Continuous Defoliation Stress in Vegetative and Reproductive Stages of Soybean Genotypes

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A B S T R A C T
Soybean yield depends on photosynthesis generated by leaves, so any factor that interferes with their leaf area could affect the yield. Among these, defoliating insect attacks cause significant decreases in yield by its direct reduction of the leaf area, thereby reducing the total photosynthesis of the plant. The agronomic traits of cultivars can influence the tolerance level of the plant to this kind of stress, if it is continuous. Thus, the aim of this work was to evaluate the effect of continuous defoliation in vegetative and reproductive stages in soybean cultivars with different traits. The research was carried out in an experimental randomized complete block design in a 2×5×6 factorial scheme with four replications. Factors consisted of defoliation stages (vegetative and reproductive), defoliation levels (0, 16.7, 33.3, 66.6 and 100%) and cultivars (M 7211 RR, TMG 123 RR, TMG 1176 RR, M 7908 RR, TMG 127 RR, TMG 7188 RR). The following variables were evaluated: number of pods/plant, number of seed/plant, 100-seed weight and seed yield/plant. From the obtained results, it was observed that defoliation has a negative effect on all cultivars yield components with greater decrease when it occurs in the reproductive stage. Continuous defoliation from 16% in both the vegetative and reproductive stage significantly decreases the soybean yield. Regardless of agronomic characteristics such as growth type, maturity group and leaf shape, the effect of stress by defoliation in soybean cultivars is similar.

Key words: Glycine max, yield components, leaf area

INTRODUCTION
The soybean crop is the largest seed production in Brazil and in the season (2012/2013) reached 81.4 million tons in an area of 27.7 million ha. The average yield was 2938 kg ha⁻¹ (CONAB., 2014). The soybean yield potential is high, but stress that occurs during the crop cycle can decrease productivity, which is the result of photosynthesis generated by the leaves under environmental conditions. Thus, any factor that affects the leaf area could affect the production. Among these are the defoliating insects that cause accentuated decrease in seed yield by its direct action in reducing leaf area, thereby reducing total plant photosynthesis (Diogo et al., 1997).

Research has found that less than 50% defoliation before flowering usually does not reduce seed yield, while this amount of defoliation during the reproductive stage has caused greater reductions in soybean yield (Bueno et al., 2010; Salvadori and Corseuil, 1979; Pickle and Caviness, 1984; Diogo, 1997; Peterson et al., 1998; Campelo and Sediyama, 1999; Fontoura et al., 2006). However, the ability of the soybean crop to avoid reduced productivity after being...
subjected to defoliation depends on some factors: the defoliation intensity, the growth stage at which it occurs, the ability of the cultivar to tolerate or compensate the defoliation (Costa et al., 2003; Parcianello et al., 2004) and also environmental factors, including solar radiation (Andrade et al., 2002).

According to the recommendation of EMBRAPA (2010), the control of defoliating insects must be done when the soybean plant reach a maximum of 30% defoliation in the vegetative stage and 15% in the reproductive stage to prevent yield reduction. In the reproductive stage, the plant is more sensitive to defoliation since assimilates are directed to formation of flowers, pods and seeds.

Barros et al. (2002) observed that the greatest reduction in yield occurred when the plants had their fully formed pods (R4 stage) and when the leaves were fully removed. Diogo (1997) found reduction of approximately 80% when there was total plant defoliation at the stage of pod filling. In the vegetative stage, Peluzio et al. (2004) observed that the maximum level of defoliation delayed the soybean flowering, plant height, number of pods per plant, 100-seed weight and seed yield. The yield reduction will also depend on other factors, such as the cultivar itself and duration of injury; because cultivars with larger cycles have more time for formation of leaves and can tolerate greater loss of leaf area.

The works done so far to quantify defoliation loss have studied a specific defoliation. In other words, the artificial defoliation was performed in certain stages of crop development. Thus, conclusions are based from the leaf area loss in a single developmental stage (Bahry et al., 2013; Fontoura et al., 2006; Ribeiro and Costa, 2000). However, maintaining the same defoliation level for a long period of crop development, we could obtained conflicting results.

