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Post-flowering leaflet removals increase pod initiation in soybean canopies

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ABSTRACT

Much of the yield variation in soybean (*Glycine max* L. Merrill) crops is related to changes in pod and seed number. Pod number is the result of pod initiation and pod abscission while seed number is the result of potential seed per pod and seed abortion. However, the physiological regulation of these processes is not well understood. A field experiment was conducted to investigate the role of post-flowering changes in source size and canopy structure on pod initiation, pod abscission and seed abortion in soybean. Two soybean genotypes: DM48 and A7409 (maturity groups IV and VII, respectively) were used. Leaflet removal treatments (L) consisted of removing none (L0), one (L1) or two (L2) lateral leaflets of every developed trifoliate leaf present. Leaflet removals were applied twice: the first at full bloom and the second shortly after the beginning seed stage. Crop growth rate (CGR), leaf area index (LAI), light interception (LI), and relative leaf growth rate, were determined during the periods in which numerical components are established. For the period between the first and the second leaflet removal, CGR remained unchanged among L treatments in both genotypes because LAI reductions were compensated through an increase in the net assimilation rate of the remaining leaves. The first leaflet removal increased the relative leaf growth rate and the number of pods initiated (PI) and these increases were inversely related to the remaining LAI in both genotypes. Moreover, the inverse relationship between LAI and PI was sustained at LAI below and above critical (i.e., LAI for 95% LI) and was not related to CGR or LI. The number of pod abscised also increased with the level of leaflet removal during the first and main abscission period in both genotypes and the percentage of pod abscission was directly related to the seed growth rate per unit leaf area during the abscission period. Seed abortion was inversely related to LAI after the second leaflet removal. Only the highest level of leaflet removals (i.e., L2) was able to reduce seed size in both genotypes. Whereas pod abscission, seed abortion and seed size could be related to indicators of canopy assimilatory capability pod initiation was not, suggesting that other physiological mechanism/s operate in the regulation of pod initiation. In addition, our results suggest that early (i.e., at flowering) canopy closure may negatively impact pod initiation in soybean. To the best of our knowledge, this study is the first to document that the number of initiated pods is inversely related to LAI in soybean canopies.

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1. Introduction

The primary components of soybean yield are seed number (seed/m^2) and seed size (mg/seed). Improvements in agronomic practices or genetic gains could increase future yields by increasing seed number, seed size, or both components. Pod number has been shown to be highly associated with seed number because the actual number of seeds per pod shows low environmental variation (Egli, 1998). Assimilate availability has been considered to be the main factor that regulates pod and seed number changes (Board and Tan, 1995; Jiang and Egli, 1993). Variations in leaf area index (LAI), light interception (LI), or crop growth rate (CGR), which is an index usually used as an estimator of canopy photosynthesis, have been associated with differences in pod and seed number (Herbert and Litchfield, 1984; Ramseur et al., 1985; Board and Harville, 1994; Board and Tan, 1995). Linear relationships between LI or CGR measured from full flowering to the beginning of seed growth, and pod or seed number at maturity were then considered as evidence that pod and seed number are source-limited (Egli and Zhen-wen, 1991; Board et al., 1995; Board and Harville, 1998). These models explain well the relationship between pod or seed number and CGR when CGR is abruptly reduced through defoliation or shading, but the association becomes weak when additional genetic or environmental factors are involved (Egli and Bruening, 2000). On the other hand, there are situations where CGR increases significantly while seed number remains unchanged, which indicate that higher CGR does not necessarily mean higher

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partition of assimilates to reproductive structures (Quijano et al., 1998).

Pod number is determined by pod initiation and pod abscission, and seed number is determined by the potential number of seeds per pod and seed abortion. These components are sequentially established and partially overlap during development. They may also have different physiological and/or environmental requirements for reaching their maximum potentials. In addition to assimilates, other environmental and/or internal signals may affect soybean pod initiation and abscission (Heindl and Brun, 1983; Myers et al., 1987; Kokubun and Honda, 2000) as well as seed number and filling (Morandi et al., 1988, 1990).

In this study we investigated the effects of changes in post-flowering source size and canopy structure on pod initiation, pod abscission, and seed abortion in soybean. Evidence will be presented showing that the number of pods initiated was inversely related to LAI while it was not related to CGR or LI, suggesting that other physiological mechanisms, not directly related to assimilatory capability, were involved in the regulation of pod initiation in soybean canopies.

2. Materials and methods

A field experiment was conducted at the research field of the Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Zavalla, Argentina (33°01′ S, 60°52′ W) during the 2001–2002 growing season. The emergence date was December 1st, 2001. Soybean genotypes were DM48 (Maturity Group (MG) IV) and A7409 (MG VII), both of which had an indeterminate growth habit. These genotypes were commercial varieties that have been extensively sown in Argentina. Genotypes were over-seeded and thinned to a final seed density of 32 and 26 plants/m^2 for DM48 and A7409, respectively. Individual plots were 6 m long and 3.2 m wide (eight rows with 0.40 m between rows). The plots were irrigated when necessary to avoid water deficits. Pests, diseases and weeds were permanently controlled during the experiment. Phenological stages were defined according to Fehr and Caviness (1977). Full bloom (R2), beginning seed (R5), beginning maturity (R7), and full maturity (R8) occurred at 42, 66, 121 and 130 days after emergence (DAE), respectively, for DM48 and at 66, 87, 128 and 140 DAE, respectively, for A7409. Leaflet removal treatments consisted of removing none (L0), one (L1) or two (L2) lateral leaflets of every trifoliate leaf at R2 and again shortly after R5. Thus, the canopy structure was modified by homogeneous removal of leaflets of all developed leaves that were present at the time of treatment. The LAI was estimated using regression functions that related leaf weight with LAI data from another experiment conducted in the same field, which used the same genotypes and leaflet removal treatments. Coefficients of determination (R^2) for these functions ranged from 86% to 99% and were statistically significant in all cases ($P < 0.05$).

A radiation sensor (pyranometer), an air temperature sensor and a bucket rain gauge were connected to a LI-COR LI-1200 Data set recorder to measure incident global solar radiation, temperature and rainfall. Sensor instruments were placed near the experimental plots. Statistical differences between climatic variables were determined by *t*-tests using standard errors.

The experimental design was a split plot with three replicates. Genotypes (G) were the main plots, and they were arranged in a randomised, complete block design. Leaflet removal treatments were randomised within each main plot and applied two times (T1 and T2). For DM48, leaflet removals were performed at phenological stages R2 (T1) and R5 + 7 d (T2), which were 42 and 73 DAE, respectively. For A7409, leaflet removals were performed at R2 + 4 d (T1) and R5 + 13 d (T2), which were 70 and 100 DAE, respectively. The period from T1 to T2 was named Per1, and the period from T2 to R7 was named Per2.

