Field Crops Research 176 (2015) 45–55

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Post-flowering photoperiod and radiation interaction in soybean yield determination: Direct and indirect photoperiodic effects

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ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form 18 February 2015
Accepted 18 February 2015
Available online 12 March 2015

Keywords:
Photoperiod extension
Radiation levels
Seed number
Yield
Soybean
RUE

ABSTRACT

Soybean (*Glycine max* (L.) Merrill) exposure to long days during the post-flowering phase increases total biomass, nodes, pods and seeds per plant, and also the post-flowering duration, increasing the radiation offer. This work aims to identify the main mechanisms responsible for yield increases in response to long days, separating direct photoperiodic effects on yield determination, from the indirect effect associated with changes in cumulative radiation when the crop cycle is modified by photoperiod. Two field experiments were conducted with an indeterminate soybean cultivar. A factorial combination of two radiation levels (unshaded and shaded), and two or three photoperiod regimes (control, extended 1.5 and 3 h) was imposed from flowering to maturity. Yield tended to be reduced by shade and increased by extended photoperiod mainly through their effects on nodes per m^2, and thereby affecting pods and seeds per m^2. Photoperiod extension increased node number due to both increased cumulative radiation (indirect effect) and delayed reproductive development (direct effect). As a result, more pods were established per unit of cumulative radiation under extended photoperiod. The results suggest that photoperiod extension enhanced yield radiation use efficiency due to the alleviation of intra-nodal interferences. The direct post-flowering photoperiodic effect on node number and the resultant effects on pod and seed number, provide evidence of direct photoperiodic effects on soybean yield determination.

1. Introduction

Soybean yield is mainly determined during the post-flowering phase (R1 stage onwards; Fehr and Caviness, 1977), throughout flowering, pod setting and seed filling (Board et al., 1995; Egli, 2010a; Jiang and Egli, 1993, 1995). During that period, often called 'critical period' due to its importance for yield determination (Egli, 1998), limitations in daily assimilate supply caused by shading, defoliation or water stress, reduce seed number per unit area, which is the main yield component (Egli and Yu, 1991; Jiang and Egli, 1995). As a consequence, a positive relationship between seed number or yield and plant or crop growth rate during post-flowering phases has been widely found in the literature (Board et al., 1995; De Bruin and Pedersen, 2009; Egli, 1998; Egli and Yu, 1991; Jiang and Egli, 1995).

Post-flowering photoperiodic effects on soybean seed number and yield have been previously proved evident in experiments that manipulated day length during different reproductive subphases using growth chambers (Board and Settimi, 1986; Cober et al., 1996), night interruptions (Cure et al., 1982; Guiamet and Nakayama, 1984a, 1984b; Morandi et al., 1988; Raper and Thomas, 1978; Thomas and Raper, 1976, 1983) and day length extensions (Han et al., 2006; Kantolic and Slafer, 2001, 2005, 2007; Kumudini et al., 2007). Previous studies found that long photoperiods during the post-flowering phase increase total biomass and nodes, pods and seeds per plant, irrespective of the experimental procedure applied to manipulate photoperiod (Guiamet and Nakayama, 1984a; Morandi et al., 1988; Kantolic and Slafer, 2001; Kantolic et al., 2013).

Abbreviations: iPAR, daily incident photosynthetically active radiation; PAR_{R1-R7}, cumulative photosynthetically active radiation intercepted during post-flowering; PAR_{VE-R6}, cumulative photosynthetically active radiation intercepted from emergence to full seed stage; $RUE_{Y,PAR}$, photosynthetically active radiation use efficiency to produce yield.

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http://dx.doi.org/10.1016/j.fcr.2015.02.018
0378-4290/

Photoperiodic effects on soybean yield and its components are accompanied by the well-known developmental response to photoperiod during post-flowering (Guiamet and Nakayama, 1984b; Summerfield et al., 1998; Thomas and Raper, 1976). Long days delay reproductive development and physiological maturity, extending the post-flowering phase and the duration of the critical period. A positive relationship has been found between the duration of the critical period and yield (Dunphy et al., 1979) or seed number per unit area (Egli and Bruening, 2000; Kantolic and Slafer, 2001, 2005, 2007). Although the mechanisms controlling these relationships are not completely understood, the exposure of the plant community to a prolonged incident radiation offer should result in more growth during the phase when pods and seeds are produced (Kantolic and Slafer, 2005). Therefore, the post-flowering photoperiodic effect on yield could be an indirect effect associated only to the increase in cumulative incident radiation resulting from the extended post-flowering phase, as the direct environmental factor controlling yield would be the photosynthetically active radiation available for growth. However, Egli and Bruening (2000) had previously suggested that most of the benefit of the longer period should not come from simply exposing the plant community to more incident radiation and that both crop growth and phase length may have some independent effect on seed number.