Another factor is that the results of research regarding the effect of defoliation have used cultivars only with ovate leaf types, because these leaf kinds were always preferential in breeding programs. However, in the last years, cultivars with lanceolate leaves have become more popular. There are no studies of defoliation tolerance for these genotypes, especially in the reproductive period in which the soybean crop is much more sensitive to this stress. Furthermore, soybeans belong to different maturity groups, which may result in varying tolerance degrees according to the cycle.

Given these observations, the objective of this study was to evaluate the effect of continuous defoliation levels in vegetative and reproductive stage in soybean cultivars with different characteristics.

### MATERIALS AND METHODS

The experiment was conducted at the Federal University of Viçosa, Minas Gerais State, Brazil in a greenhouse in the Agronomy Department (20°45'29" S and 42°52'11" W). The soil had the following chemical characteristics:

| Soil characteristic | Value |
|---------------------|-------|
| pH (H2O)            | 4.9   |
| P (Mehlich 1)       | 1.8 mg dm⁻³ |
| K                   | 14 mg dm⁻³ |
| Ca                  | 0.6 cmol dm⁻³ |
| Mg                  | 0.2 cmol dm⁻³ |
| A1                  | 1.2 cmol dm⁻³ |
| H⁺Al                | 9.40 cmol dm⁻³ |
| Matéria Orgânica    | 3.2 g dm⁻³ |
| CTC (pH7.0)         | 10.24 cmol dm⁻³ |
| Saturation (V%)     | 8     |

The soil had clay texture. According to these results we applied 2 g of calcitic lime kg⁻¹ of soil and as fertilizer we used 300 and 150 mg of P and K kg⁻¹ of soil, respectively. The average temperature in the greenhouse during the conducting of the experiment was of 30°C and 20°C during the day and night, respectively.

The experimental design was a completely randomized block design. The treatments resulted from the combination of three factors (2×5×6) with four replications. The factors consisted of defoliation stages (vegetative and reproductive), defoliation levels (0, 16.7, 33.3, 66.6 and 100%) and cultivars (M 7211 RR, TMG 123 RR, TMG 1176 RR, M 7908 RR, TMG 127 RR, TMG 7188 RR). Each plot consisted of a pot of 2.5 L of soil with two plants. The cultivars used in this study have the following important agronomic characteristics (Table 1).

| Cultivar   | Growth type | Maturity group | Shape leaflet |
|------------|-------------|----------------|---------------|
| M 7211 RR | Indeterminated | 7.2           | Pointed oval  |
| TMG 123 RR| Determinated  | 7.4            | Lanceolate    |
| TMG 1176 RR| Determinated  | 7.6            | Lanceolate    |
| TMG 127 RR| Indeterminated | 7.2           | Oval          |
| M 7908 RR | Determinated  | 7.9            | Oval          |
| TMG 7188 RR| Determinated  | 8.8            | Oval          |

Table 1: Agronomic characteristics of soybean cultivars

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The sowing was carried out on December, 6th 2011. Five seeds/pot were planted and at V1 stage, thinning was done by keeping the two most vigorous plants. From the V2 stage (fully developed trifoliate leaf at node above the node of unifoliate leaves), the plants were submitted to defoliation levels until flowering (R1 stage). For analysis of defoliation in the reproductive stage, new plants were submitted to stress from the opening of the first flower bud (R1), keeping each defoliation level until the last leaf in each plant was opened.

The variables number of pods/plant, number of seeds/plant, 100-seeds weight and seeds yield/plant were evaluated. The data was subjected to analysis of variance, comparisons of means by Tukey test (5% probability) and regression analysis by the Genes program (Cruz, 2013). In regression analysis, the procedure of polynomial joint regression and identity test model procedure were used, considering 5% probability to choose the best method and to reject the hypothesis of similarity between the statistical models. Besides the significance of the regression by F test, the coefficient of determination (R2) was considered.