Light interception (photosynthetic active radiation, PAR) was measured immediately after T1 and T2 with a LI-COR Line Quantum sensor (LI-COR, Lincoln, NE) connected to an LI-1000 data logger. Percentage of light interception was determined in each plot from readings made above the canopy and at ground level. Measurements were taken on clear day, between 1130 and 1400 h solar time. Line quantum was placed on the ground diagonally between the plot rows. In each plot, two readings were taken at different random positions. The LI values on the other dates were estimated by a function developed from the relationship between LI and LAI for DM48 ($R^2 = 0.97$, $P < 0.0001$) and A7409 ($R^2 = 0.95$, $P < 0.0001$).

The extinction coefficient of the canopy (k) was obtained from the Lambert-Beer's law, such that, $k = -\ln(I_i/I_0)/\text{LAI}$, where ln means natural logarithm, I_i is PAR at soil level and I_0 is PAR above the canopy (Gardner et al., 1985).

Between T1 and T2, four destructive samples of 0.25 m^2 were taken for both genotypes. Between T2 and R7, three destructive samples were taken for DM48, and two destructive samples were taken for A7409. For each sample, all plants were separated into stems, leaves (petioles plus leaflets), pods and seeds (when present). Samples were dried to a constant weight at 60 °C in a forced-air dryer, and dry matter was expressed as grams per square meter (land basis).

The number of pods was counted in all sampled plants at 54, 72, 83, 97 and 111 DAE for DM48 and at 81, 87, 94, 103 and 115 DAE for A7409. A pod was counted when it was visible (≥2 mm). The number of pods initiated (PI) was defined as the maximum number of pods in the flush minus the minimum number of pods before the flush. The number of pods abscised (PA) was defined as the maximum number of pods in the flush minus the minimum number of pods after the flush.

Total dry matter (TDM) per land area during the R5 to R7 period was increased to account for the photosynthate requirement of seed production [2 g of photosynthate required to produce 1 g of seed dry weight (Sinclair and de Wit, 1975)]. Total dry matter and LAI were regressed against time (Hunt and Parsons, 1981) to obtain the following indices: CGR [g/m^2 (land area)/d], NAR [net assimilation rate, g/m^2 (leaf area)/d] and relative leaf growth rate [m^2/m^2 (leaf area)/d]. Linear, quadratic and cubic components of each regression equation were successively tested for significance and included in the equation if they significantly reduced the residual sum of squares. Significant differences were determined by *t*-tests using standard errors calculated by the regression program. Seed growth rate (SGR) was calculated as the slope of the regression function of increased seed dry matter over time during the linear seed-growth period. The date of initiation of linear seed growth was estimated by dividing the origin ordinate of the regression function by SGR.

At maturity, a 1-m^2 area was harvested by cutting the plants at ground level. Harvested plants were separated into main stems, branches, seeds and pod walls. Seed size (mg/seed) was calculated as the mean dry weight of 480 randomly sampled seeds. Maturity seed number (seed/m^2) was calculated as seed yield (g/m^2) divided by seed size. Maturity pod number (pod/m^2) was calculated as the final seed number divided by the actual seeds per pod at maturity.

A sample of 10 plants was used to determine the branch number, the main stem and branch node number and the number of pods in the main stem and branches. The potential seed number per pod was established by counting the number of pods with 2 (loc2), 3 (loc3) and 4 (loc4) loculi and then resolving the following equation: [(loc2 × 2) + (loc3 × 3) + (loc4 × 4)/total pod number]. A loculus was counted if a remnant of seed structure was visible in a partially or fully developed pod loculus. Potential seed number was determined by the potential seed number per pod multiplied by the pod number at maturity. Seed abortion was calculated as the actual seed number divided by the potential seed number. The

Table 1
Mean daily photoperiod, temperature and incident radiation during the first (Per1) and the second (Per2) periods, for genotypes DM48 and A7409.

Genotypes	Per1[A]			Per2		
	Photoperiod (h/d)	Temperature (°C/d)	Radiation (MJ/m²/d)	Photoperiod (h/d)	Temperature (°C/d)	Radiation (MJ/m²/d)
DM48	14.7a	24.6a[B]	24.3a	13.4a	23.7a	18.5a
A7409	13.9b	25.0a	20.4b	12.8b	20.6b	15.3b

[A] Per1 is the period between the first (T1) and the second (T2) leaflet removal treatments and Per2 is the period between T2 and beginning maturity (R7). T1 was done at full bloom (R2) and T2 shortly after the beginning seed stage (R5).
[B] Different letters indicate significant differences according to *t*-test ($P < 0.05$) between genotypes.

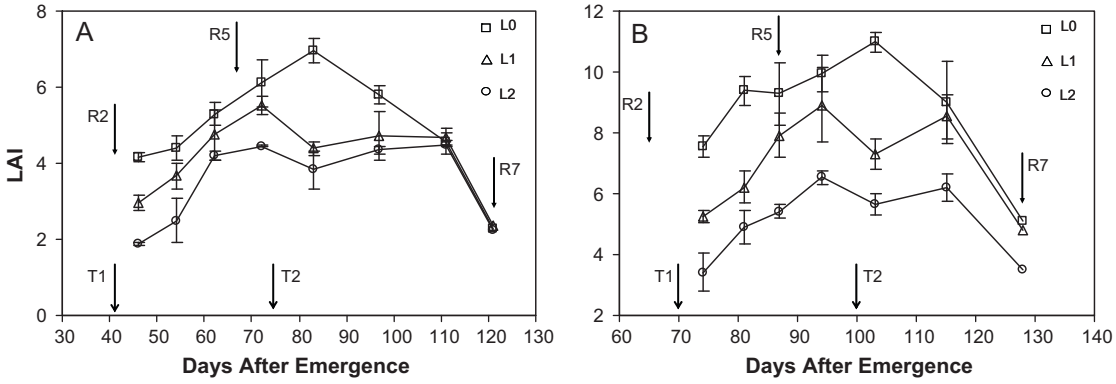

Fig. 1. Evolution of the leaf area index (LAI) as a function of the days after emergence (DAE) for soybean genotypes DM48 (A) and A7409 (B) subjected to different levels of leaflet removal treatments, consisting in removing none (L0), one (L1) or two (L2) lateral leaflets of every trifoliate leaf. Squares, triangles and circles indicate L0, L1 and L2 treatments, respectively. Points represent the mean ± SE of three replicates. Arrows indicate the dates of full bloom (R2), beginning seed (R5), beginning maturity (R7), first leaflet removal (T1), and second leaflet removal (T2).

actual seeds per pod were calculated as potential seeds per pod multiplied by seed abortion.

