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Assessment of soybean yield in plants subjected to artificial defoliation simulating the defoliation caused by insects at different crop development stages in a cloudy and low-latitude environment

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Abstract: The damage caused by defoliating insects is one of the main factors limiting soybean productivity. It is crucial to define a level of defoliation that does not cause significant yield reduction, defined as the economic threshold. Therefore, in this study, we aimed to evaluate the effects of artificial defoliations, simulating insect damage, on soybean leaf area index (LAI), total aboveground dry biomass (TAGB), and yield in low-latitude regions. The experiment was performed in a 4 × 3 factorial design with 12 treatments, including four levels of artificial defoliation (0, 17, 33, and 67%) at the 8th-trifoliolate (V8), full flowering (R2), and beginning of seed filling (R5) stages. The LAI and TAGB were evaluated immediately after defoliation and at the fully formed seed stage (R6), along with an evaluation of yield components at the full maturity stage (R8). Linear models were fitted to determine the effects of the defoliation treatments and their interactions with the different phenological stages. The LAI and TAGB declined as defoliation levels increased to 17, 33, and 67%, with a higher impact when defoliation occurred at the R5 stage compared to the other stages. Defoliation at the V8 stage did not affect yield (3,837 kg ha^{-1}) as the crop recovered its growth and yield. However, a positive effect was observed with defoliation levels of 27 and 16% at the R2 and R5 stages, increasing the yield to 4,314 and 3,959 kg ha^{-1}, respectively. This increase was related to a higher grain number and mass because of LAI recovery after defoliation, which was associated with leaf expansion and better solar radiation interception through the canopy under cloudy ambient conditions. The maximum accepted defoliation levels in the R2 and R5 stages were 46 and 35%, respectively, to achieve at least 95% of the maximum yield. The relationships between yield and LAI after defoliation and between yield and LAI were significant at R5 and R6, respectively. Thus, variations in the stages and levels of defoliation differentially affect soybean crop yield and can be used to optimize defoliator management for maximum yield and sustainability.

Keywords: leaf area index; leaf losses; integrated pest control; maximum defoliation level; pest management.

Introduction

According to the Food and Agriculture Organization (FAO) of the United Nations, crops pests lead to losses of aproximately US$ 70 billion annually in agricultural areas worldwide (FAO, 2019). Defoliator pests are among the main factors limiting high soybean yields. Defoliators reduce the leaf area index (LAI), which is critical for soybeans to reach their potential yield (Battisti et al., 2018). Furthermore, the LAI is affected by interaction between the cultivar, plant density, and sowing date in various ways in different environments (Liu et al., 2008; Tagliapietra et al., 2018; Sampaio et al., 2021). An LAI below optimal values leads to lower solar radiation interception, transpiration, and photosynthesis, reducing plant capacity for translocating storage nutrients during later stages in the development cycle with a higher demand for grain filling (Owen et al., 2013). *Chrysodeixis includens* (Walker), *Anticarsia gemmatalis* (Hübner), and *Spodoptera spp.* are the main caterpillars responsible for soybean defoliation in Brazil (Bortolotto et al. 2015; Horikoshi et al., 2021). The use of Cry1Ac soybean has provided Brazilian farmers with 8 years of consistent protection against damage from the primary lepidopteran soybean pests (*C. includens* and *A. gemmatalis*). However, it has also led to an increase in the relative abundance of the larvae of non-target *Spodoptera spp*. in both non-Bt and Cry1Ac soybeans.

Most of the control is achieve using chemical pesticides. However, the use of non-selective pesticide products reduces the population of the natural enemies of plant pests, leading to an imbalance in the environment (Carmo et al., 2010; Sosa-Gómez et al., 2003) by indirectly increasing the number of pests by decreasing their natural enemies (Bueno et al., 2017). Therefore, integrated pest control is an efficient strategy for controlling pests in agricultural areas (Batistela et al., 2012).

