crops when not exceeding 20 kg.ha<sup>-1</sup> to ensure the efficiency of nodulation (Uchôa et al., 2009). However, nitrogen supply via mineral fertilization can adversely affect BFN in legumes, since plants can directly absorb the nitrogen present in the soil (Oliveira et al., 2004; Brito et al., 2011). Thus, the substitution or low use of chemical nitrogen fertilizers to increase cowpea productivity and sustainability may lower the risks to farmers.

Plant nutrition directly influences insect-plant relationships (Primavesi, 2003) and the adequate supply of N can increase plant resistance and decrease pest damage. Moura et al. (2014) determined the level of economic damage by defoliators as 60% and 47% for the vegetative and reproductive stages, respectively. However, these authors did not evaluate the effect of nitrogen fertilization on plant response to defoliation. Despite the extensive knowledge of the benefits of adequate N supply in cowpea, the relationship between N source and plant resistance to defoliators has not been examined. Since the harvest of pods and seeds

are the main focus of cowpea production, these traits need to be evaluated when plants are treated with different N sources and defoliation levels.

Traits with non-normal distributions have variations that are functions of the means and, therefore, are by definition heterogeneous. In contrast, the transformation of these variables would not be effective because stable variations. In addition, the transformation of variables can be problematic for regression adjustments in which it also affects the functional relationship between explanatory variables and the response variable (Gbur et al., 2012). Therefore, the use of generalized linear models avoids these issues because the data is not transformed; instead, a function of the means is modeled as a linear combination of explanatory variables.

This study was aimed at evaluating the effect of artificial defoliation and different nitrogen sources on production traits of cowpea using mixed generalized linear models under the edaphoclimatic conditions of the municipality of Bom Jesus-PI.

## 2. Methodology

The study was carried out in two phases at the Professor Cinobelina Elvas Campus (CPCE) of the Federal University of Piauí (UFPI) in Bom Jesus in the state of Piauí. The first phase consisted of a greenhouse experiment conducted in 2016 and in the second phase, an assessment was conducted in the field in an experimental area during the 2017/2018 year/crop, with cowpea seeds supplied by EMBRAPA Meio-Norte.

This research integrates elements from various methods, aiming for a comprehensive and in-depth investigation of the phenomenon in question. This approach enables the achievement of more robust and reliable results, allowing for the exploration of multiple aspects of the problem under study. According to Pereira et al. (2018), scientific research should seek answers to problems through rigorous and systematic methods. The justification for each methodological approach adopted ensures the understanding of the research strategy and its appropriate application to achieve the established objectives (Koche, 2011; Gil, 2017).

The municipality of Bom Jesus is part of the southwestern mesoregion of Piauí, an ecotone between Cerrado and Caatinga, in the Upper Middle Gurgueia microregion. The climate is hot and humid, classified by Köppen as Awa (tropical rainy climate with dry winter and mean air temperature of the hottest month above 22 °C). The mean altitude is 277 m, with mean rainfall ranging between 900 and 1200 mm.year<sup>-1</sup>, and mean air temperature of 26.2 °C, according to Inmet (2019).

### 2.1 Greenhouse experiment

The soil used in the first experiment was collected at a depth of 0-20 cm in areas where cowpea is grown in Bom Jesus-PI. Fertilization was carried out based on the soil analysis (Table 1) and liming was conducted to reduce soil acidity thirty days before sowing.

Cowpea seeds were sowed in seedling bags with a capacity of 2 kg of soil. Three seeds were placed per bag and thinning was performed ten days after emergence (DAE), leaving one plant per bag. The plants were watered with micro sprinklers and pest control was carried out with the chemical insecticide Malathion 500.