Analysis of variance (ANOVA) with mean separation by LSD was used to analyse the data. Regression analysis was applied to the relationship between variables. All statistical analyses (i.e., ANOVA, regression, *t*-tests and LSD tests, $P < 0.05$) were performed using SAS v 8.00 statistical software.

3. Results

3.1. Effects of leaflet removal on crop growth parameters

Table 1 shows the environmental conditions that each genotype was exposed to during the studied period. Genotype differences in photoperiod and temperature sensitivity determined differences in the date of flowering, the length of the vegetative period and the size of the leaf area at flowering. Therefore, the LAI of A7409 was 40% higher than the LAI of DM48 at the first leaflet removal (Fig. 1 and Table 2, L0).

The first leaflet removal reduced LAI and LI for both genotypes (Table 2) and increased the subsequent relative leaf growth rate during Per1. This increase in the relative leaf growth rate was inversely related to the remaining LAI in both genotypes (Fig. 2). There were no differences between the two genotypes in the slope of relative leaf growth rate as a function of LAI, which indicated that DM48 and A7409 had a similar leaf area recuperation response after leaflet removal. For the same level of LAI, however, the relative leaf growth rate of A7409 was always higher than the relative leaf growth rate of DM48 (Fig. 2). In both genotypes, the increased relative leaf growth rate in the leaflet removal treatments progressively reduced the differences in LAI during Per1, especially between L0 and L1 (Fig. 1).

Critical LAI was about five for both genotypes (Fig. 3). Although the leaflet removal methodology removed a similar proportion of leaf area in both genotypes, LI was always superior in A7409 because this genotype had a higher LAI before flowering, which was due to a longer vegetative period. Despite these differences, leaflet removal did not change the CGR of DM48 or A7409 (Table 2). Indeed, LAI reductions were compensated through the increase in the relative leaf growth rate (Fig. 2) and the increase in the net assimilation rate of the remaining leaves (data not shown). No relationship was found between CGR and LI for DM48 ($R^2 = 0.09$,

Table 2
Leaf area index (LAI) and light interception (LI) measured immediately after the first (T1) and the second (T2) leaflet removal treatments, and mean crop growth rate (CGR) during the first (Per1) and the second (Per2) periods, for soybean genotypes DM48 and A7409 and three leaflet removal levels.

Genotype	Leaflet removal	LAI_{T1}	LI_{T1} (%)	CGR_{Per1} (g/m²/d)[B]	LAI_{T2}	LI_{T2} (%)	CGR_{Per2} (g/m²/d)
DM48	L0[A]	4.2a[C]	88a	16.3a	7.0a	95a	23.4a
	L1	2.9b	71b	14.0a	4.4b	94a	17.3b
	L2	1.9c	53c	15.3a	3.8b	81b	14.9b
A7409	L0	7.0a	98a	33.4a	11.0a	99a	14.8a
	L1	5.3b	91b	34.5a	7.3b	98a	17.6a
	L2	3.4c	76c	31.1a	5.0c	93b	12.8a

[A] L0, L1 and L2, indicate removal of none, one and two lateral leaflets, respectively, of every trifoliate leaf. Leaflet removal was done twice: at full bloom (T1) and again shortly after the beginning seed stage (T2).
[B] Per1 is the period between T1 and T2, and Per2 is the period between T2 and beginning maturity (R7).
[C] Different letters indicate significant differences according to *t*-test ($P < 0.05$) among treatments within genotypes.

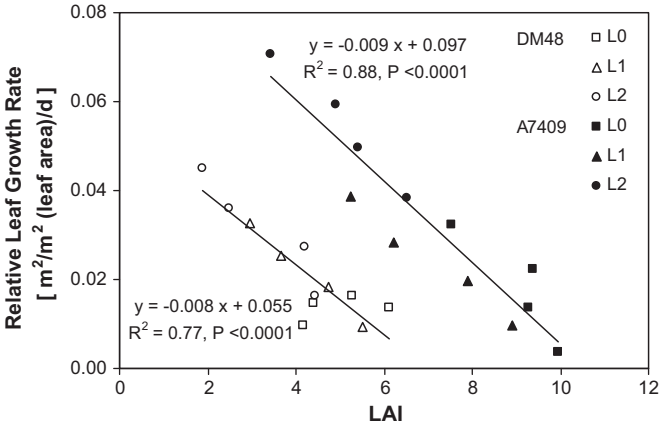

Fig. 2. Relationship between the relative leaf growth rate, and leaf area index (LAI) after the first leaflet removal for soybean genotypes DM48 (open symbols) and A7409 (close symbols). Squares, triangles and circles indicate L0, L1 and L2 leaflet removal treatments, respectively, as described in Fig. 1.

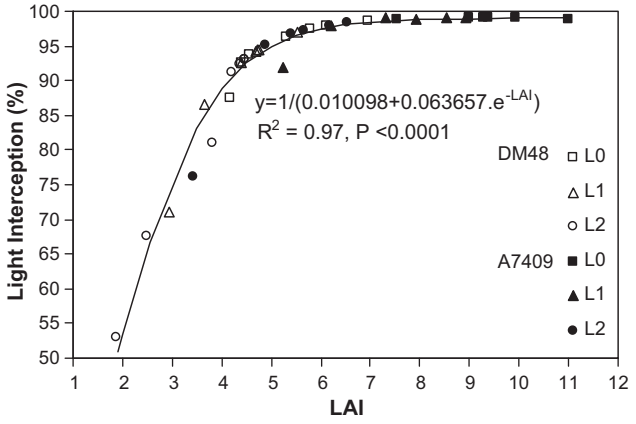

Fig. 3. Light interception as a function of leaf area index (LAI) for soybean genotypes DM48 (open symbols) and A7409 (close symbols) subjected to different levels of leaflet removal. Squares, triangles and circles indicate L0, L1 and L2 treatments, respectively, as described in Fig. 1.

$P > 0.05$) or A7409 ($R^2 = 0.002$, $P > 0.05$). In addition, no relationship was found between CGR and LAI for DM48 ($R^2 = 0.20$, $P > 0.05$) or A7409 ($R^2 = 0.31$, $P > 0.05$).

After the second leaflet removal (T2) the pattern of LAI evolution was changed in L1 and L2 treatments compared to the control (L0). The LAI of L0 increased until 83 and 103 DAE for DM48 and A7409, respectively, and then declined continuously during the rest of Per2. Differently, the LAI of L1 and L2 was transiently maintained from 83 to 111 DAE for DM48 and from 103 to 115 DAE for A7409 before start to decline in both treatments (Fig. 1).

