

# Are economic thresholds for IPM decisions the same for low LAI soybean cultivars in Brazil?

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## Abstract

**BACKGROUND:** Economic thresholds (ETs) are well-established for defoliation of soybean, *Glycine max*, and have been updated for many of the newer cultivars; however, there is increasing grower adoption of cultivars with a reduced leaf area index (LAI). It is of theoretical and practical interest to determine low LAI cultivar tolerance to defoliation. We conducted experiments during two consecutive crop seasons (2017/2018 and 2018/2019) using three soybean cultivars (NS 5959 IPRO, NS 5445 IPRO, and DON MARIO 5.8i) and three defoliation levels (0%, 16.7%, and 33.3%) to evaluate the tolerance of reduced LAI soybean cultivars under different defoliation levels.

**RESULTS:** We observed differences among cultivar's LAI during plant development during both years. Soybean LAI was reduced with increasing defoliation intensity. Tested continuous defoliation levels from plant development stages of V2 to R6 reduced the weight of 1000 seeds and yield but did not impact oil or protein content.

**CONCLUSIONS:** Despite our findings that current ET for defoliators in soybean (30% defoliation during vegetative stage and 15% defoliation during reproductive stage) are valid, it is important to consider that continuous defoliation injury impacts the capacity of the plant to respond to injury and must be further evaluated for ET refinement in future research.

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**Keywords:** action threshold; defoliation; grow tissue removal; pest management

## 1 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the main oilseed crops produced and processed around the world. In the 2018/2019 crop season, approximately 122.57 million ha were sown, with a production of 358.77 Mt, accounting for a 4.78% increase over the previous worldwide crop season.<sup>1</sup> However, this production could be even higher if quality and quantity losses caused by pests were mitigated. Pests lower soybean production by an estimated 26–30% annually depending upon region. These losses can be reduced with implementation of integrated pest management (IPM).<sup>2–4</sup>

Many IPM programs are based on the concepts of economic injury level (EIL), defined as the lowest pest density that cause economic damage, and economic threshold (ET), the timing when the control should begin to prevent pest population density or injury from reaching the EIL.<sup>5</sup> Preventative applications of insecticides, in the absence of sufficient pest numbers, result in inconsistent economic returns, and can cause pest resistance and environmental damage.<sup>6,7</sup>

The ETs can be established for pest density or degree of injury and are influenced by many factors including pest species, cultivars, climate and other different agroecosystem properties.<sup>8</sup> As a result, the ETs for soybean defoliation can differ by country or even inside the same country. In Brazil, chemical control applications are recommended when defoliation percentage reaches 30% in the vegetative or 15% during the reproductive stages.<sup>9,10</sup> In contrast, in the United States, ETs for defoliators vary among

states. For example, in Georgia, ETs for defoliators are the same as those used in Brazil. Differently, in Ohio, treatment is triggered only when defoliation exceeds 40% prior to bloom, 15% from bloom to pod-fill, or 25% after pod-fill to plant yellowing.<sup>11</sup> In contrast, chemical control is recommended when 35% defoliation is attained during vegetative and 20% during the reproductive stages in Mississippi.<sup>12</sup> These differences could result from differences in light interception, photosynthetic efficiency or leaf area index (LAI) among cultivars and therefore, can produce different levels of tolerance to injury.<sup>13–15</sup>

Defoliation ET has been recently re-evaluated for newer cultivars belonging to early maturity groups with indeterminate growth habits.<sup>9</sup> Development and increasing adoption of cultivars with reduced LAI has increased the need for study of how low LAI cultivars respond to defoliation.<sup>16</sup> Therefore, this study aimed to evaluate LAI of new soybean cultivars to determine the impacts of defoliation.

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## 2 MATERIAL AND METHODS

Experiments were carried out under field conditions during two consecutive soybean seasons (2017/2018 and 2018/2019) at Embrapa, in the municipality of Londrina (S 23° 11' 11.7"; W 51° 10' 46.1") in the northern state of Paraná (PR), Brazil. The experiment was carried out in a 3 × 3 factorial randomized block design; three cultivars (NS 5959 IPRO, NS 5445 IPRO, and DON MARIO 5.8i) and three defoliation levels (0%, 16.7%, and 33.3%); with four replicates (six 5-m-long soybean rows). Cultivars' features were indeterminate growth habit with maturity group 5.9 (NS 5959 IPRO), 5.4 (NS 5445 IPRO), and 5.5 (DON MARIO 5.8i).