An approach combining two radiation scenarios and contrasting photoperiods has shed some light on the comparison of these two environmental factors' effects on wheat yield (Gonzalez et al., 2005), and more recently on soybean (Kantolic et al., 2013). Kantolic et al. (2013) showed that seed number was closely related to radiation accumulated during pod setting (from stage R3 to R6; Fehr and Caviness, 1977), irrespective of the factor that increased cumulative radiation (higher daily radiation or longer phase duration). These results suggest that long photoperiod increased pods and seeds established per unit land area, mainly through increasing resource availability during part of a phase that is critical for yield determination. Nevertheless, more detailed observations revealed that photoperiodic and radiation effects were not alike at individual nodes: while shading reduced pod number in all plant positions, long photoperiod increased pods per node only in those nodes that flowered after treatment initiation (Kantolic et al., 2013). The authors suggest that long photoperiod failed to promote pod setting at nodes that flowered before treatment initiation (under short photoperiod) because some hierarchical relationships between pods might have already been established. This is relevant, given the fact that age-related interferences among pods within a node seem to be stronger than those among pods produced on different nodes (Egli and Bruening, 2002a, 2002b, 2006a, 2006b).

At the same time, long days or photoperiod extension induce other changes in plant development that may also be directly associated with yield increases. Pod number is related to the number of flowers (Egli, 2005; Jiang and Egli, 1993) and nodes (Egli, 2013). A long flowering period increases flower production and the duration of that period is under control of photoperiod (Guiamet and Nakayama, 1984b; Summerfield et al., 1998) and is rather independent of the assimilate availability (Dybing, 1994). Additionally, biomass partitioning to reproductive structures may be also altered by photoperiod (Cure et al., 1982; Raper and Thomas, 1978). All these effects, and perhaps some additional ones, can be considered "direct effects", as they require the perception of photoperiod and are, at least partially, independent of photosynthetically active radiation.

Mechanisms that increase yield in a way not related to cumulative radiation enhance the efficiency with which radiation is used to produce yield. Thus, identifying these mechanisms and understanding their interaction with other yield-forming traits is of great interest to increase crop yield potential. As soybean post-flowering photoperiod sensitivity is under control of a relatively low number of genes (Cober et al., 1996; Summerfield et al., 1998), direct post-flowering photoperiodic effects that increase yield could be traits that can be rapidly introduced in breeding programs.

In the present work we further analyse soybean yield determination in response to photoperiod extension under contrasting incident radiation scenarios to understand the interaction between photoperiod and radiation effects on yield when the whole post-flowering phase is lengthened. The objective of the present work was to identify the mechanisms responsible for increasing yield under extended photoperiod, separating indirect photoperiodic effects (i.e. those associated with the increase in cumulative radiation caused by the prolongation of the reproductive phase) from the direct effects (i.e. those independent of increases in cumulative radiation and dependent on photoperiod induced changes in crop structure and function).

2. Materials and methods

2.1. Culture

The commercial indeterminate soybean cultivar NA 5009 RG (Nidera Argentina) was grown under field conditions at the experimental field of the School of Agronomy, University of Buenos Aires (34°35′S, 58°29′W) during the 2008/09 (Exp1) and 2009/10 (Exp2) growing seasons. Sowing dates were January 25th and October 25th in Exp1 and Exp2, respectively. Seeds were inoculated with *Bradyrhizobium* liquid inoculant and sown at a high-planting rate in field plots. When the unifoliate leaves were expanded, the plots were hand-thinned to obtain a uniform plant population of 40 plants per m^2. Plots consisted of six rows, 2.5 m long, with 0.35 m row spacing. Weeds, pests and diseases were chemically controlled as needed following local agronomic practices. Rainfall was complemented throughout the crop cycle with a drip system. When necessary, plants were tied up to avoid lodging.