During Per2, CGR was reduced by leaflet removal for DM48 but not for A7409 (Table 2). The CGR_{Per2} value was significantly related to LI and LAI for DM48 ($R^2 = 0.67$, $P < 0.01$, and $R^2 = 0.87$, $P < 0.001$, respectively), but these relationships were not significant for A7409 ($R^2 = 0.36$ and $R^2 = 0.32$ for LI and LAI, respectively, $P > 0.05$).

3.2. Effects of leaflet removal on pod initiation

Two pod initiation flushes (PI1 and PI2) were identified in both genotypes. The PI1 flush began at R2 and peaked at 72 and 87 DAE in DM48 and A7409, respectively. The PI2 flush, which was a minor one, began after R5 and peaked at 111 and 115 DAE in DM48 and A7409, respectively (Fig. 4).

The PI1 represented 86%, 83% and 89% of the total initiated pods in L0, L1 and L2, respectively, for DM48, and 85%, 77% and 88% in L0, L1 and L2, respectively, for A7409. The PI2 represented 14%, 17% and 11% of the total initiated pods in L0, L1 and L2, respectively, for DM48, and 15%, 23% and 12%, in L0, L1 and L2, respectively, for A7409. Thus, for both genotypes, the total number of initiated pods was mainly the result of the pods initiated in the first flush (i.e., PI1) (Table 3). The PI1 occurred between the first and second leaflet removal (i.e., after T1 and before T2). Surprisingly, the number of PI1 increased with the increase in the level of leaflet removal (i.e., with the decrease of LAI) for both genotypes (Fig. 5). In addition, PI1 was negatively associated with LI in both genotypes ($R^2 = 0.81$, $P < 0.01$ for DM48 and $R^2 = 0.81$, $P < 0.01$ for A7409). No association between PI1 and CGR was observed for DM48 ($R^2 = 0.04$, $P > 0.05$) or A7409 ($R^2 = 0.35$, $P > 0.05$).

The second pod initiation flush was not altered by leaflet removal or genotype. Therefore, the total number of initiated pods increased with the level of leaflet removal because of the effect of leaflet removal on PI1 (Table 3).

3.3. Effects of leaflet removal on pod abscission

The first pod abscission (PA1) flush occurred shortly after R5, and the second pod abscission (PA2) flush occurred shortly before R7 (Fig. 4).

In both genotypes, PA1 increased as the level of leaflet removal increased (Table 3). The relationship of PA1 with LAI and LI was analysed during the first pod abscission period. This period went from 72 to 97 DAE for DM48 and 87 to 103 DAE for A7409. The

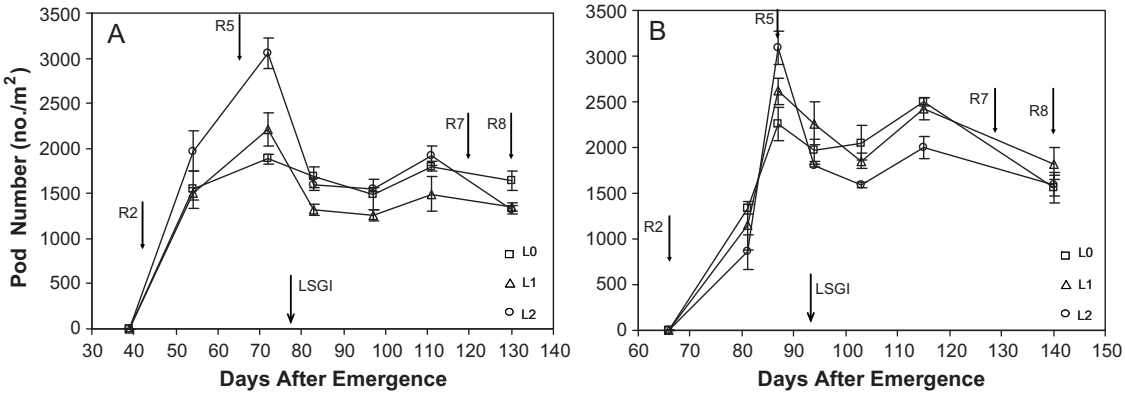

Fig. 4. Evolution of pod number for soybean genotypes DM48 (A) and A7409 (B) subjected to different leaflet removal treatments. Squares, triangles and circles indicate L0, L1 and L2 treatments, respectively, as described in Fig. 1. Points represent the mean ± SE of three replicates. Arrows indicate the dates of full bloom (R2), beginning seed (R5), beginning maturity (R7), full maturity (R8), and linear seed growth initiation (LSGI).

Table 3
Number of initiated and abscised pods in the first and second flush, total initiated and abscised pods, and pods at maturity for DM48 and A7409 genotypes and three leaflet removal levels.

Genotype (G)	Leaflet removal (L)	First flush		Second flush		Total initiated pods	Total abscised pods	Pods at maturity
		Initiated pods (no./m^2)	Abscised pods (no./m^2)	Initiated pods (no./m^2)	Abscised pods (no./m^2)			
DM48	L0[A]	1883	391	309	152a[B]	2192	543a	1649a
	L1	2208	948	349	255a	2557	1203b	1354b
	L2	3059	1514	372	603 b	3431	2117c	1314b
A7409	L0	2263	211	453	946a	2716	1157a	1559 a
	L1	2620	770	575	602b	3195	1372a	1823b
	L2	3092	1497	402	414b	3494	1910b	1584a
Mean for genotypes								
DM48		2384	951	343	337	2726	1288	1438
A7409		2658	826	476	653	3134	1480	1655
Mean for leaflet removal								
	L0	2073a	301a	381	549	2454a	850	1604
	L1	2414b	859b	462	428	2876b	1288	1588
	L2	3075c	1505c	387	508	3462c	2014	1449
ANOVA								
G		ns[C]	ns	ns	**	ns	*	ns
L		***	***	ns	ns	***	**	**
G × L		ns	ns	ns	**	ns	*	***

[A] L0, L1 and L2, indicate removal of none, one and two lateral leaflets, respectively, of every trifoliate leaf. Leaflet removal was done twice: at full bloom and again shortly after the beginning seed stage.
[B] Different letters indicate differences among treatments according to LSD, for $P < 0.05$.
[C] ns, non-significant differences.
* Significant difference at 0.05 probability level, in ANOVA.
** Significant difference at 0.01 probability level, in ANOVA.
*** Significant difference at 0.001 probability levels, in ANOVA.

mean LAI values for L0, L1 and L2 during this period were 5.9, 5.1 and 4.4, respectively, for DM48 and 10.1, 7.6 and 5.5, respectively, for A7409. The mean LI values for L0, L1 and L2 during the same period were 98%, 95% and 89%, respectively, for DM48 and 99%, 99% and 97%, respectively, for A7409. For both genotypes, PA1 was inversely correlated with LAI ($R^2 = 0.52$, $P < 0.05$ for DM48, and $R^2 = 0.80$, $P < 0.01$ for A7409) and LI ($R^2 = 0.65$, $P < 0.01$ for DM48, and $R^2 = 0.48$, $P < 0.05$ for A7409). The CGR values for L0, L1 and L2 during the first abscission period were 24.9, 19.7 and 22.3 g/m^2/d, respectively, for DM48 and 32.9, 37.8 and 34.0 g/m^2/d, respectively, for A7409. The first pod abscission flush was not associated with CGR for DM48 ($R^2 = 0.20$, $P > 0.05$) or A7409 ($R^2 = 0.01$, $P > 0.05$).