### RESULTS

There were significant factors studied for all production variables (Table 2). The interaction between defoliation stages and defoliation levels was significant at 1% probability for all

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Table 2: Summary of variance analysis to yield components: number of pods/plant, number of seeds/plant, 100-seeds weight and seeds yield/plant

| Source of variation | GL Platform | Pod/plant | Seed/plant | 100-Seed | Seed yield |
|---------------------|-------------|-----------|------------|-----------|------------|
| Block               | Medium square | 473.684  | 2491.579   | 14.7824   | 38.2211    |
| Stage (S)           | 1           | 193.501*  | 1542.801** | 220.3592**| 107.1739** |
| Defoliation (D)     | 4           | 10334.01**| 64448.01** | 206.1235**| 2409.634** |
| Cultivar (C)        | 5           | 1543.994**| 6918.274** | 249.9702**| 43.248**   |
| SxD                 | 4           | 603.7068**| 2763.71**  | 107.4973**| 85.8306**  |
| SxC                 | 5           | 32.806ns  | 238.8885ns | 8.3572**  | 11.5049*   |
| DxC                 | 20          | 103.8626**| 488.5532** | 5.8162**  | 5.656ns    |
| Residue             | 177         | 39.3051   | 193.0414   | 2.3315    | 4.2193     |
| Media               | 37.7        | 88.11     | 17.90      | 15.90     | 12.91      |
| CV(%)               |             |           | 16.59      | 6.69      | 5.64       | 12.91      |

*Significant at 5% probability by F test, **Significant at 1% probability by F test, ns: Not significant

Table 3: Number of pods/plant of soybeans cultivars subjected to defoliation levels in vegetative and reproductive stage

| Defoliation levels (%) | Stage | 0   | 16.7 | 33.3 | 66.7 | 100  |
|------------------------|-------|-----|------|------|------|------|
| Vegetative             |       | 48.72a | 43.04a | 44.58a | 37.66a | 19.31a |
| Reproductive           |       | 48.72a | 46.14a | 43.85a | 40.34a | 5.18a |

Table 4: Number of pods/plant of soybeans in relation on the level of defoliation and cultivar

| Defoliation levels (%) | Cultivar | 0   | 16.7 | 33.3 | 66.7 | 100  |
|------------------------|----------|-----|------|------|------|------|
| M 7211 RR              | 57.6e    | 48.2e | 46.2e | 40.1e | 10.8e |
| TMG 123 RR             | 44.8e    | 38.5e | 38.3e | 32.8e | 8.8e  |
| TMG 1176 RR            | 47.0e    | 45.3e | 46.9e | 42.1e | 14.4e |
| M 7908 RR              | 36.6e    | 39.7e | 38.9e | 35.8e | 11.1e |
| TMG 127 RR             | 43.5e    | 39.1e | 38.4e | 33.0e | 13.1e |
| TMG 7188 RR            | 62.7e    | 56.5e | 56.5e | 50.3e | 15.1e |

Means followed by an uppercase letter in the column do not differ at 5% probability by Tukey test

Table 5: Number of soybean seeds/plant subjected to defoliation levels and cultivar

| Defoliation levels (%) | Cultivar | 0   | 16.7 | 33.3 | 66.7 | 100  |
|------------------------|----------|-----|------|------|------|------|
| M 7211 RR              | 115.0e   | 94.1e | 94.0e | 80.5e | 20.4e |
| TMG123 RR              | 124.1e   | 110.2e | 107.8e | 93.3e | 20.0e |
| TMG1176 RR             | 136.1e   | 122.6e | 128.5e | 111.8e | 30.9e |
| M 7908 RR              | 93.3e    | 89.8e | 89.8e | 88.8e | 74.8e | 26.1e |
| TMG127 RR              | 136.1e   | 119.0e | 119.0e | 103.6e | 27.0e |

Means followed by an uppercase letter in the column do not differ at 5% probability by Tukey test

characteristics, demonstrating that the effect of defoliation is dependent upon the stage at which it occurs. The variable 100-seed weight and seed yield/plant were also affected between the interaction of the cultivar and the defoliation stage. The interaction between defoliation levels and cultivars was not significant for yield/plant.