Integrated pest control was proposed by Stern et al. (1959), and one of its principles is that chemical pesticides must be applied only when pests reach their threshold that, leading to adverse economic impacts. This threshold is related to the minimum pest population that can cause economic losses. In Brazil, up to15 and 30% defoliation are accepted to be the limits for the reproductive and vegetative stages of soybean plants, respectively, which affect the economic threshold (Batistela et al., 2012). However, these values can vary depending on the environmental conditions and soybean management strategies (Hayashida et al., 2021). The economic threshold can change when considering cultivar tolerance and the capacity to recover leaf area, crop stage, sowing date, plant growth rate, established plant density after emergence, leaf area dynamics, and photosynthetic solar radiation (Batistela et al., 2012; Gregorutti et al., 2012; Glier et al., 2015; Raza et al., 2019; Durli et al., 2020).

The interactions in these systems lead to different levels of impact on soybean yield based on defoliation levels, where the soybean maturity group, sowing date, and potential yield can define the maximum level of defoliation. Durli et al. (2020) verified a reduction in soybean yield at defoliation levels higher than 16% in the vegetative and reproductive stages, with the impact on yield being dependent on the soybean maturity group at defoliation during the vegetative stage. Thrash et al. (2021) observed relatively high yield losses with delayed sowing dates at a 100% defoliation rate during the vegetative stage. Defoliation can increase biomass translocation to seeds and reduce the impact of self-shading, and Raza et al. (2021) verified that 15% defoliation at the beginning pod emission (R3) stage resulted in maximum soybean yield. These studies used artificial defoliation and observed that natural and artificial defoliation exhibited similar responses, making artificial defoliation applicable tool for evaluating the impacts of pest-induced defoliation on crops (Ostlie and Pedigo, 1984).

Adjusting soybean tolerance to defoliation is essential to achieve maximum yield with the sustainable use of pesticides based on integrated pest control. In this scenario, the advancement of soybean cultivation to northern Brazil demands a comprehensive elucidation of interactions between the climate, soil, sowing date, and soybean maturity groups at defoliation in this region. The region has a low range in daylength associated with low water deficit, with sowing dates after the summer solstice (January – February) and maturity groups higher than 8.0 (Battisti and Sentelhas, 2014; Sampaio et al., 2021). Currently, farmers adopt zero tolerance to defoliation, which leads to excessive pesticide use due to their low application cost. However, the economical threshold recommended is defoliation levels of up to 15 and 30% during the reproductive and vegetative stages, respectively (Batistela et al., 2012), with this threshold defined for traditional soybean production regions at relatively high latitudes experiencing more frequent water deficit (Battisti and Sentelhas, 2019).

Based on this information, we hypothesized that soybean plants exhibit a higher level of defoliation tolerance under the edaphoclimatic conditions of northern Brazil compared to those observed in their traditional production regions. Therefore, in this study, we aimed: to (1) quantify soybean yield in response to the artificial defoliation levels of 0, 17, 33, and 67% at three different crop development stages, i.e., eighth trifoliolate (V8), full flowering (R2), and beginning of seed filling (R5), simulating defoliation caused by insects; (2) evaluate the correlation between LAI and soybean yield for defoliation treatments at the different defoliation levels and development stages; and (3) identify the maximum defoliation level accepted by a crop development stage without significant yield reduction in the region as a contribution to integrated pest control.

Results

LAI and total aboveground dry biomass (TAGB)

The different defoliation levels and crop development stages exhibited statistically significant ($p < 0.05$) interactions for the LAI and TAGB measured after defoliation at the R6 and R8 stages, respectively, and for crop yield (Table 1). Defoliation exhibited isolated effects on TAGB at the R6 stage and grain number per unit area (Table 1). Grain weight showed the isolated effects of defoliation treatments at the different defoliation levels and crop development stages, whereas node number on the main stem, total node number on the plant, and total branch number at the R8 stage did not show significant effects of the different treatments (Table 1).

For the 0% defoliation treatment, the mean values of LAI were 1.6, 2.6, 4.7, and 3.9, at stages V8, R2, R5, and R6, respectively (Figure 1a). In this treatment, soybeans reached an LAI of 3.0 around 40 days after sowing (DAS), when pod formation began. The LAI had reduction rates of 0.0133, 0.027, and 0.048 $m^2\ m^{-2}$ by the percentage of defoliation at stages V8, R2, and R5, respectively (Figure 1b). For defoliation treatment at the R5 stage of development, the LAI value dropped below the reference value of 3.0 at defoliation levels exceeding 33% (Figure 1b). At 67% defoliation level, the LAI values for defoliation treatments at the R2 and R5 stages of development were almost similar (Figure 1b).