The second pod abscission flush (i.e., PA2) showed an interaction between genotype and treatment. Although PA2 increased in L2 for DM48, it was decreased in L1 and L2 for A7409 (G x L, $P < 0.01$, Table 3). Except for the L0 and L1 treatments in DM48, PA2 was almost equal to or higher than PI2, indicating that most of the pods from PA2 were lost. In addition, the number of abscised pods in DM48-L2 and A7409-L0 greatly surpassed the number of pods initiated during the second flush, which indicated that part of the pods that were lost came from pods initiated during the first flush.

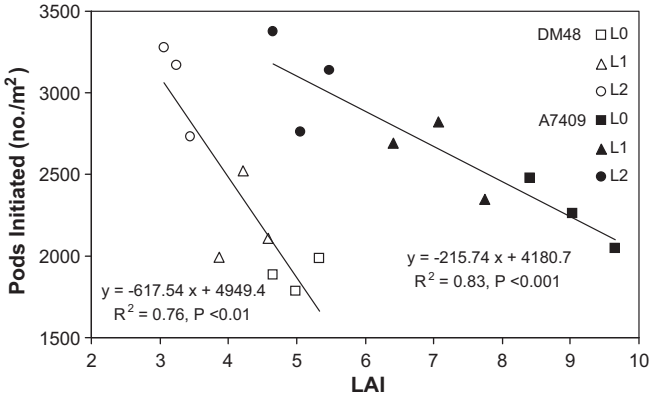

Fig. 5. Number of pods initiated in the first flush (PI1) as a function of leaf area index during the pod initiation period, for soybean genotypes DM48 (open symbols) and A7409 (close symbols). Squares, triangles and circles indicate L0, L1 and L2 treatments, respectively, as described in Fig. 1.

Although total abscised pods (i.e., PA1 + PA2) increased with the level of leaflet removal for DM48, they only increased in L2 for A7409 (G × L, $P < 0.05$, Table 3). The total abscission/initiation ratio (PA/PI) was enhanced by the level of leaflet removal for DM48 (25%, 47% and 62% in L0, L1 and L2, respectively, $P < 0.05$), but it remained unchanged for A7409 (43%, 43% and 55% in L0, L1 and L2, respectively, $P > 0.05$).

3.4. Effects of leaflet removal on pod number at maturity

The number of pods at maturity showed a strong genotype by leaflet removal interaction (G × L, $P < 0.001$, Table 3). Indeed, compared with L0, the pod number at maturity was reduced in L1 and L2 for DM48. For A7409, however, the number of pods at maturity was increased in L1 and remained unchanged in L2 (Table 3). Despite the increase in total initiated pods, the number of pods at maturity in L1 and L2 decreased for DM48 because of the increased total pod abscission in these treatments. Conversely, the increased pod number at maturity observed in A7409-L1, compared with A7409-L0, was due to the increase in the number of pods initiated. The lack of difference in the pod number at maturity between A7409-L2 and A7409-L0 occurred because the higher number of initiated pods was compensated by a higher number of abscised pods in A7409-L2 (Table 3).

3.5. Effects of leaflet removal on the distribution of nodes and pods between the main stem and branches at maturity

Although the number of branch nodes (BN) increased in the L2 treatment for both genotypes, the number of nodes in the main

Table 4
Total nodes, main stem nodes (SN), branch nodes (BN) and BN/SN ratio, main stem pods (SP), branch pods (BP) and BP/SP ratio at maturity for DM48 and A7409 genotypes (G) and leaflet removal treatments (L).

Genotype (G)	Leaflet removal (L)	Total nodes (no./m^2)	Stem nodes (SN) (no./m^2)	Branches nodes (BN) (no./m^2)	BN/SN	Stem pods (SP) (no./m^2)	Branches pods (BP) (no./m^2)	BP/SP
DM48	L0[A]	1132	571	561	1.0	786	863a	1.1a
	L1	1029	444	585	1.3	666	688a	1.0a
	L2	1301	587	713	1.2	673	641a	1.0a
A7409	L0	1451	639	812	1.3	723	836a	1.2a
	L1	1488	572	916	1.6	495	1328b	2.7b
	L2	1791	599	1192	2.0	516	1068c	2.1b
Mean for genotypes								
DM48		1154a[B]	534	620a	1.2a	708	730	1.1
A7409		1577b	604	973b	1.6b	578	1077	1.9
Mean for leaflet removals								
	L0	1292a	605	686a	1.2a	754a	850	1.2
	L1	1259a	508	750a	1.5b	580b	1008	2.2
	L2	1546b	593	953b	1.6b	595b	854	1.6
ANOVA								
G		** [C]	ns	**	**	ns	ns	ns
L		*	ns	**	**	*	*	**
G x L		ns	ns	ns	ns	ns	**	**

[A] L0, L1 and L2, indicate removal of none, one and two lateral leaflets, respectively, of every trifoliate leaf. Leaflet removal was done twice: at full bloom and again shortly after the beginning seed stage.
[B] Different letters indicate differences among treatments according to LSD, for $P < 0.05$.
[C] ns, non-significant differences.
* Significant difference at 0.05.probability level, in ANOVA
** Significant difference at 0.01 probability level, in ANOVA

stem (SN) was not changed by leaflet removal. Thus, the ratio of branch nodes to main stem nodes (BN/SN) was primarily increased in leaflet removal treatments because of the increase in the number of BN (Table 4). Moreover, in the case of the L2, the increase in BN caused an increase in the total number of nodes per square meter in both genotypes (Table 4). In addition, A7409 had a higher total number of nodes when compared with DM48, which was due to a higher number of BN (Table 4).

The number of pods on the main stem (SP) was reduced by leaflet removal treatments in both genotypes. The number of pods on the branches (BP), as well as the BP/SP ratio, showed an interaction between genotype and leaflet removal. Indeed, compared with L0, BP and the BP/SP ratio were increased in L1 and L2 treatments for A7409. Both of these values remained unchanged, however, for DM48 (Table 4).