2.2. Treatments

Treatments consisted of the factorial combination of different shade and photoperiod levels applied from the beginning-bloom stage (R1, as described by Fehr and Caviness, 1977) to beginning-maturity stage (R7). In both experiments shading treatments were achieved by installing commercial shade nets (35% radiation reduction) over the plots to reduce canopy photosynthesis (called "shaded" throughout the text). The shade nets changed photosynthetic photon flux density without changing the spectral composition of light. Control plots were maintained without the shading nets ("unshaded"). In Exp1, two photoperiod treatments were imposed: (i) plots were either kept under natural photoperiod ("control") or (ii) exposed to artificially 3.0 h-extended photoperiod in relation to natural photoperiod ("3 h") (Kantolic and Slafer, 2001). In Exp2, an intermediate photoperiod treatment extended by 1.5 h in relation to natural photoperiod was added ("1.5 h"). To extend photoperiod in the field plots, portable lighting structures were used. Each structure combined incandescent and fluorescent lamps that provided an extremely low photosynthetic photon flux density (400–700 nm) of 4 $\mu mol\ m^{-2}\ s^{-1}$ (measured on top of the canopy using a LI-COR Inc. quantum sensor) and a red to far-red ratio of 1.17 (measured using a SKR 110 660/730 sensor, Skye Instruments Ltd.). Lights were automatically switched-on before sunset and switched-off at the required time depending on the length of extension. Lighting structures and shade nets were always kept 20–30 cm above the canopy.

2.3. Data collection and analysis

Daily incident photosynthetically active radiation (iPAR) and mean air temperature were registered 200 m away from the experimental site (Vantage Pro2, Davis Instruments, California, USA). Weekly, on clear days and at noon, incident and transmitted photosynthetically active radiation to ground level were measured with a linear radiometer (Bar-Rad 100, Cavadevices, Buenos Aires, Argentina) placed perpendicular to the rows to estimate the proportion of iPAR intercepted by the canopy. Cumulative intercepted photosynthetically active radiation (PAR_{R1-R7} or PAR_{VE-R6}), was estimated as the iPAR sum, affected by shading treatments when corresponding, and by the proportion of the incoming radiation intercepted by the canopy that day.

Phenological stages and the number of nodes with expanded leaves on the main stem were recorded at 1–3 day intervals when 50% of the plants reached the stage described by the scale proposed by Fehr and Caviness (1977). Duration of VE-R1 (emergence to beginning-bloom), R1–R3 (beginning-bloom to beginning-pod), R3–R7 (beginning-pod to beginning-maturity) phases was corrected by temperature and expressed in days corrected by temperature (thermal days) using a three-segmented linear function (Piper et al., 1996). Cardinal temperatures used to compute development rates at different stages were taken from Piper et al. (1996). Photoperiod sensitivity was estimated as the slope of the regression line adjusted to phase duration (in thermal days) as a function of mean photoperiod during that phase. The rate of leaf appearance was estimated as the slope of the regression line adjusted to leaf number as a function of time (in thermal days).

Aerial biomass was hand sampled at R3 and R6 (full-seed stage) on 0.21 m^2 and at R7 on 0.35 m^2. All measurements and samples were taken from two of the central four rows of each plot, and at least a 0.3 m border was maintained between sampled areas and the edge of the plot. Buds, flowers, pods and seeds were excised from plants and dried separately. Samples were dried at 60 °C until constant weight for at least 72 h before biomass was determined. After weighting, the maturity sample was hand-threshed and seeds weighted to calculate crop yield. Harvest index was estimated as the ratio of seed yield to total above ground biomass at R7.

Partitioning was estimated as the ratio of reproductive biomass (flowers, pods and seeds) to total above-ground biomass at R3, R6 and R7 per m^2. The time of beginning of partitioning and partitioning rate were estimated from the X-intercept and slope of the regression line adjusted to partitioning as a function of time (in thermal days), respectively.

The source/sink relationship at crop level was estimated at R6 as the ratio between aboveground vegetative biomass (stems and leaves) and the number of reproductive structures (flowers and pods) per m^2. The source/sink relationship at node level was estimated as the ratio between PAR_{R1-R7} and the number of nodes per m^2. Radiation use efficiency to produce yield ($RUE_{Y,PAR}$) was estimated as the ratio between yield and PAR_{R1-R7}.

Seed, pod and node number were counted in main stems and branches separately, from the R7 sample. Individual seed weight, seeds per pod and pods per node were calculated as the ratio between yield and seeds, seeds and pods, and pods and nodes per m^2, respectively.