**Table 1** - Physical and chemical characteristics of the soil at 0-20 cm in depth in Bom Jesus - PI

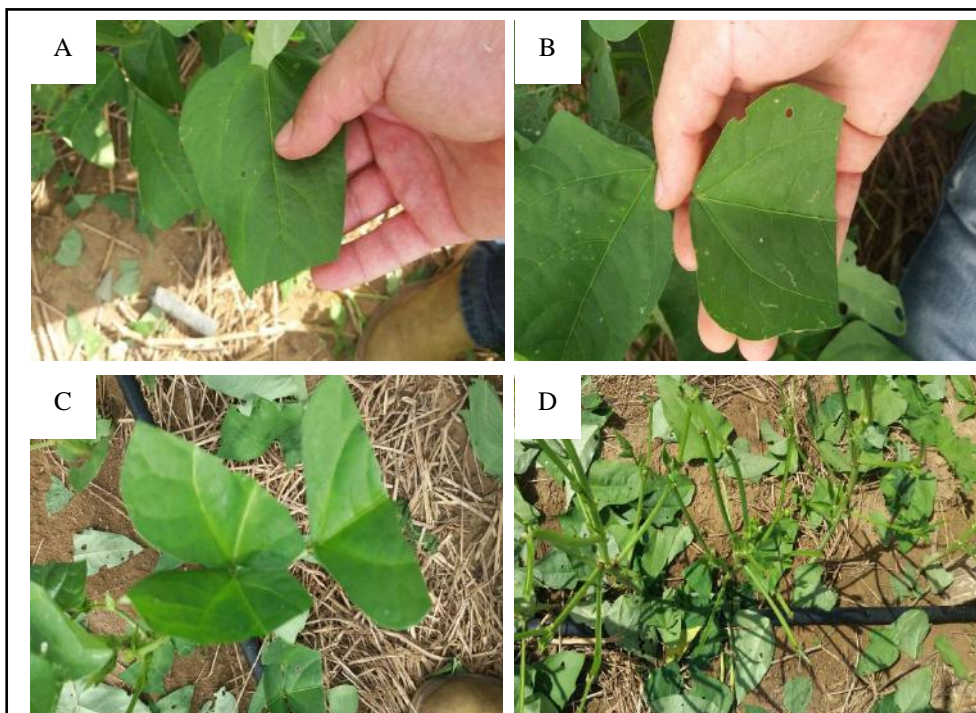
| Soil Characteristics                         | Depth 0–20 (cm) |
|--|-----------------|
| pH (H <sub>2</sub> O)                        | 5.60            |
| Ca <sup>2+</sup> (cmolc.dm <sup>-3</sup> )   | 3.33            |
| Al <sup>3+</sup> (cmolc.dm <sup>-3</sup> )   | 0.00            |
| H+Al <sup>3+</sup> (cmolc.dm <sup>-3</sup> ) | 2.24            |
| K <sup>+</sup> (cmolc.dm <sup>-3</sup> )     | 0.67            |
| Mg <sup>2+</sup> (cmolc.dm <sup>-3</sup> )   | 1.04            |
| S (mg.dm <sup>-3</sup> )                     | 5.04            |
| P (mg.dm <sup>-3</sup> )                     | 30.50           |
| Clay (g.kg <sup>-1</sup> )                   | 260.00          |
| Silt (g.kg <sup>-1</sup> )                   | 184.00          |
| Sand (g.kg <sup>-1</sup> )                   | 556.00          |
| Organic matter (%)                           | 13.50           |
| Index of base saturation (%)                 | 69.20           |
| Index of aluminum saturation (%)             | 0.00            |

Source: Authors.

## 2.2 Evaluated traits

The variables analyzed were: Number of pods produced per plant (VP); number of seeds produced per pod (GV); number of seeds produced per plant (GP); mean length of pods in cm (CMV); mean weight of 100 seeds in grams (P100); estimated production per hectare in kilograms (PHA). Analyzed at different defoliation levels (Figure 1).

**Figure 1** - Artificial defoliation levels in cowpea: 25% defoliation (A); 50% defoliation (B); 75% defoliation (C) and 100% defoliation (D).



Source: Authors.

### 2.3 Experimental design and statistical model

The experiment followed a 3x5x2 factorial arrangement: three nitrogen sources (soil with no added nitrogen; nitrogen fertilization with 2.5g of ammonium sulfate, and inoculation of seeds with the *Rhizobium* strain INPA 03 11B. For the latter, the bacterial mass was diluted in water to the recommended ratio, creating a paste that was added to seeds in a bowl 20 minutes before planting), five levels of artificial defoliation (0%; 25%; 50%; 75%, and 100%) performed with the aid of scissors (Figure 1) and two defoliation times (25 and 40 days after planting - DAP), with four repetitions, totaling 120 stands distributed in randomized block design.

For the analyses, a mixed generalized linear model was used, described as a matrix based on the  $y$  vector by the expression:

$$y = X\gamma + Zu + \varepsilon$$

in which:  $y$  is the vector of the observed data for the trait under analysis;  $X$  is the matrix of fixed effects;  $\gamma$  is the vector of fixed effects to be estimated;  $Z$  is the incidence matrix of random effects;  $u$  is the vector of random effects to be estimated and  $\varepsilon$  is the vector of unobservable random errors.

$$E \begin{bmatrix} Y \\ \varepsilon \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$Var \begin{bmatrix} Y \\ \varepsilon \end{bmatrix} = \begin{bmatrix} G & \phi \\ \phi & R \end{bmatrix}$$

with  $Z = \phi$  and  $R = \sigma^2 I$ . The covariance structures for the  $G$  and  $R$  matrices are specified as:

$$Var(\gamma) = G; Var(\varepsilon) = R; Cov(\gamma, \varepsilon') = \phi$$

$$Var(y) = Var(Z\gamma) + Var(\varepsilon) = ZGZ' + R$$

$$Cov(y, \gamma') = Cov(Z\gamma, \gamma') = ZVar(\gamma) = ZG$$

$$Cov(y, \varepsilon') = Cov(\varepsilon, \varepsilon') = Var(\varepsilon) = R$$

For countable traits (VP; SGS; and GP), data were evaluated with an analysis of variance using Poisson distribution and the natural logarithm as link function. Therefore, the conditional mean for the countable traits on the scale can be described as:

$$\eta_{ijkl} = g(E[y_{ijkl}|b_l]) = \log(E[y_{ijkl}|b_l]) = \mu + N_i + D_j + E_k + (ND)_{ij} + (NE)_{ik} + (DE)_{jk} + (NDE)_{ijk} + b_l + e_{ijkl}$$