3.6. Effects of leaflet removal on potential seeds per pod, actual seeds per pod and seed abortion

The number of actual seeds per pod is the result of potential seeds per pod and seed abortion. Although the number of potential seeds per pod was similar between genotypes and was not affected by leaflet removal, seed abortion increased with the level of leaflet removal. Thus, the decreased number of actual seeds per pod was

Table 5
Potential seed per pod, actual seed per pod, seed abortion, seed number and seed size for DM48 and A7409 genotypes and three leaflet removal levels.

Genotype (G)	Leaflet removal (L)	Potential seed per pod (no.)	Actual seed per pod (no.)	Seed abortion (%)	Seed number (no./m^2)	Seed size (mg/seed)
DM48	L0[A]	2.8	2.2	23	3591a[B]	172
	L1	2.8	2.0	29	2740b	173
	L2	2.8	1.7	40	2340c	166
A7409	L0	2.5	2.3	8	3614a	133
	L1	2.6	2.0	23	3656a	139
	L2	2.6	1.9	26	3064b	123
Mean for genotypes						
DM48		2.8	2.0	31a	2890	170a
A7409		2.6	2.1	19b	3445	132b
Mean for leaflet removals						
	L0	2.7	2.3a	16a	3602	153a
	L1	2.7	2.0b	26b	3198	156a
	L2	2.7	1.8c	33c	2702	145b
ANOVA						
G		ns[C]	ns	**	**	**
L		ns	**	**	***	*
G × L		ns	ns	ns	*	ns

[A] L0, L1 and L2, indicate removal of none, one and two lateral leaflets, respectively, of every trifoliate leaf. Leaflet removal was done twice: at full bloom and again shortly after the beginning seed stage.
[B] Different letters indicate differences among treatments according to LSD, $P < 0.05$.
[C] ns: non-significant differences.
* Significant difference at 0.05 probability level, in ANOVA.
** Significant difference at 0.01 probability level, in ANOVA.
*** Significant difference at 0.001 probability level, in ANOVA.

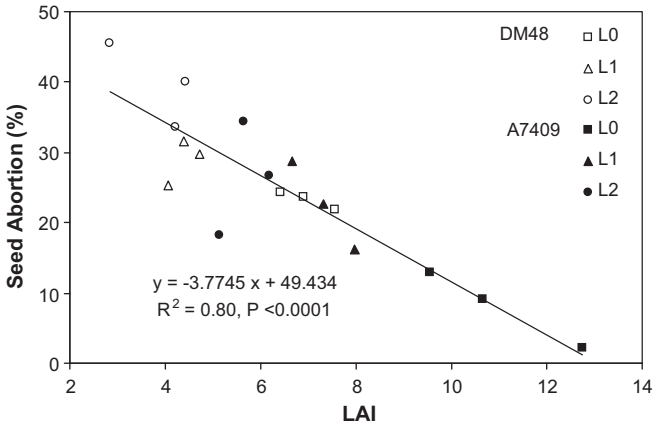

Fig. 6. Relationship between seed abortion percentage and leaf area index (LAI) measured after the second leaflet removal for soybean genotypes DM48 (open symbols) and A7409 (close symbols). Squares, triangles and circles indicate L0, L1 and L2 leaflet removal treatments, respectively, as described in Fig. 1.

a consequence of the effect of leaflet removal on the abortion of developing seeds. Also, seed abortion was higher for DM48 than for A7409 (Table 5). Seed abortion was inversely related to LAI after the second leaflet removal in both genotypes (Fig. 6).

The final seed number (seed/m^2) showed a genotype by leaflet removal interaction. Indeed, the final seed number for DM48 decreased as the level of leaflet removal increased (L0 > L1 > L2). The final seed number for A7409, however, was only reduced in the L2 treatment (L0 = L1 > L2) (G × L, $P < 0.05$, Table 5).

3.7. Effects of leaflet removal on seed size

DM48 had larger seed size than A7409 (Table 5). Despite genetic differences in seed size, only the highest level of leaflet removal (i.e., L2) was able to reduce seed size in both genotypes (Table 5). When the CGR during the linear seed growth period was standardised to account for differences in seed number among treatments, seed size was directly related to CGR per seed in both genotypes ($R^2 = 0.91$, $P < 0.01$).

3.8. Effects of leaflet removal on seed yield

The seed yield in L0, L1 and L2 were 617.7, 474.0 and 388.4 g/m^2, respectively, for DM48 and 480.7, 508.2 and 376.9 g/m^2, respectively, for A7409. There was a genotype by leaflet removal interaction for yield. For DM48, the yield was significantly decreased in L1 and L2 compared with L0 ($P < 0.05$). Not all components that contributed to the seed yield, however, were equally affected by leaflet removal treatments. Indeed, DM48-L1 reduced seed number without changing seed size while DM48-L2 reduced both seed number and size (Table 5).

For A7409, the yield remained unchanged in L1 and was decreased in L2, compared with L0 ($P < 0.05$). Although there were no changes in seed number or size in A7409-L1, both components were reduced by leaflet removal in A7409-L2 (Table 5).

4. Discussion

4.1. Leaflet removal and crop growth parameters

Under our experimental conditions, the critical LAI (i.e., the LAI necessary for 95% LI) was equal to 5.1. No direct relationship was observed between LI and CGR during Per1 even though LI was reduced below critical by the first leaflet removal (Table 2). The LAI reduction might have been compensated through the increase in the relative leaf growth rate induced by leaflet removal during this period (Fig. 2) and/or by the increase in the net assimilation rate of the remaining leaves. The net assimilation rate of the remaining leaves may have increased because mutual shading of the leaves in the canopy was decreased and/or vegetative sink demand was increased by the growth of new leaves in leaflet removal treatments. These results are consistent with previous reports in which defoliation stimulate compensatory leaf re-growth and enhanced the photosynthetic rate of the remaining leaves during reproductive growth in soybean canopies (Klubertanz et al., 1996; Haile et al., 1998). In our conditions, the compensatory leaf area recuperation was due to the growth of new leaves in branch nodes. The higher relative leaf growth rate of A7409 compared with DM48 can then be explained by the fact that A7409 had 37% more nodes than DM48 (Table 4).

The second leaflet removal induces a delay in the start of rapid LAI decay during Per2 in L1 and L2 compared to L0 (*cf.,* L1 and L2 with L0 in Fig. 1). These responses are consistent with previous reports about the effects of defoliation delaying leaf senescence in soybean (Klubertanz et al., 1996; Haile et al., 1998). However, when the LAI after the second leaflet removal was lowered below the critical value, the CGR was reduced during Per2 (e.g., DM48-L1 and DM48-L2, Table 2). Results of Per2 contrasted with the results of Per1 in which a large reduction of LAI did not affect CGR. It is worth to note that during Per2 seeds were the dominant sink and seed growth rate was the main component of CGR. In addition, during this period there was no possibility for new leaf area development. Thus, the delay in leaf senescence observed in leaflet removal treatments was not enough to compensate for the reduction of the LAI below the critical value during the seed filling period.