The LAI was above 3.0 at the R6 stage until the defoliation level of 17% for the defoliation treatments at the V8, R2, and R5 stages of development (Figure 1c). At 33% defoliation, defoliation at the R5 stage had a lower performance than defoliation at V8 and R2, with an LAI below 3.0. The rates of reduction in the LAI at the R6 stage based on the percentage of defoliation for the defoliation treatments at stages V8, R2, and R5 were 0.0140, 0.0255 and 0.0340 $m^2\ m^{-2}$, respectively. This led to relatively high differences in LAI values between the V8, R2, and R5 stages at 67% defoliation, where only the V8 stage exhibited an LAI near the reference value of 3.0 (Figure 1c).

The maximum TAGB was observed at the R6 stage, reaching 6,665 kg ha^{-1} at the 0% defoliation level (Figure 1d). Defoliation led to a significant reduction in all crop stages, with defoliation at the R5 stage showing the highest rate of TAGB reduction

Table 1. Analysis of variance, mean, and coefficient of variation (CV) for variables measured at different artificial defoliation levels and crop development stages.

Variables[1]	p values based on factors and variables			Mean	CV (%)
	Development stage (S) at defoliation	Level (L) of defoliation	S x L		
LAI after defoliation (m^2 m^{-2})	<0.0001	<0.0001	<0.0001	2.2	60.2
LAI at R6 (m^2 m^{-2})	0.004	<0.0001	<0.001	2.9	20.4
TAGB after defoliation (kg ha^{-1})	<0.0001	<0.0001	<0.0090	1,994.0	81.4
TAGB at R6 (kg ha^{-1})	0.861	<0.0001	0.153	5,961.0	15.2
TAGB at R8 (kg ha^{-1})	0.036	<0.001	0.022	5,593.0	13.3
Grain number (nº m^{-2})	0.274	<0.0001	0.445	2,519.0	4.3
Grain weight (g x 100)	0.007	0.001	0.403	13.2	6.6
Grain Yield (kg ha^{-1})	0.003	0.001	0.004	3,818.0	13.5
Node number on the main stem	0.059	0.061	0.672	11.3	5.7
Total node number on the plant	0.691	0.914	0.475	22.5	12.7
Total branch number	0.776	0.510	0.113	3.7	15.1

[1]LAI is the leaf area index, TAGB is the total aboveground dry biomass, R6 is the crop stage with fully formed seeds, and R8 is the crop stage at full maturity.

Table 2. Pearson correlation between the analyzed growth variables in soybean crops after artificial defoliation treatments at different defoliation levels and crop development stages.

Variables[1]	LAI at R6	TAGB at R8	Soybean yield	Grain weight	Seed number
LAI after defoliation (n = 48)	0.21	0.29*	0.23	0.14	0.18
LAI after defoliation at V8 (n = 16)	0.47	0.39	0.30	0.03	0.28
LAI after defoliation at R2 (n = 16)	0.82*	0.58*	0.49	0.38	0.29
LAI after defoliation at R5 (n = 16)	0.95*	0.48	0.55*	0.69*	0.34
LAI at R6 (n = 48)		0.47*	0.53*	0.45*	0.36*
TAGB at R8 (n = 48)			0.98*	0.30*	0.88*
Soybean yield (n = 48)				0.33*	0.90*
Grain weight (n = 48)					-0.12

[1]LAI is the leaf area index, TAGB is the total aboveground dry biomass, V8 is the eighth trifoliolate, R2 is the full flowering stage, R5 is the stage at the beginning of seed filling, R6 is the crop stage with fully formed seeds, and R8 is the crop stage at full maturity, * correlation significant at 5% of probability.

(24.45 kg ha^{-1} per percent of defoliation) (Figure 1e). The rates of reduction in the TAGB based on the percentage of defoliation at the V8 and R2 stages of development were 5.18 and 7.19 kg ha^{-1}, respectively. There were no significant differences in the TAGB at the R6 stage between the defoliation treatments at the different crop development stages, showing a response only as a function of defoliation levels, with a reduction rate of 25.63 kg ha^{-1} based on the percentage of defoliation (Figure 1d). The TAGB at the R8 stage exhibited a quadratic response to the level of defoliation, with defoliation at the R5 stage showing a statistically significant difference from those at the other development stages only at the 67% defoliation level (Figure 1f).