where:  $y_{ijkl}$  represents the observed value of the trait for the  $i$ th nitrogen source in the  $j$ th level of artificial defoliation of  $k$ -th defoliation time of the  $l$ -th plant of the block;  $\mu$  is the constant inherent to all observations (overall mean of the experiment for each trait);  $N_i$  is the effect of the  $i$ th source (with  $i = 1, 2, \text{ and } 3$ );  $D_j$  is the effect of the  $j$ th defoliation level (with  $j = 1, 2, 3, 4, \text{ and } 5$ );  $E_k$  is the effect of the  $k$ th defoliation time (with  $k = 1 \text{ and } 2$ );  $(ND)_{ij}$  is the effect of the interaction between the  $i$ th nitrogen source and the  $j$ th level of artificial defoliation;  $(NE)_{ik}$  is the effect of the interaction between the  $i$ th nitrogen source and the  $k$ th defoliation time;  $(DE)_{jk}$  is the effect of the interaction between the  $j$ th level of artificial defoliation and the  $k$ th defoliation time;  $(NDE)_{ijk}$  is the effect of the triple interaction among the factors;  $b_l$  is the effect of  $l$ th block (with  $l = 1, 2, 3, \text{ and } 4$ ); and  $e_{ijkl}$  is the experimental error associated with the observations  $y_{ijkl}$ . In the model, in addition to the experimental error, the effect of the blocks was assumed as random. The effects of nitrogen source, artificial defoliation level, defoliation time, and interactions were considered as fixed.

The matrix model can be described as:

$$\eta = \log(E[Y|u]) = X\gamma + Zu$$

The distribution of random effects was assumed as multivariate normal, described as  $u \sim MVN(0, G)$ . The covariance matrix (G) has a block-diagonal structure. The conditional covariance matrix of Y, given  $u$  (R matrix), does not contain additional parameters, as the mean and variance of a Poisson distribution are the same.

The mixed generalized linear model for the traits (CMV; P100; and PHA) considering a normal distribution with interactions can be described as:

$$y_{ijkl} = \mu + N_i + D_j + E_k + (ND)_{ij} + (NE)_{ik} + (DE)_{jk} + (NDE)_{ijk} + b_l + e_{ijkl}$$

## 2.4 Statistical analysis

The GLIMMIX procedure was used because it allows the probability distribution of the response variable to take the appropriate distribution model. This approach does not require data transformation, as they are treated according to the indicated distribution (in the theory of classical linear models it is assumed, in general, that errors have normal distribution, if not, data transformation is necessary). In addition, the assumption regarding the normality of the data for countable traits (PV; SGS; and GP) is not followed, then automatically two assumptions are no longer met. This occurs because non-normal data results in a heterogeneity of variance, and vice versa. And rejecting the assumptions of the model can lead to erroneous estimates.

Significant values were found with the  $F$  test. The means were adjusted with the lsmeans command (*least-squares means*) and compared using the Tukey-Kramer test. Significance of the analyses was set at  $p \leq 0.05$ . The analyses were performed using the *Software SAS - Statistical Analysis Systems* (v.9.4, Cary, North Carolina).

## 2.5 Field experiment

An area of 150m<sup>2</sup> was prepared for planting and soil analysis and correction were carried out following recommendations from Embrapa (1997), except for Nitrogen. Twenty kilograms of limestone was added in the area based on the soil chemical analysis.

The experimental design and statistical model were applied according to the greenhouse experiment. For the chemical fertilization with a nitrogen source, ammonium sulfate was applied at a depth of 5 cm. Each block consisted of five rows of six meters of planting area, keeping two edge rows, the stand consisted of a linear meter of the crop with a spacing of 0.5 x 0.1m. Three seeds per hole were used and thinning was performed ten days after emergence (DAE), leaving one plant per hole, to ensure uniformity in the stand.

The same traits evaluated in the greenhouse experiment were assessed. The area was irrigated to keep soil moisture close to field capacity. Pest control was performed by spraying with tobacco extract insecticide (100 g of rope tobacco + 100 g of coconut soap + 500 ml of alcohol + 500 ml of water). The traits evaluated and the statistical analysis were the same as those described for the greenhouse experiment.

## 3. Results and Discussion

### 3.1 Greenhouse experimental

The measurement of residual variability in the marginal distribution of the data obtained with the ratio between the generalized chi-square statistics and its degrees of freedom were close to 1.00 (Table 2). This indicates that data variability has

been adequately modeled and that there is no residual overdispersion (variance greater than the mean). Thus, the distributions used provided a reliable adjustment. It should be pointed out that reasons of chi-square values and their degrees of freedom greater than two are potential indicators of overdispersion (Gbur et al., 2012).

A significant effect was observed in blocks ( $p < 0.05$ ) for all traits evaluated. For greenhouse experiments in which the blocks represent locations, testing their variability may be the most appropriate, because not considering this effect would result in misinterpretations.

No significant effect was observed for interactions among the factors evaluated ( $p > 0.05$ ) in the greenhouse experiment (Table 2).