The total photoperiod extension effect on nodes per m^2 was calculated as the number of nodes per m^2 under control photoperiod minus the number of nodes per m^2 under extended photoperiod for each block. Each total photoperiod extension effect was separated into indirect and direct photoperiodic effects. Indirect photoperiodic effects were considered those caused by the increased cumulative radiation and were estimated using a linear model adjusted to nodes per m^2 vs. PAR_{VE-R6} (for plots under control photoperiod). Direct photoperiodic effects were considered those independent of the cumulative radiation intercepted during VE-R6 and were calculated as the difference between total and indirect photoperiodic effects.

2.4. Experimental design and statistical analysis

The experimental design was a randomized complete-block design with three replicates. Treatment effects and their interactions on measured and calculated variables were evaluated by analysis of variance (ANOVA) for a linear mixed model in each experiment separately using InfoStat Profesional v1.1 (Di Rienzo et al., 2011). Radiation, photoperiod and their interaction were considered to be fixed effects, while block was considered a random effect. Tukey tests were used to determine significant differences ($\alpha = 0.05$) between means. In the text, mean ± 1 standard error is presented. Relationship between variables was analysed by regression and correlation analysis using GraphPad Prism v5.01 (Graphpad Software, San Diego, USA). The mean of the replicates was used for linear regression and correlation analyses. When comparing the slopes and *Y*-intercepts between treatments, a dummy variable regression model was used with orthogonal contrasts in InfoStat Profesional v1.1 (Di Rienzo et al., 2011). The *X*-intercepts were compared through their 95% confidence intervals using GraphPad Prism v5.01 (GraphPad Software, San Diego, USA).

3. Results

3.1. Environmental conditions

As a result of contrasting sowing dates, mean temperatures explored by control treatments during the vegetative phase (VE-R1) in Exp1 were higher (25.0 °C) than in Exp2 (21.4 °C) while mean temperatures during the reproductive phase (R1–R7) had the opposite trend: 21.3 °C in Exp1 and 24.6 in Exp2. Within each experiment, as a consequence of the effects of photoperiod extension treatments on crop development (see Section 3.2), mean temperatures during the reproductive phase were lower when photoperiod was extended, with respect to the control: 19.1 °C (Exp1, 3 h), 24.1 °C (Exp2, 1.5 h) or 23.2 °C (Exp2, 3 h).

The combination of sowing dates and treatments produced a relative wide range of photoperiod (12.4–16.9 h) and incident radiation explored by the crop during R1–R7 (Table 1). In control treatments, mean photoperiod during post-flowering phases in Exp1 was shorter than in Exp2, while the 3 h photoperiod treatment of Exp1 explored similar mean photoperiod to the control photoperiod treatment of Exp2. Daily incident radiation during reproductive phases was lower in Exp1 than in Exp2, and thereby shaded treatments of Exp2 received similar radiation levels to that of the unshaded treatments of Exp1.

3.2. Crop development

The duration of the vegetative phase (VE-R1) – prior to treatment initiation – was 30.6 and 37.0 thermal days in Exp1 and Exp2, respectively. Photoperiod extension treatments increased post-flowering phase duration when expressed both in calendar and thermal days. Shading treatments slightly delayed crop development and maturity; however effects were only significant in Exp2 and under control and 3 h extended photoperiod treatments (Table 1).

When duration of the R1–R7 phase was plotted against mean phase's photoperiod, a common function was fit for data from all treatments and both experiments. The slope of this function, which indicates the photoperiod sensitivity of the reproductive phase, was 13.2 ± 1.1 td h^{-1} ($r^2 = 0.94$ $p < 0.01$). Within the

Table 1
Mean photoperiod and mean daily incident photosynthetically active radiation (iPAR) explored by the crop during post-flowering (R1–R7). Post-flowering (R1–R7) duration is expressed in calendar (d) and thermal days (td).

	Photoperiod (h)	iPAR ($MJ\,m^{-2}\,d^{-1}$)	Post-flowering duration	
			(d)	(td)
Exp1				
Unshaded				
Control	12.4 b	4.1 a	63.0 b	57.3 b
3 h	15.0 a	3.4 b	92.3 a	80.2 a
Shaded				
Control	12.4 b	2.5 c	64.3 b	58.5 b
3 h	14.9 a	2.0 d	96.7 a	83.5 a
Photoperiod	**	**	**	**
Shading	ns	**	ns	ns
Photoperiod × shading	ns	ns	ns	ns
Exp2				
Unshaded				
Control	14.5 c	8.5 a	88.0 e	84.3 e
1.5 h	16.0 b	8.0 b	110.0 c	100.6 c
3 h	16.9 a	7.4 c	123.0 b	114.7 b
Shaded				
Control	14.4 c	5.1 d	92.0 d	88.1 d
1.5 h	15.7 b	4.8 d	106.0 c	100.3 c
3 h	16.8 a	4.3 e	131.0 a	121.1 a
Photoperiod	**	**	**	**
Shading	ns	**	**	**
Photoperiod × shading	ns	ns	**	**