The number of pods/plant (Table 3) revealed that, for the defoliation level within the stage, there was a significant difference only with 100% defoliation. Regarding the number of pods/plant depending on the levels of defoliation (Fig. 1a) and regardless of cultivars, it was observed that there was a linear decline in this yield component when defoliation was performed both in vegetative as well as in the reproductive stage. However, in the reproductive stage, the number of pods had greater decrease according to the increased level of continuous defoliation. Among the soybean yield components, the number of pods varied the most with environmental stress. Regarding cultivars depending on defoliation, all had similar behavior, adjusting to a common equation (Fig. 2a). The number of seeds/plant in relation to the defoliation stage and defoliation levels had a similar behavior to the number of pods in the same conditions, smaller only when there was 100% defoliation at the reproductive stage (Table 3).

Looking into interactions between cultivars and defoliation levels (Table 4), there was no difference among cultivars with 100% defoliation, i.e., there is a high decrease in the number of pods independent of the cultivars characteristics. However, for other levels of defoliation, the TMG 7188 RR cultivar was the one that maintained the highest number of pods.

For the cultivars within each defoliation levels (Table 5), the TMG 1176 RR cultivar had the highest seed average/plant regardless of defoliation level. This is due to this cultivar producing a considerable amount of four-seed pods, a characteristic genetically linked to lanceolate leaf type. The cultivar M 7908 RR produced less seeds per plant, but it has a higher seed weight than other cultivars. Similarly, for the number of pods/plant, there was no significant difference among the cultivars in the number of seeds/plant at 100% defoliation.
In relation, the behavior according to the defoliation level shows that the insensitivity to the defoliation level at 66% is due to the linear decrease for the both defoliation stages (Fig. 1b). By observing the number of seeds/plant of cultivars in relation to the defoliation levels (Fig. 2b), there was a similar result in the number pods/plant, adjusting to a common equation by identity model test with linear decrease according to increasing defoliation.

All cultivars showed a decrease in 100-seeds weight when there was defoliation in the reproductive stage (Table 6), except TMG 7188 RR. In the comparison of cultivars within each level (Table 7), the M 7908 RR cultivar maintained seed weight at all defoliation levels, while TMG 123 RR and TMG 1176 RR cultivars had the lowest averages. This result is exactly contrary to the number of seeds/plant, demonstrating a compensatory effect on the cultivars, i.e., those that produce...
For the 100-seed weight depending on the defoliation levels (Fig. 2c) a decrease was observed in all cultivars. By an identity model test, the TMG 127 RR and TMG 7188 RR cultivars (5 and 6) showed similar effects due to higher seed weight, while M 7211 RR cultivar (1) showed intermediate seed weight with decrease less pronounced than the others. The TMG 123 RR, TMG 1176 RR and TMG 7188 RR (2, 3 and 6) cultivars were grouped in a common equation, being cultivars that produce smaller seeds. Most of the lanceolate leaf cultivars have this characteristic in common.

Regarding seed yield/plant to defoliation levels within the stages (Table 3), there was only a significant difference with 100% defoliation. However, in the observing the defoliation stages depending on the levels, there was a decline in yield in both the vegetative stage and the reproductive. The defoliation caused the largest decrease in the reproductive stage (Fig. 1d). This result disagrees with most parts of the work, showing that more seeds produce smaller seeds than those that produce fewer seeds/plant. In the Fig. 1c, this behavior can be observed: With defoliation in the vegetative stage, 100-seed weight remained constant, while in the reproductive stage there is a linear decrease.
from 16.7%, there has been a decrease in seed yield/plant in the vegetative stage. Regarding cultivars with defoliation in the reproductive stage, it is observed in Table 6 that the TMG 1176 RR maintained high yield. On the other hand, with defoliation at the reproductive stage, it was observed that the TMG 7188 RR cultivar had higher yield.