The source of nitrogen fertilization had a significant effect only on VP and GP in cowpea. The treatment without a nitrogen source had similar results to those obtained with the inoculant (Table 3), but the numbers of pods and seeds per plant (VP and GP) decreased with the use of ammonium sulfate. For the remaining production traits, the effect of nitrogen fertilization did not have a significant effect.

The low production index observed in the treatment with ammonium sulfate corroborates the results reported in the literature, suggesting that in order to use the mineral nitrogen fertilization satisfactorily, the soil characteristics must be taken into account, because in some cases, nitrogen-fixing plants may decrease the pH of the soil (Lacerda, 2015), as well as the use of mineral fertilizers.

Costa et. al (2011) studied the cultivar BR 17 Gurguéia and evaluated the agronomic efficiency of symbiotic diazotrophic bacterial strains in cowpea yield. These authors reported the presence of efficient native nodulating bacteria, not requiring the use of chemical nitrogen fertilization.

Nitrogen is an element affected by a complex dynamics and fertilization does not have direct residual effects, making the management of nitrogen fertilization challenging (Raij, 1991). Thus, based on the cost/benefit ratio and social characteristics of the majority of cowpea growers in the study region, mostly family farmers, we recommend the cultivation of this crop without mineral nitrogen sources, especially in soils with a previous history of cowpea.

**Table 2** - Summary of the analysis of variance of the effects evaluated with mixed generalized linear models in greenhouse and field experiments on cowpea, variety Tumucumaque, in Bom Jesus-PI.

| Trait                        | Effect          | DF | Greenhouse  |      |         | Field       |       |         |
|------------------------------|-----------------|----|-------------|------|---------|-------------|-------|---------|
|                              |                 |    | $\chi^2/DF$ | F    | p-value | $\chi^2/DF$ | F     | p-value |
| Pods produced per plant      | Nitrogen (N)    | 2  |             | 5.71 | 0.0048  |             | 0.61  | 0.5471  |
|                              | Defoliation (D) | 4  |             | 6.64 | 0.0001  |             | 0.78  | 0.5407  |
|                              | Stage (E)       | 1  |             | 1.95 | 0.1658  |             | 0.50  | 0.4818  |
|                              | N*D             | 8  | 0.18        | 1.77 | 0.0945  | 0.78        | 0.20  | 0.9902  |
|                              | N*E             | 2  |             | 2.36 | 0.0658  |             | 0.37  | 0.6902  |
|                              | D*E             | 4  |             | 1.89 | 0.1192  |             | 0.23  | 0.9233  |
|                              | N*D*E           | 8  |             | 1.78 | 0.0931  |             | 0.17  | 0.9945  |
| Seeds produced by pod        | Nitrogen (N)    | 2  |             | 0.51 | 0.6005  |             | 0.39  | 0.681   |
|                              | Defoliation (D) | 4  |             | 1.62 | 0.1760  |             | 3.19  | 0.0492  |
|                              | Stage (E)       | 1  |             | 0.26 | 0.6099  |             | 0.19  | 0.6652  |
|                              | N*D             | 8  | 0.14        | 1.12 | 0.3560  | 0.26        | 0.79  | 0.6085  |
|                              | N*E             | 2  |             | 0.24 | 0.7848  |             | 0.18  | 0.8328  |
|                              | D*E             | 4  |             | 1.70 | 0.1569  |             | 1.27  | 0.2885  |
|                              | N*D*E           | 8  |             | 1.92 | 0.1287  |             | 1.42  | 0.2213  |
| Seeds produced by plant      | Nitrogen (N)    | 2  |             | 3.36 | 0.0394  |             | 4.81  | 0.0106  |
|                              | Defoliation (D) | 4  |             | 3.50 | 0.0108  |             | 3.17  | 0.0179  |
|                              | Stage (E)       | 1  |             | 1.87 | 0.1754  |             | 4.17  | 0.0443  |
|                              | N*D             | 8  | 0.24        | 1.10 | 0.373   | 0.57        | 1.19  | 0.3164  |
|                              | N*E             | 2  |             | 2.09 | 0.1301  |             | 2.61  | 0.0797  |
|                              | D*E             | 4  |             | 1.57 | 0.1782  |             | 4.59  | 0.0021  |
|                              | N*D*E           | 8  |             | 1.83 | 0.2100  |             | 0.13  | 0.7991  |
| Average pod length           | Nitrogen (N)    | 2  |             | 0.57 | 0.5663  |             | 0.57  | 0.5704  |
|                              | Defoliation (D) | 4  |             | 4.05 | 0.0047  |             | 4.12  | 0.0043  |
|                              | Stage (E)       | 1  |             | 0.83 | 0.3659  |             | 0.86  | 0.3558  |
|                              | N*D             | 8  | 6.59        | 2.25 | 0.0673  | 1.02        | 2.30  | 0.0281  |
|                              | N*E             | 2  |             | 2.67 | 0.0512  |             | 1.23  | 0.3064  |
|                              | D*E             | 4  |             | 1.19 | 0.3223  |             | 3.71  | 0.0285  |
|                              | N*D*E           | 8  |             | 1.16 | 0.334   |             | 1.18  | 0.3204  |
| Average weight of 100 grains | Nitrogen (N)    | 2  |             | 0.57 | 0.5698  |             | 1.84  | 0.1654  |
|                              | Defoliation (D) | 4  |             | 0.29 | 0.8842  |             | 4.14  | 0.0003  |
|                              | Stage (E)       | 1  |             | 0.37 | 0.5444  |             | 0.74  | 0.3911  |
|                              | N*D             | 8  | 1.44        | 1.53 | 0.1602  | 1.70        | 0.51  | 0.5915  |
|                              | N*E             | 2  |             | 0.38 | 0.6833  |             | 1.28  | 0.2824  |
|                              | D*E             | 4  |             | 2.36 | 0.0596  |             | 5.22  | 0.0008  |
|                              | N*D*E           | 8  |             | 1.84 | 0.0803  |             | 0.72  | 0.5807  |
| Production per hectare       | Nitrogen (N)    | 2  |             | 0.28 | 0.7534  |             | 11.80 | < 0001  |
|                              | Defoliation (D) | 4  |             | 1.09 | 0.3692  |             | 61.40 | < 0001  |
|                              | Stage (E)       | 1  |             | 0.23 | 0.6323  |             | 1.61  | 0.1984  |
|                              | N*D             | 8  | 1.79        | 1.04 | 0.4133  | 1.32        | 0.77  | 0.9801  |
|                              | N*E             | 2  |             | 1.73 | 0.1828  |             | 0.98  | 0.8709  |
|                              | D*E             | 4  |             | 0.25 | 0.9076  |             | 10.61 | < 0001  |
|                              | N*D*E           | 8  |             | 1.98 | 0.0592  |             | 0.66  | 0.992   |