### 2.1 Experimental defoliation

Trials were planted on October 17, 2017 (2017/2018) and on October 22, 2018 (2018/2019) with 15 seeds per linear meter and 0.50 m row spacing. Artificial defoliation was carried out twice a week by manually removing the number of leaflets corresponding to each treatment with the aid of scissors, following the method of Gazzoni and Moscardi.<sup>17</sup> This procedure was performed on all leaves of the plant and on all plants in the plot from phenological stage V2 through R5/R6.<sup>18</sup>

To prevent interference from natural defoliators, insecticides and fungicides were applied on a 20-day interval on the plots, using a carbon dioxide (CO<sub>2</sub>) pressurized backpack sprayer (Herbicat®, Catanduva, São Paulo, Brazil) set for a spray volume of 150 L ha<sup>-1</sup>. Herbicides were applied during the third and sixth weeks after emergence of soybeans. All pesticides were applied equally over the total area of all treatments, including the control area without defoliation.

### 2.2 Assessments

Throughout soybean development, samples were collected from one linear meter and measured for foliar area, using a leaf area meter (Model 3000, LI-COR, Lincoln, NE, USA). The LAI is the ratio between leaf area and the corresponding land area and was calculated from collected material. At the R8 development stage, the two 2-m-long central rows of each plot were separately harvested and threshed for evaluation. The weight and moisture content of each sample was recorded (moisture meter G800, Gehaka Agri, São Paulo, SP, Brazil) and were then corrected to obtain the productivity for 13% seed moisture. In addition to yield, the weight of 1000 seeds was measured, and oil and protein content was quantified using an Antaris II FT-NIR infrared spectroscope (Thermo Fisher Scientific, Dublin, Ireland).

### 2.3 Statistical analysis

Results were submitted to exploratory analysis to verify the assumptions of normality of residuals, homogeneity of treatment variance, and additivity of the model to allow for analysis of variance (ANOVA). When data did not meet ANOVA assumptions, transformations were performed:  $\sqrt{x+1}$  (1000 seed mass of 2017/2018 season). When significant differences were detected, they were identified using the Tukey test at 5% probability.<sup>19</sup> The cultivar growth equation was made using the polynomial quadratic regression for the LAI development data ( $R^2$  more than 89%).

## 3 RESULTS

For all evaluated cultivars, the LAI increased over the season until R5.3 and decreased in the last evaluation (R5.5/R6) (Table 1). Defoliation impacted the LAI development for all cultivars during both

seasons (2017/2018 and 2018/2019). For both seasons, the quadratic equations closely described LAI with  $R^2$  between 0.89 and 0.94 (Fig. 1(A, B)) for control and defoliated treatments.

LAI differences were observed in both study seasons during crop development among the tested cultivars. In the first season (2017/2018), LAIs were similar among cultivars in the first three evaluations (V4/V5 and, R2/R3) but NS 5959 IPRO showed higher LAI in the last evaluation (R5.5/R6). In contrast, during the second season (2018/2019), NS 5959 IPRO had lower LAI and was similar to DON MARIO 5.8i in the first three evaluations (V2, V4/V5 and, R2/R3) and no differences among cultivars were recorded in the last two evaluations (R5.3 and R5.5/R6). NS 5445 IPRO had the highest LAI in the first three evaluations (V2, V4/V5 and, R2/R3). In the last two evaluations (R5.3 and R5.5/R6), NS 5959 IPRO always had the highest LAI, but only differed significantly for the first season (2017/2018).

Regarding the impact of defoliation over soybean LAI values, plots with 33.33% defoliation had significantly lower LAI than the undefoliated controls from V2 to R5.3 and from V4/V5 to R5.5/R6 in 2017/2018 and 2018/2019 crop seasons, respectively. Plots with 16.7% defoliation had intermediate LAI, being similar to 33.3% defoliation at V2, V4/V5, R5.3, and R5.5/R6 (2017/2018) and V2, V4/V5, R2/R3, and R5.3 (2018/2019) and similar to control (0% defoliation) at V4/V5, R2/R3, R5.3, and R5.5/R6 (2017/2018) and V2, R5.3, and R5.5/R6 (2018/2019) (Table 1).