Different letters indicate significant differences between means ($p < 0.05$) according to Tukey's multiple comparison test within experiments. Not significant (ns), $p < 0.01$ (**).

whole reproductive phase, duration of the sub-phase R1–R3, was the most sensitive to photoperiod and thereby the phase that showed the longest delay due to photoperiod extension (Fig. 1). In fact, photoperiod sensitivity of the R1–R3 phase was higher ($11.0 \pm 2.3\,td\,h^{-1}$; $r^2 = 0.75$ $p < 0.01$) than the sensitivity of the R3–R7 phase ($2.6 \pm 1.9\,td\,h^{-1}$; $r^2 = 0.19$ $p = 0.21$).

3.3. Growth and yield

Both photoperiod and shading treatments modified cumulative photosynthetically active radiation intercepted during post-flowering (PAR_{R1-R7}) (Table 2). As complete radiation interception was achieved simultaneously in all plots before treatments were applied (at R1), photoperiod extension increased PAR_{R1-R7} due to the prolongation of the R1–R7 phase, while shading decreased PAR_{R1-R7} due to the reduction in daily incident radiation during that phase.

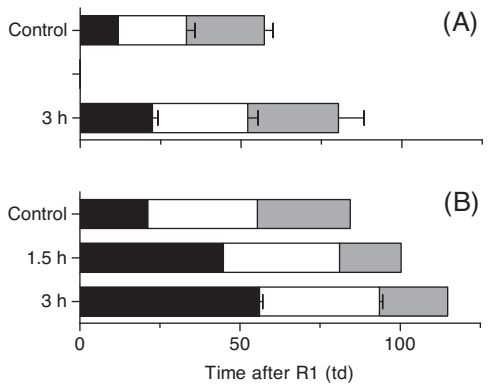

Fig. 1. Duration of reproductive phases from beginning-bloom to beginning-pod stage (R1–R3, black bars), beginning-pod to full-seed stage (R3–R6, white bars) and full-seed to beginning-maturity stage (R6–R7, grey bars) expressed in thermal days (td) for unshaded plants grown under control photoperiod (control) or extended photoperiod (1.5 or 3 h) during R1–R7 phase in Exp1 (A) and Exp2 (B). Error bars, representing the standard deviation, are shown when larger than the line width.

Above-ground biomass at maturity was associated with photosynthetically active radiation accumulated during the whole crop cycle, across all experiments and treatments ($r = 0.90$ $p < 0.01$). In both experiments, photoperiod extension increased above-ground biomass with respect to the control (Table 2). In Exp1, 3 h photoperiod extension significantly increased above-ground biomass by 51% ($p < 0.05$) independently of the shading treatment. In Exp2, there was a significant interaction ($p < 0.05$) between photoperiod and radiation treatments on above-ground biomass. Consequently, the magnitude of the biomass increment under extended photoperiod depended on the shading treatment. In unshaded plots, 1.5 and 3 h photoperiod extension increased above-ground biomass by 43 and 26%, respectively, while the increases under shade were over two-fold with respect to the shaded control photoperiod treatment. In Exp2, shading significantly reduced above-ground biomass by 48% when plants were grown under control photoperiod, while biomass tended to be reduced by 20% and 8% when photoperiod was extended by 1.5 and 3 h, respectively. In Exp1, the same tendencies were observed, although the interaction between shading and photoperiod treatments was not significant ($p = 0.33$). Thus, photoperiod extension increased biomass by 30 and 80% in unshaded and shaded treatments, respectively; and shading tended to reduce above-ground biomass by 28% when the crop was grown under control photoperiod, but had no effect when plots were exposed to extended photoperiod.

Harvest index was not significantly affected by either shade or photoperiod extension (Table 2). Nevertheless, biomass partitioning to reproductive structures through time differed between photoperiod treatments (Fig. 2). In Exp1, photoperiod extension delayed (although not significantly in statistical terms) by 8 thermal days the beginning of partitioning to reproductive structures and significantly ($p < 0.05$) diminished partitioning rate. Similarly, in Exp2 photoperiod extension significantly delayed the beginning of partitioning by more than 20 thermal days, but had no significant effect on partitioning rate to reproductive organs. Shading had no effect on the beginning nor on the rate of partition to reproductive structures.