$\chi^2$  – chi-square; DF - degree of freedom. Source: Authors.



**Table 3** - Agronomic variables of cowpea, variety Tumucumaque, cultivated in a greenhouse, treated with different nitrogen sources, defoliation levels and times in Bom Jesus – PI.

| Factor            | Levels             | Estimate (Normal scale)       |                               |                                | Normal scale        |                     |                      |
|-------------------|--------------------|-------------------------------|-------------------------------|--------------------------------|---------------------|---------------------|----------------------|
|                   |                    | VP                            | GV                            | GP                             | CMV(cm)             | P100 (g)            | PHA (Kg)             |
| Nitrogen Source   | Ammonium sulfate   | 0.2899 (1.3363) b             | 1.893 (6.6396) <sup>ns</sup>  | 2.1466 (8.5556) b              | 15.19 <sup>ns</sup> | 19.74 <sup>ns</sup> | 184.77 <sup>ns</sup> |
|                   | Inoculant          | 0.4178 (1.5186) a             | 1.9420 (6.9727) <sup>ns</sup> | 2.3288 (10.2656) a             | 15.76 <sup>ns</sup> | 18.97 <sup>ns</sup> | 196.81 <sup>ns</sup> |
|                   | No nitrogen source | 0.4802 (1.6164) a             | 1.8633 (6.4447) <sup>ns</sup> | 2.3039 (10.0131) a             | 15.68 <sup>ns</sup> | 18.12 <sup>ns</sup> | 200.52 <sup>ns</sup> |
| Defoliation level | 0%                 | 0.4210 (1.5235) ab            | 2.0215 (7.5496) <sup>ns</sup> | 2.4105 (11.1396) a             | 16.64 a             | 19.25 <sup>ns</sup> | 216.71 <sup>ns</sup> |
|                   | 25%                | 0.5068 (1.6600) a             | 1.8560 (6.3980) <sup>ns</sup> | 2.3352 (10.3313) ab            | 15.36 as            | 17.88 <sup>ns</sup> | 198.42 <sup>ns</sup> |
|                   | 50%                | 0.5313 (1.7011) a             | 1.7829 (5.9571) <sup>ns</sup> | 2.2809 (9.7852) ab             | 15.25 ab            | 19.91 <sup>ns</sup> | 210.90 <sup>ns</sup> |
|                   | 75%                | 0.3187 (1.3753) bc            | 1.9099 (6.7523) <sup>ns</sup> | 2.1987 (9.0131) bc             | 16.70 a             | 18.53 <sup>ns</sup> | 171.45 <sup>ns</sup> |
|                   | 100%               | 0.2020 (1.2239) c             | 1.9269 (6.8683) <sup>ns</sup> | 2.0736 (7.9532) c              | 14.76 b             | 19.13 <sup>ns</sup> | 172.70 <sup>ns</sup> |
| Defoliation stage | Vegetative         | 0.3487 (1.4173) <sup>ns</sup> | 1.9161 (6.7945) <sup>ns</sup> | 2.2170 (9.1800) <sup>ns</sup>  | 15.76 <sup>ns</sup> | 19.33 <sup>ns</sup> | 189.67 <sup>ns</sup> |
|                   | Reproductive       | 0.4432 (1.5577) <sup>ns</sup> | 1.8828 (6.5716) <sup>ns</sup> | 2.23025 (9.9991) <sup>ns</sup> | 15.32 <sup>ns</sup> | 18.55 <sup>ns</sup> | 198.40 <sup>ns</sup> |

Number of pods produced per plant (VP); Number of seeds produced per pod (GV); Number of seeds produced per plant (GP); Mean pod length (CMV); Mean weight of 100 seeds (P100); Estimated production per hectare (PHA); a Means followed by the same lowercase letter in the column, for each factor, do not differ from each other by the Tukey-Kramer test ( $p < 0.05$ ); ns Not significant. Source: Authors.