Defoliation significantly reduced yield for both 16.7% and 33.3% defoliation levels during both crop seasons. However, there were no significant differences between treatments. Cultivars also influenced yield. During the first season (2017/2018), NS 5959 IPRO was the most productive (5051.5 ± 100.3 kg ha<sup>-1</sup>), NS 5445 IPRO was the least productive (4816.8 ± 93.0 kg ha<sup>-1</sup>) and cultivar DON MARIO 5.8i showed intermediate yield (4841.0 ± 42.2 kg ha<sup>-1</sup>), but were not significantly different. In 2018/2019 season, the cultivar NS 5959 IPRO showed the lowest yield (3104.4 ± 152.6 kg ha<sup>-1</sup>) and cultivars DON MARIO 5.8i and NS 5445 IPRO had similar higher yields (3561.9 ± 136.9 kg ha<sup>-1</sup> and 3740.7 ± 133.5 kg ha<sup>-1</sup>, respectively) (Table 2).

Neither defoliation nor cultivar impacted oil content, which was similar among cultivars and among defoliation levels in both seasons. Protein (%) was also similar among plants with different defoliation levels, but higher for DON MARIO 5.8i than the other studied soybean cultivars. NS 5445 IPRO had intermediate protein (%) but only different from NS 5959 IPRO at the second studied season (2018/2019) (Table 2).

In contrast to protein and oil content, the weight of 1000 grains was impacted by both cultivar and defoliation levels. Higher defoliation resulted in the lowest weight of 1000 seeds in the first season and both 33.3% and 16.7% defoliation reduced grain weight during the 2018/2019 trial. For seed weight, the cultivar's response varied. During 2017/2018, DON MARIO 5.8i had the lowest weight of 1000 grains while the lowest wither of 1000 grains was recorded for NS 5959 IPRO in the second season (Table 2).

## 4 DISCUSSION

Globally, soybean IPM is based on extensive data that shows soybean plants can tolerate some amount of leaf injury without economically relevant yield reductions.<sup>6,11</sup> Despite this tolerance to defoliation, the response of plants to injury can vary among cultivars, developmental stage of plants, and the timing of exposure to defoliation.<sup>14</sup> Newer soybean cultivars have lower LAI and it is

**Table 1.** Leaf area index (LAI) of three soybean cultivars under different levels of defoliation measured throughout different developmental stages