Soybean yield

Soybean yield had a mean value of 3,837 kg ha^{-1} for the treatment without defoliation (Figure 1g) and did not show significant adjustment as a function of defoliation at V8. The defoliation level of 17% resulted in an improvement in yield to 4,258 kg ha^{-1} regardless of the development stage at defoliation; however, the yield was reduced at higher defoliation levels (Figure 1g). Defoliation at the R2 and R5 stages showed a quadratic response, with maximum yields at 27 and 16% defoliation, respectively. At the maximum rate of defoliation (67%), defoliation at the R5 stage was reduced to 2,599 kg ha^{-1}. The yield results can be explained by a quadratic response as a function of defoliation for grain weight (Figure 1h) and number of grains per area (Figure 1i). Both these parameters showed a reduction in values at defoliation levels higher than 20%. There were no statistically significant differences between grain weight after the defoliation treatments at the V8 and R2 stages at 13.16 and 13.23 g for 100 grains, respectively; however, it reduced to 12.78 g for 100 grains after the defoliation treatment at the R5 stage.

Correlations between yield, LAI, and TAGB

The impact of defoliation treatments at the different defoliation levels and development stages on yield can be verified through the results of correlation analysis (Table 2). Soybean yield was significantly correlated with LAI after defoliation at R5, LAI at R6, and with TAGB at R8 (Table 2). The LAI values after defoliation at the R5 and R2 stages showed a high correlation with the LAI value at the R6 stage (0.82 and 0.95, respectively). Of the variables that correlated significantly with soybean yield, the LAI after defoliation at the R5 stage and the LAI at the R6 stage correlated significantly with grain weight, whereas only the LAI at the R6 stage correlated significantly with seed number. The LAI values after defoliation at