The effect of the defoliation level was significant ( $p < 0.05$ ) on VP, GP, and CMV, while for the remaining traits, no significant differences were observed. Thus, leaf damage of up to 50% does not affect the number of VP and GP. Regarding CMV, the tolerable damage is up to 75%. Also damage level of 100% of leaf area does not influence GV, P100, and PHA. These results differ from those obtained by Moura et al. (2014) in a field study with the cultivar BR 17-Gurguéia, reporting higher losses at the 100% defoliation level for the traits examined, except for number of seeds per pod.

Our findings suggest that the cultivar BRS Tumucumaque is more resistant to higher leaf loss levels compared to the cultivar BR 17-Gurguéia, as defoliation may have caused a stimulus for higher production of reproductive buds, since plants modified their photosynthetic indexes and consequently concentrated and stimulated the production of photo-assimilates directly to pods.

No significant effect ( $p > 0.05$ ) was observed regarding the defoliation stage (Table 3). Whether the damage occurs at 25 or 45 DAP, the productive response will be the same for the traits evaluated in cowpea plants. Thus, understanding the effect of resistance to leaf area loss of the cultivar to be used and soil fertility is essential as the latter provides the necessary nutrients to plants, making them more resistant to pest attacks without drastically reducing indexes of productivity.

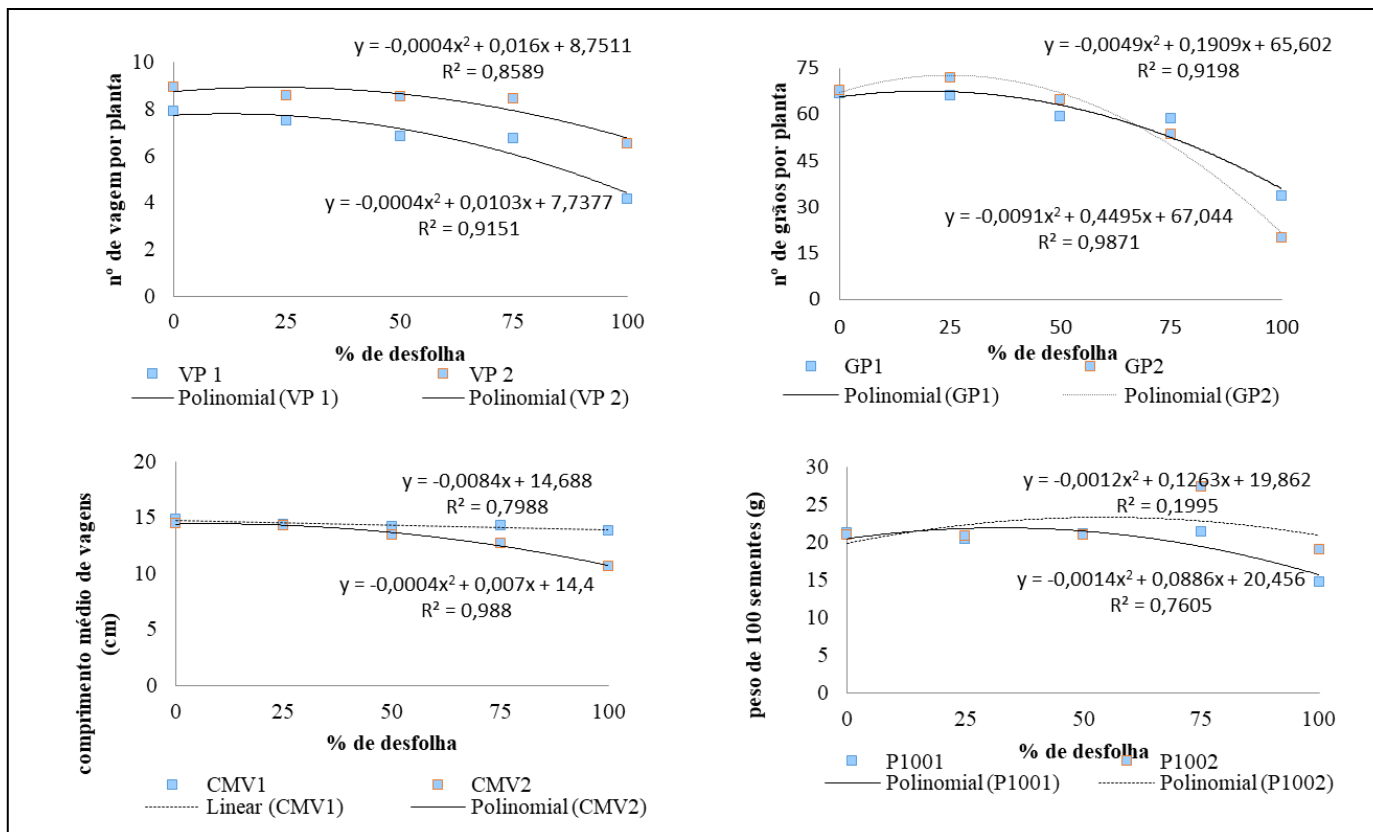
### 3.2 Field experiment

In the field experiment, a significant effect ( $p < 0.05$ ) was observed regarding the interaction between the factors DxEx and the traits VP, GP, CMV, and P100 (Table 2). It should be pointed out that there was a significant effect of defoliation percentage on all evaluated characteristics.

When the plant loses leaf area during the reproductive stage, PV is higher (Figure 2a), and production can reach maximum levels in defoliation levels of up to 20%. However, when the injury occurs in the vegetative stage, the plant sustain losses of leaf area above 13% without affecting the mean number of pods per plant. This indicates the regeneration capacity of cowpea plants that despite suffering a high reduction in leaf area, produces a favorable number of pods per plant. The damage causes some

stress, as plants with defoliation levels above 75% have significantly reduced yields in all traits (Table 4).

**Figure 2** - Agronomic traits: a) Number of pods per plant (VP); b) Number of seeds per plant (GP); c) Mean pod length (CMV); and d) Weight of 100 seeds (P100), as a function of the percentage of artificial defoliation in the vegetative (1) and reproductive (2) stages of cowpea, variety Tumucumaque, cultivated in the field, in Bom Jesus - PI.



Source: Authors.

**Table 4** - Agronomic variables of cowpea, variety Tumucumaque, cultivated in the field, subjected to different levels of artificial defoliation in Bom Jesus-PI.

| Defoliation level | Estimate (Normal scale) |                 |                  | Normal scale       |                    |                     |