Parameter	Soybean developmental stage (Fehr <i>et al.</i> <sup>18</sup> )						
	V2	V4/V5	R2/R3	R5.3	R5.5/R6		
2017/2018	Cultivar	NS 5959 IPRO	0.17 ± 0.01 b	0.47 ± 0.02 <sup>ns</sup>	1.70 ± 0.12 <sup>ns</sup>	4.58 ± 0.22 a	3.70 ± 0.46 a
		NS 5445 IPRO	0.22 ± 0.01 a	0.58 ± 0.04	1.96 ± 0.23	3.82 ± 0.16 b	3.05 ± 0.47 b
		DON MARIO 5.8i	0.21 ± 0.02 ab	0.54 ± 0.06	1.84 ± 0.14	4.00 ± 0.21 ab	2.96 ± 0.53 b
	Defoliation (%)	0	0.23 ± 0.01 a	0.59 ± 0.04 a	2.19 ± 0.19 a	4.51 ± 0.24 a	3.45 ± 0.66 <sup>ns</sup>
		16.7	0.19 ± 0.01 b	0.52 ± 0.04 ab	1.88 ± 0.15 a	4.08 ± 0.15 ab	3.26 ± 0.60
		33.3	0.18 ± 0.01 b	0.47 ± 0.04 b	1.44 ± 0.10 b	3.79 ± 0.22 b	3.00 ± 0.41
	Statistics	$P_{\text{cultivar}}$	0.03	0.07	0.32	0.01	<0.01
		$P_{\text{defoliation}}$	0.01	0.04	<0.01	0.02	0.05
		$P_{\text{cultivar*defoliation}}$	0.25	0.15	0.34	0.66	0.72
		$F_{\text{cultivar}}$	4.29	3.01	1.18	5.75	10.95
		$F_{\text{defoliation}}$	5.92	3.56	9.87	4.82	3.30
		$F_{\text{cultivar*defoliation}}$	1.44	1.88	1.20	0.60	0.52
	2018/2019	Cultivar	NS 5959 IPRO	0.22 ± 0.01 b	0.84 ± 0.07 b	2.31 ± 0.20 b	2.84 ± 0.22 <sup>ns</sup>
NS 5445 IPRO			0.30 ± 0.02 a	1.04 ± 0.09 a	2.70 ± 0.19 a	2.64 ± 0.19	2.48 ± 0.13
DON MARIO 5.8i			0.20 ± 0.01 b	0.77 ± 0.06 b	2.21 ± 0.21 b	2.71 ± 0.22	2.65 ± 0.19
Defoliation (%)		0	0.26 ± 0.02 <sup>ns</sup>	1.09 ± 0.09 a	2.93 ± 0.21 a	3.17 ± 0.21 a	3.04 ± 0.16 a
		16.7	0.24 ± 0.02	0.84 ± 0.06 b	2.22 ± 0.15 b	2.71 ± 0.20 ab	2.77 ± 0.18 a
		33.3	0.23 ± 0.02	0.72 ± 0.04 b	2.08 ± 0.16 b	2.31 ± 0.13 b	2.22 ± 0.15 b
Statistics		$P_{\text{cultivar}}$	<0.01	<0.01	<0.01	0.72	0.05
		$P_{\text{defoliation}}$	0.28	<0.01	<0.01	0.01	<0.01
		$P_{\text{cultivar*defoliation}}$	0.81	0.26	0.17	0.43	0.14
		$F_{\text{cultivar}}$	14.14	6.71	6.82	0.34	3.39
		$F_{\text{defoliation}}$	1.34	12.35	21.49	6.04	13.14
		$F_{\text{cultivar*defoliation}}$	0.40	1.42	1.77	0.99	1.93

Londrina, Paraná, Brazil (S 23° 11' 11.7"; W 51° 10' 46.1"). Crop seasons 2017/2018 and 2018/2019. Means (± standard error) followed by the same letter in the column for each parameter and crop season do not differ statistically from each other by the Tukey test ( $P > 0.05$ ). ns, ANOVA non-significant.

tempting to assume that the newer cultivars will be more sensitive to defoliation.<sup>11</sup>

The results generated in this study show that despite having shorter maturity periods and lower LAIs than other cultivars cropped in Brazil, the response to defoliation was similar to that of cultivars with higher LAI.<sup>11,20,21</sup> Overall, the results for both crop seasons indicate that continuous soybean defoliation of 16.7% and 33.3% significantly reduced yield, as a result of reduced LAI, and that these results are most pronounced during the reproductive developmental stage.<sup>16,22</sup>

It is important to note that LAI varies during soybean development and higher or lower LAI soybean cultivars may still behave similarly. For example, a cultivar with higher LAI can be more vulnerable to leaf self-shading, which can trigger earlier leaf senescence relative to lower LAI cultivars. As a consequence, when the plant is at the R5 growth stage, when LAI is most important, newer low LAI cultivars may actually have higher LAI compared to older cultivars because of their ability to retain leaves for longer periods.<sup>11,13</sup> Therefore, in addition to LAI, light interception should also be taken into consideration.<sup>23</sup>

Soybean sensitivity to defoliation usually peaks at the early R5 growth stage and decreases linearly down to less than 10% of

cultivars) but also for production and development of reproductive structures, including flowers, pods and seeds. Usually, defoliation during the vegetative and early reproductive stages has less impact on yield, because of leaf regrowth and delayed leaf senescence of remaining tissues that compensate for losses.<sup>25–29</sup> Importantly, even during the reproductive period, yield sensitivity to defoliation declines as the seed filling period progresses from stages R5 to R7.<sup>24</sup>