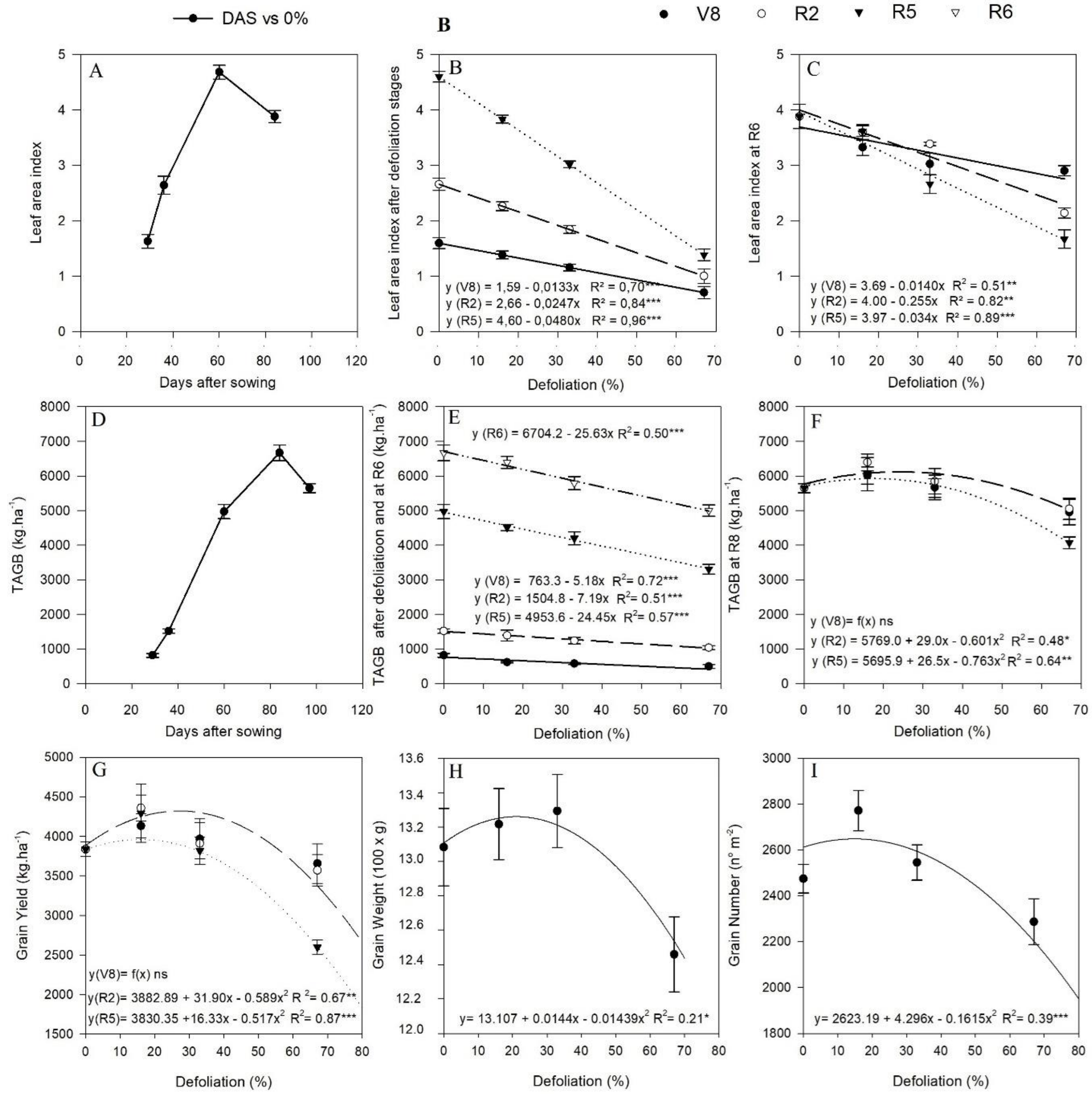

Figure 1. Analyses of the effects of different levels of defoliation at different development stages on various plant growth parameters. A - leaf area index across crop cycle, B - leaf area index after defoliation at different development stages, C - leaf area index at the R6 stage, D - total aboveground dry biomass (TAGB) across crop cycle, E - TAGB after defoliation at different development stages, F - TAGB at the R8 stage, G - soybean grain yield, H - soybean grain weight, and I - soybean grain number. The V8 stage represents the eighth trifoliolate, R2 represents the full flowering stage, R5 is the development stage representing the beginning of seed filling, R6 is the crop stage representing full seed development, and R8 represents the stage of fully mature crop. DAS, says after sowing.

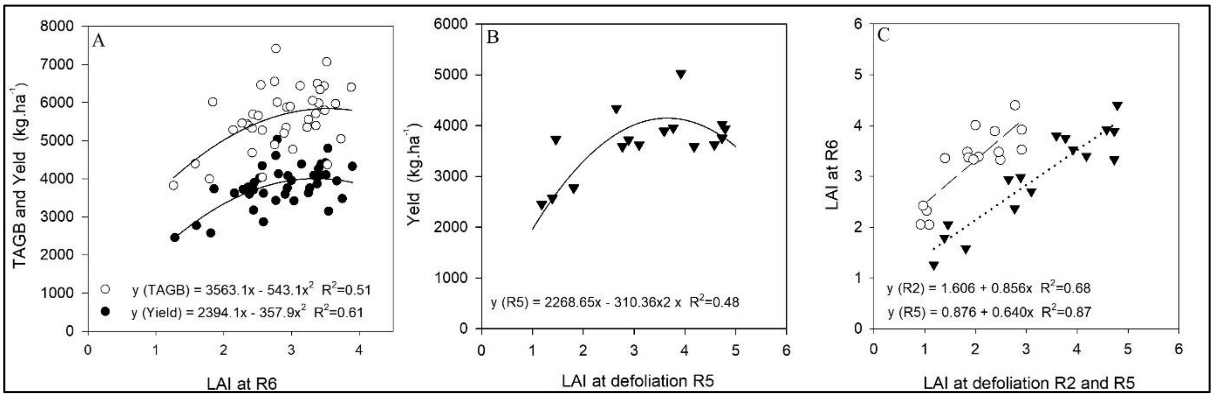

Figure 2. Analyses of the effects of the leaf area index (LAI) values immediately after defoliation and at the R6 stage on various plant growth parameters. A - yield and total aboveground dry biomass (TAGB) at R8 in function of soybean LAI at stage R6; B - yield in function of soybean LAI after defoliation at stage R5; and C - LAI at stage R6 in function of LAI after defoliation at stages R5 and R2. R2 is the full flowering stage, R5 is the beginning of the seed filling stage, R6 is the full seed stage, and R8 is the crop stage at full maturity.

the V8 and R2 stages showed no correlation with soybean yield; however, the LAI value after defoliation at the R2 stage correlated with the TAGB value.