|-------------------|-------------------------|-----------------|------------------|--------------------|--------------------|---------------------|
|                   | VP                      | GV              | GP               | CMV(cm)            | P100(g)            | PHA(Kg)             |
| 0%                | 2.0421 (7.70) a         | 2.1503 (8.66) a | 3.0121 (66.70) a | 14.62 a            | 21.10 <sup>a</sup> | 2763.3 <sup>a</sup> |
| 25%               | 2.0961 (7.92) a         | 2.1672 (8.70) a | 3.0200 (68.89) a | 14.30 a            | 20.61 <sup>a</sup> | 2889.7 <sup>a</sup> |
| 50%               | 2.0135 (7.21) a         | 2.1499 (8.61) a | 3.0182 (62.01) a | 13.80 <sup>a</sup> | 21.01 <sup>a</sup> | 2605.2 <sup>a</sup> |
| 75%               | 2.0021 (6.59) a         | 2.1401 (8.51) a | 3.0001 (56.09) a | 13.50 <sup>a</sup> | 21.07 <sup>a</sup> | 2358.6 <sup>a</sup> |
| 100%              | 0.8076 (3.57) b         | 2.0398 (7.47) b | 2.8071 (26.67) b | 12.19 <sup>b</sup> | 16.87 <sup>b</sup> | 871.7 <sup>b</sup>  |

Number of pods produced per plant (VP); Number of seeds produced per pod (GV); Number of seeds produced per plant (GP); Mean pod length (CMV); Mean weight of 100 seeds (P100); Estimated production per hectare (PHA); <sup>a</sup> Means followed by the same lowercase letter in the column, for each factor, do not differ from each other according to the Tukey-Kramer test ( $p < 0.05$ ). Source: Authors.

Cowpea plants subjected to leaf area loss may increase the number of seeds per pod (GV), a behavior defined as overcompensation, in both stages of development, reaching the maximum yield with defoliation level of approximately 32% (Figure 3a). This indicates that cowpea plants do not exhibit significant changes as a response to the injury, at both the vegetative

or reproductive stages. At 75% of defoliation level, however, significant reductions are observed in plants (Table 4) and farmers should start control measures.

These results corroborate those observed by Moura et al. (2014) that did not find significant differences in the number of seeds per pod, regardless of defoliation time in a field study with the cultivar BR17-Gurguéia.

The number of seeds per plant (GP) is higher when the plant is subjected to defoliation during the reproductive stage (Figure 2b), and it may even increase when the defoliation level is of up to 25%. GP was lower when plants were submitted to 100% defoliation in the vegetative stage. Some authors observed significant differences in this parameter depending on different defoliation times. Moura et al. (2014) reported significant differences in the number of seeds per pod based on defoliation times and defoliation levels. Souza et al. (2014) found that, in addition to the photosynthetic process, soybeans (*Glycine max* (L) Merrill) plants also remobilize reserves that contribute to support, at least temporary, sink organs.

Unlike the observed in the greenhouse experiment, mean pod length (CMV) is not significantly affected with the loss of leaf area during the vegetative stage, but in the reproductive stage, defoliation levels above 75% will decrease the mean length of pods (Figure 2c).