Previous published results indicate an excellent recovery capacity of some soybean cultivars.<sup>30</sup> Soybean has been documented to recover after injury of 50%, 67% and even 75% defoliation with no yield loss, showing that soybean plants are usually tolerant to defoliation.<sup>23,26,30</sup> Batistela *et al.* evaluated defoliator thresholds for IPM decisions in short-season soybeans using artificial defoliation and concluded that recommended ETs were still valid.<sup>9,12,31</sup> However, Batistela and coworkers did not measure LAI or consider the timing and duration of injury exposure which are crucial to determine possible yield loss.<sup>32,33</sup> For plant response to defoliation, when defoliation occurs, how many days the plant has to regenerate leaf area are important considerations for predicting yield.<sup>34–36</sup>

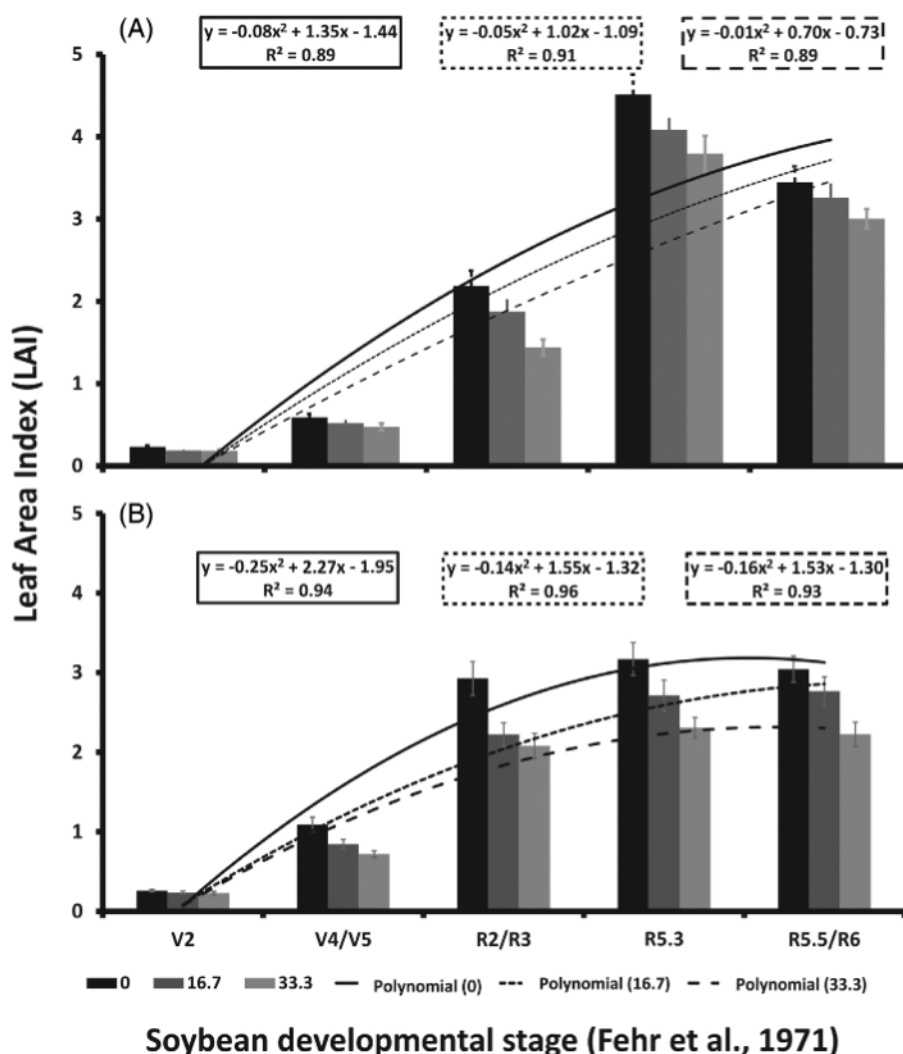
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Soybean sensitivity to defoliation usually peaks at the early R5 growth stage and decreases linearly down to less than 10% of the relative yield loss at the late R6 growth stage.<sup>24</sup> Thus, defoliation during the plant reproductive stage has been considered the most critical because photoassimilates produced in this period are intended not only for vegetative growth (in indeterminate

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In contrast to previous published work, which studied defoliation conducted on a single day or over periods during vegetative or reproductive stages or used cultivars with higher LAI, our study evaluated defoliation levels of 16.7% and 33.3% imposed twice per week from V2 up to R6.<sup>17,37,38</sup> This method imposes standardized defoliation over time.<sup>17,30,38,39</sup> We found that simulating