4.2. Leaflet removal and pod initiation

The main flush of pod initiation (PI1) was only affected by the first leaflet removal. The number of initiated pods increased proportionately with the decrease in LAI for both genotypes (Fig. 5). Remarkably, the inverse relationship between PI1 and LAI was sustained irrespective of whether the LAI was below (DM48) or above (A7409) the critical value, which indicated that soybeans can adjust pod number in response to changes in LAI before and after canopy closure. In addition, changes in PI1 were almost three times more sensitive to LAI variation in DM48 than in A7409 (*cf.* slopes of the regression lines in Fig. 5), which indicated the existence of genetic variability to this response in commercial soybean varieties.

In our experiment, the number of initiated pods increased concomitantly with the increased relative leaf growth rate (Fig. 2). Indeed, a higher relative leaf growth rate would require enhanced partition of current assimilates to vegetative sinks (i.e., new leaves). As both, relative leaf growth rate and pod initiation increased with the decrease in LAI (*cf.* Fig. 2 and Fig. 5), it was evident that competition for assimilates did not impinge on pod initiation. Previous reports have shown that developing pods require very little assimilate to sustain growth during their initial stages of development (Brun and Betts, 1984; Heitholt et al., 1986). Thus, assimilate limitation seems improbable at this early stage.

The observed increase in the number of initiated pods in leaflet removal treatments could have been a consequence of the particular defoliation methodology used. Mathew et al. (2000), however, reported an increase in pod number when the soybean canopy was artificially opened (by pushing aside adjacent rows around early flowering) without any leaf removal. In our experimental model, LAI reduction was performed by homogeneous removal of none (L0), one (L1) or two (L2) leaflets from all developed trifoliate leaves present. In addition, we allowed the leaf area to recover after treatments. Reducing LAI in this way did not change CGR during the main pod initiation flush (i.e., PI1), but it significantly decreased

the canopy extinction coefficient. Immediately after T1, the canopy extinction coefficients in L0, L1 and L2 were 0.57, 0.44 and 0.37, respectively, for DM48 and 0.62, 0.48 and 0.38, respectively, for A7409 ($P < 0.05$). Thus, leaflet removal treatments reduced the canopy extinction coefficient in both genotypes, which allowed light to penetrate deeper into the canopy and reach the strata of lower leaves. Canopy opening not only increases the quantity of light reaching the strata of lower leaves, but it also modifies the proportion of red (R) and far-red (FR) light perceived by plants. The profound morphogenetic effects that alterations in the R:FR ratio have on plants have been well documented (Smith, 1982; Ballaré and Casal, 2000). Specifically for soybeans, it has been reported that supplementary red light applied to the lower canopy strata of soybean crop enhanced pod number per node in the treated section, and this response was due to an increase in the percent of set pods rather than flower number (Heindl and Brun, 1983).

We did not measure the spectral distribution of light inside the canopy in this experiment, but studies have shown that changes in LAI are correlated with changes in the levels of morphogenetic wavelengths in canopies of many species, including soybeans (Sattin et al., 1994; Kasperbauer, 1987). Because leaves efficiently absorb red and blue but not far-red, lower LAI values correlate with higher R:FR ratios and blue light levels inside the canopies. Thus, it seems possible that photomorphogenetic effects could be involved in the modulation of the number of initiated pods in soybean canopies. This hypothesis, however, has not been demonstrated.

We would also like to point out that the peaks in the flushes of pod initiation were not shifted by leaflet removal in any genotype (Fig. 4), which indicated that the timing of pod initiation was not influenced by leaflet removal.

4.3. Leaflet removal and pod abscission

The first and main abscission period, which occurred immediately after R5, increased with the increase in the level of leaflet removal in both genotypes (Table 3). Board and Tan (1995) also reported that the main pod abscission period occurred shortly after R5. These authors considered that pod abscission was related to LI (for LI $\leq$ 95%) and suggested that pod abscission was controlled by assimilatory capacity. In our experiment, except for DM48-L2, the LI values of all treatments were above 95% during the abscission period. Besides, the minimum CGR necessary for maximum pod number in soybean was reported to be 15 g/m^2/d (Board and Harville, 1994). In the present experiment, CGR values for L0, L1 and L2 during the abscission period were 24.9, 19.7 and 22.3 g/m^2/d, respectively, for DM48 and 32.9, 37.8 and 34.0 g/m^2/d, respectively, for A7409. Thus, the CGR values were well above the minimum necessary for maximum pod number. In addition, CGR was not related to pod abscission in either genotype, which suggested that other factor might be involved in the control of pod abscission.

The seeds began to grow around the beginning of the pod abscission period (Fig. 4, R5). The linear seed growth rates in L0, L1 and L2 were 14.7, 15.1 and 13.2 g/m^2/d, respectively, for DM48 and 13.6, 13.6 and 11.5 g/m^2/d, respectively, for A7409. Although the seed growth rate was about 10% higher for DM48 than A7409, the difference was not significant. When the sink/source ratio was standardised by adjusting the sink demand (seed growth rate) relative to the size of the source (LAI), the percentage of pod abscission increased as the sink/source ratio increased in both genotypes. For similar levels of pod abscission, however, DM48 always showed a higher sink/source ratio than A7409, which indicated a better partition of assimilates to reproductive structures (Fig. 7). Despite quantitative differences between genotypes, these results support the hypothesis that competition for assimilates among reproductive structures is an important factor in the regulation of pod abscission.

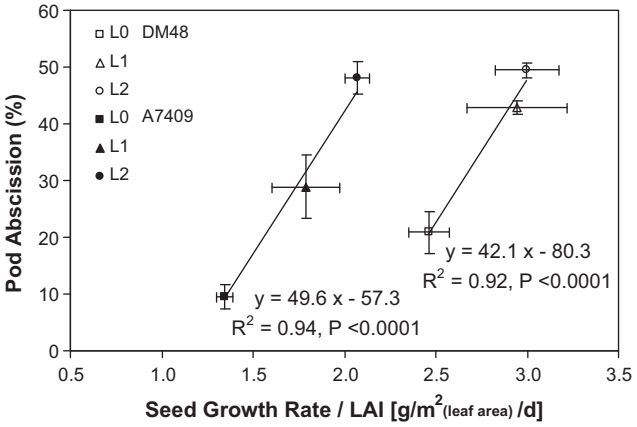

Fig. 7. Relationship between percentage of pod abscised and seed growth rate per unit of leaf area during the main abscission period for soybean genotypes DM48 (open symbols) and A7409 (close symbols). Squares, triangles and circles indicate L0, L1 and L2 treatments, respectively, as described in Fig. 1. Points represent the mean ± SE of three replicates.