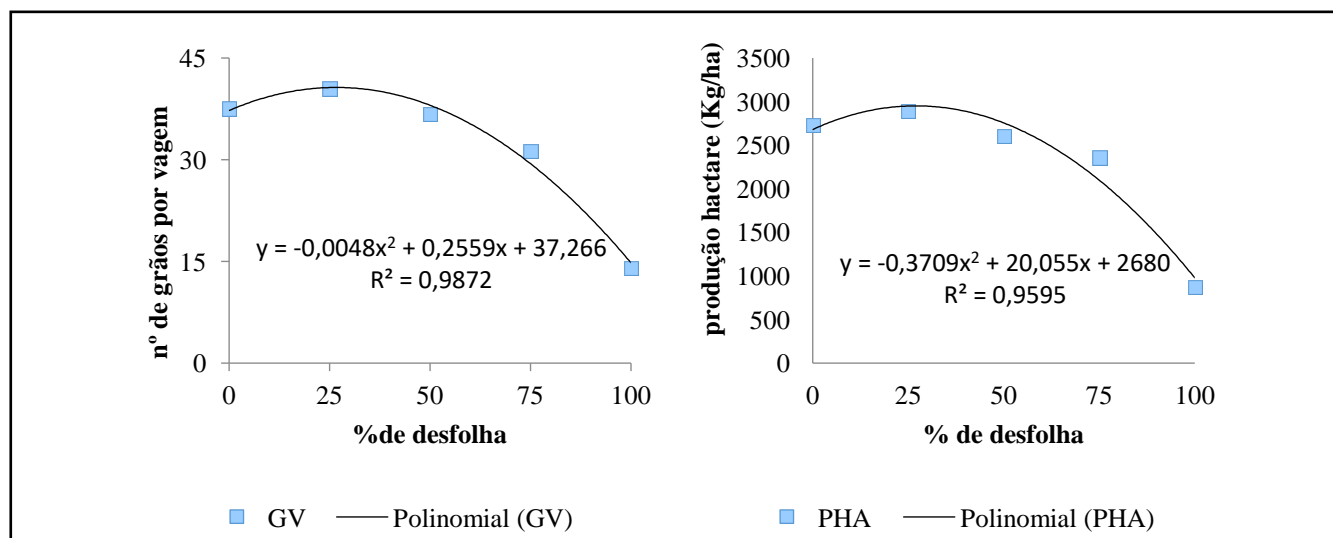
In the vegetative stage, plants subjected to defoliation levels of 25 and 100% had the highest and lowest CMV, respectively. At 40 DAP no significant differences in CMV were observed regarding the defoliation levels examined. Moura et al. (2014) observed a response in CMV depending on the treatment.

The Weight of 100 seeds (P100) is not affected when defoliation occurs during the reproductive stage, but in the vegetative stage, loss of leaf area above 50% will decrease the mean seed weight (Figure 2d). P100 was highest at the 50% defoliation level in the reproductive stage, and lowest at 100% defoliation level in the vegetative stage, possibly resulted from biomass accumulation in the plant until this stage when defoliation occurred (Table 2).

Evaluating the effect of four levels of defoliation at five different times in common bean *P. vulgaris*, Schmidt et al. (2010) observed a significant difference only for defoliation level and the decrease in P100 was more constant in plants with 100% defoliation level, a factor that is directly associated with pod size and the quantity of seeds that each plant will produce.

Production per Hectare (PHA) of plants subjected to leaf loss area can be increased. Up to 50% defoliation level, the variety Tumucumaque overcompensates in productivity, with maximum yields at 27.03% of defoliation and significant decrease above 75%, regardless of phenological stage (Figure 3a). Even with losses above these levels, this cultivar had high indexes of productivity, revealing its high resistance to leaf loss injury.

**Figure 3** – Agronomic traits Number of seeds per pod (a) and Production per hectare (b) as a function of the percentage of artificial defoliation in cowpea, variety Tumucumaque, cultivated in the field, in Bom Jesus - PI.



Source: Authors.

These results are similar to those reported by Silva et al. (2003) on the artificial defoliation in the common bean *P. vulgaris* where these authors observed that defoliation levels above 50% reduced yields regardless of plant age. Moura et al. (2014) reported a decrease in yields at 100% defoliation level and greater losses when defoliation occurred in the vegetative stage.

#### 4. Conclusion

The use of chemical nitrogen fertilization and inoculation of cowpea seeds, variety Tumucumaque with *Bradyrhizobium* is unnecessary in the edaphoclimatic conditions of Bom Jesus - PI.

The use of nitrogen fertilization with ammonium sulfate and inoculation of cowpea seeds with *Bradyrhizobium* does not influence the resistance of the tumucumaque variety to defoliation levels.

Defoliation levels above 75% impair the performance of production traits of cowpea, variety Tumucumaque.

Based on this experiment, it is possible to understand the importance and assist in the methodology of future work related to the nitrogen nutrient and defoliation levels in other crops of interest to agriculture, enabling application interest in other edaphoclimatic conditions.

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