Soybean grain yield and total aboveground biomass (TAGB) were dependent on the leaf area index (LAI) at the R6 growth stage, with their maximum values obtained at the LAI values of 3.34 and 3.28, respectively (Figure 2a). These LAI values resulted in the grain yields and TAGB of 4,004 and 5,844 kg ha^{-1}, respectively. The yield also showed a response to the LAI after defoliation at the R5 stage, and the obtained values can be used to define the impact of defoliation on yield (Figure 2b). A maximum yield of 4,146 kg ha^{-1} was obtained for an LAI above 3.65 after defoliation at stage R5 (Figure 2b). LAI at the R6 stage had a strong linear correlation with LAI after defoliation at R5 and R2 (Figure 2c), where LAI of 3.3 at R6 was reached with LAI of 3.8 and 2.1 after defoliation at R5 and R2, respectively.

Discussion

Soybean grain yield showed an interaction response to artificial defoliation level and stage of defoliation. The responses were linked to the LAI and the capacity of soybean plants to recover from defoliation. The critical LAI value has been defined as 3.0 for soybean plants to reach maximum yields in northern Brazil (Souza et al., 2013). Defoliation at stage V8 did not affect soybean yield, even at the 67% artificial defoliation level. Late vegetative defoliation made it possible for soybeans to recover their LAI, which was above three at stage R6, leading to intercepted solar radiation at an optimal level, above 95%, during grain filling (Ohnesorg and Hunt, 2015), without reducing grain weight and seed number (Figure 1h and i). Recovery was potentiated in the region by at lower water deficit during soybean growth (rainfall = 1189 mm $cycle^{-1}$, 65% at the reproductive stages), even with a short crop cycle of 98 days.

Defoliation at stage R2 had a different response than stage V8 at all defoliation levels. This treatment exhibited a positive effect on yield, with the treated plants responding in a quadratic pattern and reaching a maximum yield at 27% defoliation (Figure 1g). Plants with a higher LAI exhibit higher self-shading (Raza et al., 2021), implying that the leaves at the bottom of the canopy consume energy and create a negative photosynthetic balance. Defoliation treatments at the V8 and R2 stages exhibited an LAI > 3 at the R6 stage until 50 and 40% defoliation, respectively (Figure 1c). Therefore, defoliation can improve total canopy photosynthesis, increase grain weight and number, and reduce pod abortion (Van Roekel et al., 2015; Tagliapietra et al., 2018; Raza et al., 2021).

Defoliation at stage R5 resulted in a yield reduction at higher intensities than at other stages of defoliation. Defoliation above 35% (95% of maximum yield) resulted in an intensification of yield reduction (Figure 1g). This is linked to a higher restriction of the net photosynthetic assimilatory capacity by reduction of the LAI during the stage of intense grain growth (Board and Tan Qiang, 1995). A lower LAI leads to reduced solar radiation interception and photosynthesis. Board et al. (2010) observed a significant reduction in yield, with 18% less solar radiation being intercepted by the soybean canopy. This occurs when the LAI is below the optimal value of 3, which occurs at R6, with a defoliation of 29% at stage R5. The lower photosynthesis impact on grain weight (Board et al., 2010), with defoliation at R5 showed lower values (12.75 g by 100 grains) than V8 (13.16 g by 100 grains) and R2 (13.30 g by 100 grains).

The LAI after defoliation at the R5 stage and LAI at the R6 stage showed a significant correlation with soybean yield (Table 2). Similarly, Tagliapietra et al. (2018) verified a statistically significant correlation between the LAI and yield at the R2 stage. The LAI defines the canopy's capacity to intercept solar radiation, thereby defining net photosynthesis and potential soybean yield (Board and Tan Qiang, 1995; Ohnesorg and Hunt, 2015). Therefore, it is possible to calibrate defoliation parameters based on the optimal LAI and crop development stage. In this study, an optimal LAI of 3 at R6 was reached until the maximum defoliation of 50, 40 and 29% for defoliation at V8, R2, and R5, respectively. The recovery periods were 55, 48, and 12 days after defoliation until stage R6 for defoliation at V8, R2 and R5, respectively.