Yield tended to be increased by photoperiod extension and reduced by shading (Table 2), but these effects were only significant ($p < 0.05$) in Exp2. In Exp1 extended photoperiod treatments tended to increase yield by 33% with respect to the control, independently of the shading treatment ($p = 0.13$). In Exp2, a significant interaction between photoperiod and radiation was evident for yield determination. In unshaded treatments, the 1.5 h photoperiod extension significantly increased yield 46% with respect to the control, however, when photoperiod was extended by 3 h, yield was similar to the control. When plants were grown under shade, both photoperiod extension treatments (1.5 and 3 h) significantly increased yield by ca. 2.5 fold with respect to the control. Thus the effect of shading on yield was much stronger under control photoperiod, where it significantly reduced yield by 50%, than under extended photoperiod, where yield was not significantly reduced. Variation in yield, across all experiments and treatments, was associated with differences in above-ground biomass ($r = 0.98$ $p < 0.01$) rather than harvest index ($r = -0.35$ $p = 0.31$).

3.4. Yield components

Within yield numerical sub-components, yield variation was associated to changes in seed number per unit area across all experiments and treatments ($r = 0.97$ $p < 0.01$). In Exp1, the 3 h photoperiod extension significantly ($p < 0.05$) increased seed number per unit area by 50% with respect to the control, independently of the shading treatment (Table 2). In Exp2, the effect of shading on

Table 2
Cumulative photosynthetically active radiation intercepted during post-flowering (PAR_{R1-R7}), above-ground biomass, harvest index, yield, seed number, individual seed weight, pod number and seeds per pod.

	PAR_{R1-R7} ($MJ\,m^{-2}$)	Biomass ($g\,m^{-2}$)	Harvest index	Yield ($g\,m^{-2}$)	Seed number (m^{-2})	Seed weight (mg)	Pod number (m^{-2})	Seeds per pod
Exp1								
Unshaded								
Control	252.6 b	834 a	0.39 a	327 a	2176 a	150 a	1011 a	2.15 a
3 h	297.3 a	1086 a	0.38 a	405 a	2985 a	137 a	1342 a	2.23 a
Shaded								
Control	155.2 d	602 a	0.44 a	271 a	1816 a	146 a	807 a	2.24 a
3 h	183.9 c	1083 a	0.36 a	390 a	3018 a	128 a	1275 a	2.37 a
Photoperiod	**	*	ns	ns	*	ns	*	ns
Shading	**	ns	ns	ns	ns	ns	ns	ns
Photoperiod × shading	**	ns	ns	ns	ns	ns	ns	ns
Exp2								
Unshaded								
Control	716.3 c	1955 b	0.36 a	698 b	3544 b	197 a	2146 b	1.66 ab
1.5 h	827.5 b	2796 a	0.35 a	1017 a	5479 a	183 a	3270 a	1.68 ab
3 h	879.9 a	2464 ab	0.29 a	716 b	3965 b	180 a	2703 ab	1.47 b
Shaded								
Control	456.4 f	1009 c	0.31 a	323 c	1769 c	178 a	969 c	1.85 a
1.5 h	524.2 e	2243 ab	0.36 a	809 ab	4388 ab	184 a	2570 b	1.71 ab
3 h	571.9 d	2277 ab	0.37 a	843 ab	4447 ab	191 a	2688 ab	1.65 ab
Photoperiod	**	**	ns	**	**	ns	**	*
Shading	**	**	ns	**	**	ns	**	*
Photoperiod × shading	**	*	ns	**	**	ns	**	ns

Different letters within the column indicate significant differences between means ($p < 0.05$) according to Tukey's multiple comparison test within each experiment. Not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**).

seed number, and its significant interaction ($p < 0.05$) with photoperiod was similar to that observed on yield. In unshaded treatments, the 1.5 h photoperiod extension significantly increased seed number by 55% with respect to the control, but when photoperiod was extended 3 h, seed number was not significantly different to the control. Under shade, both photoperiod extension treatments (1.5 and 3 h) significantly increased seed number by ca. 2.5 fold with respect to the control.