**Figure 1.** Observed leaf area index (LAI), and polynomial regression of three soybean cultivars under different levels of defoliation measured different developmental stages. Londrina, Paraná, Brazil (S 23° 11' 11.7"; W 51° 10' 46.1"). (A) Crop season 2017/2018. (B) Crop season 2018/2019.

continuous defoliation from the beginning of crop development (V2) to the end of reproduction (R6), resulted in LAI recovery of defoliated plants, but reduced yield by about 14.01%. Both defoliation treatments studied here (16.7% and 33.3%) were higher than recommend ET of 30% defoliation during soybean vegetative stage and 15% defoliation during soybean reproductive stage.<sup>11</sup> Certainly, the capacity to tolerate 15% defoliation during the soybean reproductive stage is impacted by the occurrence of continuous defoliation on those plants over longer periods.

There are two methodological points that deserve highlighting. (i) In some previous studies, plant defoliation was performed on a single date and then plant recovery was observed,<sup>17,30,38</sup> while our study kept the injuries constant without allowing plants to recover from defoliation.<sup>39</sup> (ii) In the present study, defoliation was performed homogeneously over the entire plant, although defoliating insects have different feeding preferences regarding the plant parts.<sup>40</sup>

The difference observed in plant tolerance capacity in our work from the results in the literature may be attributed to the continuous defoliation imposed to the plants during a longer

period. Defoliation studied here were imposed twice a week from plant V2 to R6 development stages, which was much more intense (twice a week) and for a longer period than previously reported studies. It is important to mention that this period of defoliation is longer than soybean feeding Lepidoptera would take to complete larval stages (*ca* 19 days).<sup>41</sup> Despite the limitations of this study approach, better understanding of soybean tolerance to longer periods of injury is important not only for areas where continuous Lepidoptera pressure occurs with overlapping generations, but also where multiple defoliating pest species occurs in sequence. Other factors may also contribute to our results including: study location, plant population, climatic differences, different soil and plant fertility, sowing dates and especially, differences in the characteristics of the cultivars studied.<sup>21,42,43</sup>

In addition to LAI and overall yield, defoliation also reduced weight of 1000 seeds. It is likely that reduction in seed weight contributed to the observed reduction in yield.<sup>44</sup> It is noteworthy that the 1000 seed weight of cultivars differed between the two studied years. In the first year, NS 5959 IPRO had the highest yield, but in the second year its yield was lowest, which may indicate a

**Table 2.** Yield (kg ha<sup>-1</sup>), oil and protein content (%) and weight of 1000 grains of three soybean cultivars under different levels of defoliation