Currently, the maximum defoliation level accepted in South America is between 30 and 35% in the vegetative stage, and 15 and 20% in the reproductive stage (Board et al., 2010; Bueno et al., 2011; Batistela et al., 2012; Ohnesorg and Hunt, 2015). However, this differs from our results, where it was observed that defoliation at the V8 stage did not affect soybean yield even with 67% defoliation, whereas the reproductive stages differed between R2 and R5. The yield showed positive results for defoliation at 27 and 16% for R2 and R5, respectively. In the conservative scenario (yield > 95% of the maximum yield), the accepted defoliation rates were 46% and 35%, for defoliation at R2 and R5, respectively. The results showed different ranges owing to the growth conditions and cultivar responses. For example, Alves et al. (2020) observed a reduction in pod number when 66% defoliation was applied at the beginning of flowering and full pod emission. Gazzoni and Moscardi (1997) reported no difference in yields for defoliation treatments at the third trifolin and full flowering stages and until 33% of defoliation at the R6 stage, which is consistent with our results.

Materials and Methods

Study site

The field experiment was conducted in a farm area located in Paragominas City (Lat -3.38°, Long -47.42°, Altitude 176 m), Pará State, during the 2018 growing season. The climate in the region was classified as Aw, rainy tropical with defined dry season, mean annual air temperature of 26.5 °C, and a total rainfall of 1761 mm $year^{-1}$ (Alvares et al., 2013). In the 2018 growing season, soybean accumulated rainfall was 1,189 mm for 97 days of its development cycle, mean minimum and maximum air temperatures of 22 and 32 °C, respectively, and daily solar radiation varied from 17.3 to 19.4 MJ m^{-2} d^{-1} (INMET, 2018). The soil type was classified as Oxisol with a heavy-clay texture. The clay content ranged between 71.60 and 79.12%, pH between 5.0 and 5.3 at the surface layer, and the mean values of phosphorus, potassium, and organic matter content at 17 mg dm^{-3}, 84 mg dm^{-3} and 1.8 g kg^{-1} in the 0-20 cm layer.

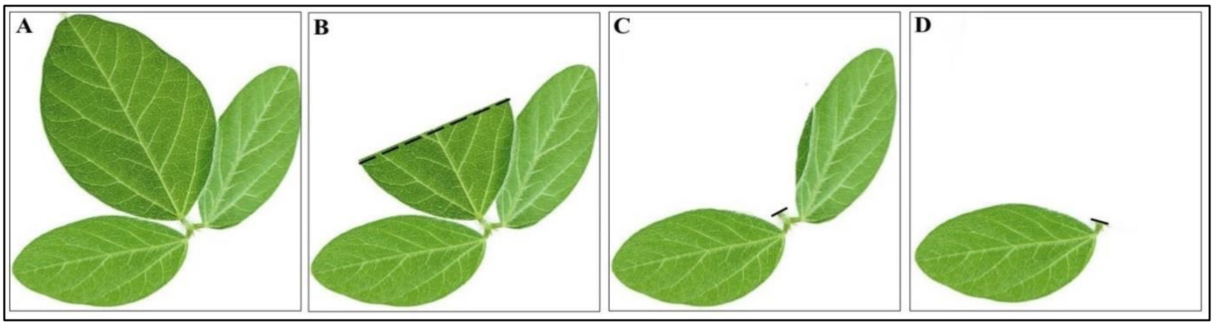

Figure 3. Images of soybean leaves representing the defoliation levels of A – 0%, B – 17%, C – 33%, and D – 67% and the methodology applied for achieving these defoliation levels at each leaf by cutting leaflets in the soybean canopy. Adapted from Gazzoni and Moscardi (1998).