**DISCUSSION**

The decline in the number of pods was because of the loss of 100% of leaf area in the reproductive stage. In the reproductive stage, the plant has no recovery ability, resulting in abortion of pods; whereas with loss of leaf area until flowering (R1), the plant also makes some trefoil, thus enabling maintenance of a number of pods. Barros et al. (2002) found that in all reproductive stages, the greatest reductions in the number of pods occurred with 100% defoliation agreeing with Diogo (1997) results.

According to Shibles et al. (1975), the defoliation changes the source-sink relationship, thus, the plant pass through a rearrangement of physiological functions. Pissaia et al. (1982) mentions that defoliation levels caused competition between reproductive and vegetative parts in the plant. The formation of new leaves occurs at the expense of carbohydrates that could be employed in forming pods. As soon as the defoliation levels increase this competition becomes more pronounced, causing greater reductions in seed yield.

Fontoura et al. (2006) observed that the component with the highest reduction due to defoliation was the number of pods/plant, especially when there was 100% defoliation at the beginning of seed filling. According to Sediyama et al. (1985), due to the increased size of the drains in flowering period and during pod formation, photosynthetic activity peaks indicating a greater need for the plant to produce assimilates during these periods. Thus, if defoliation is increased, it results in reduced pod number due to decrease in photosynthetic activity of the plant.

The most quantity of pods in TMG 7866 RR cultivar is because it has a higher maturity group than others, resulting in a longer cycle; thus, there is a greater vegetative and reproductive period, providing a higher capacity of defoliation recovery and maintenance of pods. It is worth noting that the largest number of pods does not guarantee a higher yield, since this depends on other yield components, such as seeds/plant and 100-seed weight. This component has a high correlation with the number of pods and a direct effect on yield, but the decrease depends on the number of seeds per pod as well.

The defoliation caused a negative effect on the number of pods/plant that directly reflects in the number of seeds/plant. This yield component has a greater direct effect on yield (Silva et al., 2013), thus the crop management in relation to insect defoliators must minimize the abortion of pods on the plant. Other yield components (such as number of seeds/pod and 100-seeds weight) after a certain defoliation level cannot compensate for this loss, because they are genetically determined with little influence of the environment.

Regarding the 100-seed weight, for the defoliation levels within the stages (Table 3), there was only a decrease from 66.7% defoliation at reproductive stage. At this stage, the plant is translocating assimilates for pod formation and subsequent seed filling. Very high defoliation can decrease this yield component.

The TMG 7188 RR has a large cycle, thus the plant has a longer period to recover from this stress. The plant can maintain seed weight, which is a genetic trait with little environmental variation affected only by high stress during seed filling. With continued defoliation, the plant will decrease other components, such as the number of pods and maintaining the seed weight.

Costa et al. (2003) studied the defoliation at the reproductive stage and observed that the removal of 100% of the leaves caused significant difference in R3, R4, R5 and R6 stages, compared to the R2 stage with decreases in seed yield of approximately 52, 75, 54 and 23%, respectively. Fontoura et al. (2006) found that the most critical stage for the loss of leaf area is R5, reducing the yield with the removal intensification of plant leaf area. Peluzio et al. (2004) found that defoliation of 33% at all growth stages and 66% in the early growth stages (V2-V5) did not significantly affect seed production.

The cultivars that had no significant decrease in defoliation at the reproductive stage were M 7908 RR and TMG 7188 RR, which have larger cycles, allowing greater recovery time. Bahry et al. (2013) observed that defoliation at the vegetative stage of CD 219 RR cultivar did not reduce soybean yield, considering a level of up to 66.7% of vegetative nodes without leaves in the V9 stage.

The variance analysis showed no significant interaction between cultivars and defoliation levels, which allow us to infer that the cultivars have similar decrease in yield.

**CONCLUSION**

Defoliation has a negative effect on all cultivars yield components with greater decrease when it occurs in the reproductive stage. Continuous defoliation from 16% in both the vegetative and reproductive stage significantly decreases the soybean yield. Regardless of agronomic characteristics such as growth type, maturity group and leaf shape, the effect of stress by defoliation in soybean cultivars is similar.

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