Parameter			Yield (kg ha <sup>-1</sup> )	Oil (%)	Protein (%)	Weight of 1000 seeds
2017/2018	Cultivar	NS 5959 IPRO	5051.5 ± 100.2 a	22.7 ± 0.2 <sup>ns</sup>	37.0 ± 0.2 b	185.4 ± 2.5 a
		NS 5445 IPRO	4816.8 ± 93.0 b	22.3 ± 0.2	38.3 ± 0.3 b	190.4 ± 2.9 a
		DON MARIO 5.8i	4841.0 ± 42.2 ab	22.4 ± 0.3	39.1 ± 0.3 a	176.1 ± 1.7 b
	Defoliation (%)	0	5131.3 ± 89.6 a	22.7 ± 0.3 <sup>ns</sup>	38.1 ± 0.4 <sup>ns</sup>	187.1 ± 3.0 a
		16.7	4804.1 ± 66.2 b	22.3 ± 0.3	38.2 ± 0.4	186.3 ± 2.3 a
		33.3	4774.0 ± 63.1 b	22.3 ± 0.2	38.1 ± 0.3	178.5 ± 2.8 b
		Statistics				
		<i>P</i> <sub>cultivar</sub>	0.02	0.43	<0.01	<0.01
		<i>P</i> <sub>defoliation</sub>	<0.01	0.35	0.86	0.02
		<i>P</i> <sub>cultivar*defoliation</sub>	0.08	0.44	0.27	0.35
		<i>F</i> <sub>cultivar</sub>	4.34	0.88	18.50	11.37
		<i>F</i> <sub>defoliation</sub>	10.23	1.09	0.16	4.89
		<i>F</i> <sub>cultivar*defoliation</sub>	2.36	0.98	1.39	1.18
2018/2019	Cultivar	NS 5959 IPRO	3104.4 ± 152.6 b	21.8 ± 0.2 <sup>ns</sup>	36.6 ± 0.2 c	151.4 ± 4.7 b <sup>a</sup>
		NS 5445 IPRO	3740.7 ± 133.5 a	22.2 ± 0.2	37.9 ± 0.4 b	180.0 ± 2.2 a
		DON MARIO 5.8i	3561.9 ± 136.9 a	21.5 ± 0.3	39.4 ± 0.3 a	163.9 ± 3.3 a
	Defoliation (%)	0	3929.3 ± 108.3 a	21.9 ± 0.2 <sup>ns</sup>	38.2 ± 0.5 <sup>ns</sup>	174.3 ± 3.6 a
		16.66	3376.7 ± 154.3 b	21.8 ± 0.3	38.0 ± 0.5	160.8 ± 5.7 b
		33.33	3101.0 ± 107.5 b	21.8 ± 0.2	37.6 ± 0.4	160.3 ± 4.4 b
		Statistics				
		<i>P</i> <sub>cultivar</sub>	0.00039	0.14	<0.01	<0.01
		<i>P</i> <sub>defoliation</sub>	<0.01	0.93	0.32	<0.01
		<i>P</i> <sub>cultivar*defoliation</sub>	0.561	0.55	0.52	0.10
		<i>F</i> <sub>cultivar</sub>	11.091	2.12	25.83	11.06
		<i>F</i> <sub>defoliation</sub>	18.325	0.08	1.18	17.66
		<i>F</i> <sub>cultivar*defoliation</sub>	0.761	0.78	0.83	0.83

Londrina, Paraná, Brazil (S 23° 11' 11.7"; W 51° 10' 46.1"). Crop season 2017/2018 and 2018/2019. Means (± standard error) followed by the same letter in the column for each parameter and crop season do not differ statistically from each other by the Tukey test ( $P > 0.05$ ). ns, ANOVA non-significant.

<sup>a</sup> Original means followed by statistics performed on the data transformed into  $\sqrt{x+1}$ .

strong influence of environmental/climatic conditions between years.

Both yield quantity and yield quality are essential for soybean production in order to maximize the product delivery value.<sup>44</sup> Taking this into consideration, it is important to note that tested defoliation levels did not impact oil or protein content despite records in the literature of both reduction and increase in oil and protein content based on LAI.<sup>35,45</sup> Moreover, it is noteworthy that protein content obtained in this experiment was higher than the US national average (34.1% crop 2017) and, with the exception of the cultivar NS 5959 IPRO in 2018/2019 season, it was also higher than Brazilian national average.<sup>46,47</sup>

## 5 CONCLUSION

Our results indicate that continuous defoliation injury for long periods impact soybean yield and must be further considered when developing ETs. Not only is it important for environments where Lepidoptera pressure continuously occurs during the crop season but also for fields where multiple leaf tissue feeding pest species occurs in sequence. However, lowering ETs to account for continuous defoliation injury would certainly

increase pest resistance to the pesticides.<sup>48,49</sup> Although not evaluated in this study, these possible side effects must be taken into consideration for long-term scenario evaluation. Therefore, despite our findings that the current ET for defoliators in soybean (30% defoliation during vegetative stage and 15% defoliation during reproductive stage) are valid, the need for refinement for continuous defoliation injury and low LAI cultivars should be further studied. In addition, it is important to consider that previous work using total dry weight, which has been recorded to be higher in some newer soybean cultivars, can be more closely related to soybean yield than LAI.<sup>13</sup> Dry weight was not evaluated in our work and should be further investigated in future research.

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## AUTHOR CONTRIBUTIONS

Conceptualization: RH, AFB. Bioassays development: RH, AFB. Data analysis: RH, AFB, CVG. Writing and editing: RH, AFB, WWH,

CVG. Reference analysis: RH, AFB, CVG, WWH. Final draft correction: AFB, WWH.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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