No significant differences in individual seed weight were observed among treatments (Table 2). Nevertheless, in Exp1 seeds tended to by lighter ($p = 0.10$) when photoperiod was extended; therefore, the significant increase in seed number in that experiment was not reflected in a significant increase in yield. The source/sink relationship at full-seed stage (R6) (estimated as the ratio between aboveground vegetative biomass – source – and the number of flowers and pods per m^2—sink) was 0.20 ± 0.03 in Exp1 and 0.41 ± 0.06 in Exp2 without significant photoperiod extension and shading effects.

In both experiments, increases in seed number per m^2 were closely associated with increases in pods per m^2 ($r = 0.97$ $p < 0.01$), while the relationship between seed number and seeds per pod was weak and opposite between experiments ($r = 0.56$ $p = 0.44$ Exp1, $r = -0.49$ $p = 0.33$ Exp2). Seeds per pod responded slightly to photoperiod extension in Exp2 reducing its number by 3 and 11% under 1.5 and 3 h, respectively, when compared to the control photoperiod independently of the shading treatment (Table 2). Shading significantly increased seeds per pod by 8% in Exp2 with respect to the unshaded control, independently of the photoperiod treatment.

Pod number per m^2 was positively and linearly associated with nodes per m^2 (with a slope of 1.23 ± 0.11 pods per node, $r^2 = 0.94$ $p < 0.01$) for all treatments and experiments. Photoperiod extensions and shading altered pods per m^2 as a result of their effects

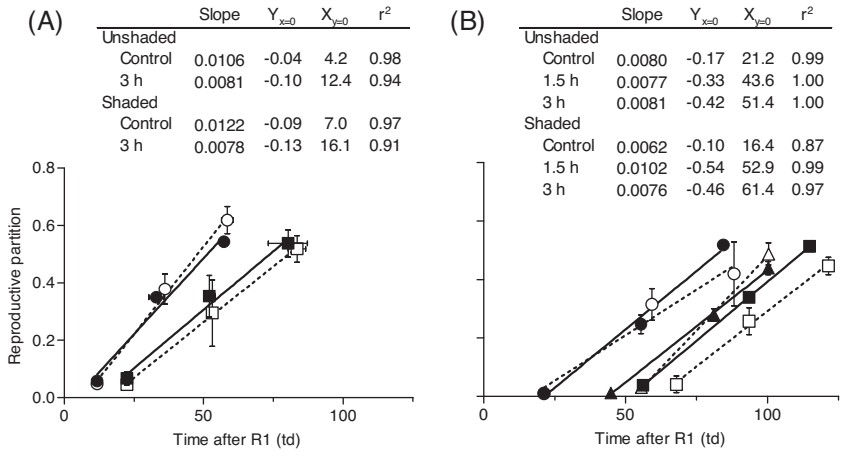

Fig. 2. Mean reproductive biomass partition to reproductive structures as a function of thermal days after flowering (R1) in Exp1 (A) and Exp2 (B). Closed symbols and full line correspond to unshaded treatments and open symbols and dotted line to shaded treatments, for control photoperiod (circles), extended 1.5 h (triangles) or 3 h (squares). Sampling was made for each treatment at stages R3, R6 and R7. Error bars represent the standard deviation. Lines were fitted by linear regression of the means for each treatment. The slope, *Y*-intercept when $X = 0$, *X*-intercept when $Y = 0$ (beginning of active reproductive partition) and coefficient of determination are presented in the inset table.

Table 3
Yield sub-components node number per m^2 and pods per node in the main stems and branches, and the total values (stems and branches).

	Mainstems		Branches		Total	
	Node number (m^{-2})	Pods per node	Node number (m^{-2})	Pods per node	Node number (m^{-2})	Pods per node
Exp1						
Unshaded						
Control	494 bc	1.18 ab	374 a	1.17 a	868 ab	1.18 a
3 h	670 ab	1.40 a	568 a	0.72 bc	1239 a	1.09 a
Shaded						
Control	465 c	1.16 b	255 a	0.98 ab	720 b	1.10 a
3 h	790 a	1.24 ab	465 a	0.62 c	1254 a	1.01 a
Photoperiod	**	*	*	**	**	ns
Shading	ns	ns	ns	ns	ns	ns
Photoperiod × shading	ns	ns	ns	ns	ns	ns
Exp2						
Unshaded						
Control	820 bc	1.44 a	948 ab	1.13 a	1768 bc	1.21 a
1.5 h	1034 a	1.47 a	1550 a	1.21 a	2584 a	1.25 a
3 h	1012 a	1.18 a	1523 a	1.13 a	2535 a	1.07 ab
Shaded						
Control	764 c	0.92 a	490 b	0.67 b	1253 c	0.78 b
1.5 h	963 ab	1.16 a	1442 a	1.10 a	2405 ab	1.07 ab
3 h	1120 a	1.26 a	1093 ab	1.33 a	2213 ab	1.23 a
Photoperiod	**	ns	**	**	**	ns
Shading	ns	*	*	*	*	*
Photoperiod × shading	*	ns	ns	**	ns	*