4.4. Leaflet removal and pod number at maturity

The number of pods at maturity was the result of the balance between two components: total initiated pods and total abscised pods. On the one hand leaflet removals had a positive effect on pod number at maturity through the increase of pod initiation. This response was mainly due to the leaflet removal effect on the first flush of pod initiation, which was not directly related to the assimilatory capability of the canopy (i.e., CGR). Conversely, except for A7409-L1, leaflet removal had a negative effect on pod number through the increase of total pod abscission.

Early predictive models of pod number (Sheldrake, 1979; Charles-Edwards et al., 1986; Egli and Zhen-wen, 1991), as well as a more recent one which incorporate the temporal profile of flowering (Egli, 2010) are assimilate-based models. Our results regarding the abscission component of the pod number could be explained by assimilate-based models. Additional factors, however, might be involved in the regulation of the pod initiation component.

4.5. Leaflet removal and the pattern of node and pod distribution between the main stem and branches at maturity

Leaflet removal induced a shift in the developmental pattern by increasing the number of nodes in the branches relative to the nodes in the main stem (BN/SN) in both genotypes (Table 4). The BN/SN ratio provided an index of the degree of correlative apical inhibition on branch growth, and higher values indicated lower inhibition. Thus leaflet removal induced a partial release of apical inhibition on branch growth in both genotypes. Interestingly, a study reported that red light pulses, applied at the end of the day, induced axillary vegetative buds to develop new branches. This effect was reversed by far-red light, which suggested a phytochrome regulated phenomenon (Kasperbauer, 1987). Moreover, in a recent study it was reported that a low R:FR ratio as well as a phytochrome B mutation have both negative effects on branching in *Arabidopsis* (Finlayson et al., 2010). Whether the partial release of correlative branch inhibition induced by leaflet removal in soybean canopies operates by a similar physiological and/or molecular mechanism remains to be demonstrated.

It seems logical to expect that the change in BN/SN ratio induced by leaflet removal would be followed by a similar change in the distribution of pods, which would increase the branch-pod to stem-pod ratio (BP/SP). The BP/SP ratio was augmented by leaflet removal in A7409 but not in DM48 (Table 4). Although we did not

discriminate between branch-abscised and stem-abscised pods, differences between genotypes in the pod abscission pattern of branches and/or main stem may account for the observed results.

4.6. Leaflet removal and seed abortion

The actual number of seeds per pod, which has been shown to have low environmental variation, is a yield component that has generally been considered to be under genetic control (Egli, 1998). Although it is hardly measured, the number of potential seeds per pod is what is actually under genetic control. In the present study, the number of potential seeds per pod did not show differences between genotypes and was not affected by the level of leaflet removal (Table 5). This result clearly indicated genetic control of the number of potential seeds per pod. The number of actual seeds per pod, however, was reduced by leaflet removal because of the increased seed abortion (Table 5). Seed abortion primarily occurred around the beginning of the linear seed-growth period (Munier-Jolain et al., 1993; Egli and Bruening, 2002). In the present study, seed abortion increased with the level of leaflet removal, and it was also higher for DM48 than for A7409. In addition, seed abortion was inversely related to the LAI after the second leaflet removal (Fig. 6), which indicated that seed set was source-limited. Source size after the second leaflet removal not only explained differences in the seed abortion ranking among treatments (e.g., L2 > L1 > L0) but also between genotypes (e.g., DM48 > A7409). This finding, together with the differential genotype response of total pod abscission to leaflet removal (Table 3), explained the significant genotype by leaflet removal interaction that was observed in the seed number (Table 5).

4.7. Leaflet removal and seed size

Although seed size (unitary seed mass) is not strictly a numerical component, it is an important component of the yield. Seed size is genetically determined (Hartwig, 1973), but it is also strongly influenced by source strength (Egli, 1998). A genetic factor was present because DM48 had larger seeds than A7409 (Table 5). Regarding the impact of leaflet removal treatments on seed size, only the more severe (i.e., L2) treatment was able to reduce seed size in both genotypes (Table 5). Because LAI decay was attenuated during the seed-filling period of leaflet removal treatments (Fig. 1), the delay in the senescence of the remaining leaflets observed in L1 could partially explain the lack of differences in seed size between this treatment and L0. Conversely, this transient delay in leaf senescence was not enough to counteract for the negative effect on seed size of the strong reduction of leaf area during the seed filling period in the case of L2 (Table 5).

Under some circumstances, an increase in the seed number of soybean crop could be partially compensated by a reduction in seed size (Sadras, 2007). In the present study we did not observe a compensatory relationship between seed number and seed size. Seed size was highly associated, however, with the CGR per seed during the seed-filling period, which indicated the strong dependence of seed size on assimilate supply.

4.8. Leaflet removal and yield

Compared with control (L0), two leaflet removals (i.e., T1 + T2) decreased the yields of DM48-L1, DM48-L2 and A7409-L2. In these genotype-leaflet-removal interactions, the positive effects of the first leaflet removal on the pod initiation component was undercompensated by either higher pod abscission and seed abortion (DM48-L1), or higher pod abscission, higher seed abortion and lower seed size (DM48-L2 and A7409-L2).

Two leaflet removals, however, were unable to reduce the yield of A7409-L1. Indeed, the A7409-L1 yield was increased, although not significantly, compared with the control (+27.5 g/m^2). In this genotype by leaflet removal combination the number of pods at maturity was increased due to the increase in pod initiation (Table 3). This response raises the question of whether it would be possible to increase yields by increasing pod initiation. For this objective to be realised, however, an increase in the number of initiated pods should not be under-compensated by the decrease in any of the subsequent yield components.

5. Conclusions

The more striking result of this work was the demonstration that the number of initiated pods increased as LAI decreased, irrespective of whether the LAI was below or above the critical value. This response showed a similar pattern for DM48 and A7409 despite large differences between genotypes in the size of the canopies and sensitivity to photoperiod. Moreover, the pattern of this response was maintained regardless of the mean radiation, temperature and photoperiod at which each genotype was exposed during the pod initiation period. Our results also suggest that early (i.e., at flowering) canopy closure may negatively impact pod initiation in soybean. Additional studies are needed to elucidate the nature of the internal and/or external signals modulating pod initiation. To the best of our knowledge, this study is the first to document that the number of initiated pods is inversely related to LAI in soybean canopies.

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