Field experiment

Soybean was sown on February 18, 2018, over millet straw, with a final plant density of 26 pl m^{-2} and a spacing of 0.5 m between lines. The cultivar used was M8644 IPRPO (maturity group 8.6 and a determined flowering habit). Seeds were treated with an insecticide and a fungicide and inoculated with a liquid biological inoculant of *Bradyrhizobium japonicum.* Soil fertilization followed recommendations based on soil analyses and potential yields (Brasil et al., 2020), and pest and disease control aimed to avoid any pest or disease attack based on the recommendations of Seixas et al. (2020).

The seeds were sown in plots of 10 m^2 (4.0 × 2.5 m) area, using a randomized block design with four repetitions. The treatments included four levels of defoliation (0, 17, 33 and 67%) at three different crop stages (eighth trifoliolate, V8; full flowering, R2; and beginning of seed filling, R5) (Fehr and Caviness, 1977). Defoliation was performed based on the percentage of total leaflets in the lower, middle and upper thirds of the plant canopy. The defoliation levels of 17, 33 and 67% were achieved by removing half of the middle leaflet in each leaf, the entire middle leaflet in each leaf, and the entire middle and one lateral leaflet in each leaf (Figure 3) (Gazzoni and Moscardi, 1998). This process was carried out at the beginning of the V8, R2, and R5 stages, which occurred at 29, 36 and 59 DAS, for defoliation treatments at the V8, R2, and R5 stages, respectively.

Growth analyses

Soybean growth analyses included total aboveground biomass, leaf, stem, and pod biomass. These were sampled from a 0.5 m^2 area by plotting at the eighth trifoliolate (V8, 29 DAS), full flowering (R2, 36 DAS), the beginning of seed filling (R5, 59 DAS), fully formed seeds (R6, 84 DAS), and full maturity (R8, 97 DAS) stages (Fehr and Caviness, 1977). The partitioned biomass was dried in a forced ventilation oven at 65 °C until constant mass was achieved. Leaf area was estimated using a digital leaf area integrator, and the LAI was calculated as the ratio between the total leaf area per sample and the sampled area. Pods were partitioned into grains and pod shells at the R8 stage, and the node and stem numbers per plant, grain weight, number of grains, and final yield, considering a residual grain humidity of 13%, were evaluated.

Statistical analyses

The measurement data were subjected to exploratory analysis to verify the assumptions of normality of the residuals and homogeneity of variance. These were analyzed considering the combined effect of defoliation (four levels) and crop stage of defoliation (three levels) as fixed factors and blocks as random factors, using a mixed model for analysis of variance. The effect and interaction of treatments were analyzed based on orthogonal contrasts using $p < 0.05$ and mean test (Tukey's) at 5% probability. First and second-degree linear models were tested using linear regression analysis based on the residual sum of squares ($p < 0.05$) and the coefficient of determination (R2). Pearson's correlation ($p < 0.05$) was used to assess the relationship between the LAI at stages V8, R2, R5, and R6 and soybean biomass, grain weight, number, and yield.

Conclusion

The results obtained in this study revealed that yields were not affected by any level of defoliation of the eighth trifoliolate stage. However, a reduction in yield was observed for defoliation treatments at the full flowering and the beginning of seed filling stages, with maximum yields observed at 27 and 16% defoliation levels, respectively. Yield was significantly correlated with LAI after defoliation at the start of seed filling and the leaf area index at the fully formed seed stages. The maximum accepted defoliation levels (95% of maximum yield) were 46 and 35% of defoliation levels at full flowering and the beginning of seed filling stages, respectively, whereas at the eighth trifoliolate stage, the maximum evaluated defoliation level of 67% did not show yield losses. These limits were similar to the defoliation levels that resulted in LAI of 3.0 at the fully formed seed stage. Furthermore, these evaluations considered a production system with a short development cycle and conditions with relatively less adaptive capacity but with a low water deficit. Thus, the results of acceptable defoliation levels are conservative, and integrated pest control must be considered to maximize yield and reduce pesticide use.

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Authors contributions: CLM and LSS: conception and design; CLM, LSS and AGS: acquisition of data; LSS, RB and FPD: analysis and interpretation of data; LSS and RB: drafting the article; RB: critical review of important intellectual content; CLM, LSS, AGS, RB and FPD: final approval of the version to be published.

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Ethical Standards: Not Applicable.

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