Different letters within the column indicate significant differences between means ($p < 0.05$) according to Tukey's multiple comparison test within each experiment. Not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**).

on node number and pods per node in the main stem and branches (Table 3). Neither photoperiod extension nor shading affected the rate of leaf appearance on the main stem; the phyllochron was 3.99 ± 0.03 and 3.50 ± 0.03 thermal days per leaf in Exp1 and Exp2, respectively.

In Exp1 and independently of the shading treatment, the 3 h photoperiod extension significantly increased ($p < 0.05$) node number by 52% and pods per node by 13% on the main stems (Table 3). At the same time, photoperiod extension increased nodes on branches by 64% but reduced pods per branch-node by 38%. As a result, the 3 h photoperiod extension significantly increased ($p < 0.05$) pod number per m^2 in main stems by 72%, but had no significant effect ($p = 0.98$) on branch-pod number. In Exp1, shading reduced branch-nodes by 24% ($p = 0.19$), and pods per node by 7 and 15% in main stems ($p = 0.11$) and branches ($p = 0.06$), respectively, independently of the photoperiod treatment, albeit none of these effects were statistically significant.

In Exp2, photoperiod extensions and shading treatments also altered pods per m^2 as a result of their effects on node number and pods per node in main stems and branches (Table 3). In this experiment there was a significant interaction ($p < 0.05$) between photoperiod and shading in determining nodes per m^2 on the main stem: the magnitude of the photoperiodic effect (23 to 47% increment) depended on the shading treatment. The number of branch-nodes was highly and significantly ($p < 0.01$) increased by 95% when photoperiod was extended, and significantly ($p < 0.05$) reduced by 25% under shading. Pods per main stem-node were significantly ($p < 0.05$) reduced 18% by shading but photoperiod treatments did not affect this yield component. There was a significant interaction between photoperiod and shade on pods per branch-node: only under shade, the photoperiod extension significantly ($p < 0.05$) increased pods per branch-node by 81% with respect to the shaded control photoperiod treatment. As a result of photoperiod effects on node number and pods per node in Exp2, the number of pods per m^2 was significantly increased ($p < 0.05$) by 39% and by 139% on main stems and branches, respectively, under extended photoperiod treatments with respect to the control, independently of the shading treatment. Also, shading significantly reduced pod number ($p < 0.05$) by 18 and 29% on main stems and branches, respectively, with respect to the control independently of the photoperiod treatment.

Nodes per m^2 increased as time to full-seed (R6) stage was delayed as shown in Fig. 3A, explaining, at least partially, how photoperiod extension increased pod number per unit area through increases in node production. Using calendar days instead of thermal days did not modify the tendency shown in Fig. 3 (data not shown). Shaded treatments responded in the same way to increases in time to full-seed, though usually with less nodes per m^2. Fig. 3B shows the relationship between nodes on main stems and branches against time to R6, showing that branches gained more importance over total node number (and therefore, on pod number), as time to R6 increased due to sowing date (differences between experiments) or photoperiod treatments. Interestingly, the number of main stem-nodes of the 3 h extended photoperiod treatment of Exp1 and the control photoperiod treatment of Exp 2 was similar, in line with the similarity in their post-flowering mean photoperiod and duration.

As a result of the high correlation within yield subcomponents, yield was positively and linearly associated with nodes per m^2 with a unique relationship for all treatments and experiments (Fig. 4A). Yield was also positively and linearly associated with PAR_{R1-R7} (Fig. 4B); however, control photoperiod treatments presented a consistent pattern of negative residuals with respect to the fitted relationship between yield and PAR_{R1-R7}. Although both relationships were significant in statistical terms, the relationship between yield and nodes per m^2 was more robust than that of yield and PAR_{R1-R7}.

Although the rate of node production and leaf appearance was not altered by photoperiod extension, long days had a direct effect on the development of the stem apex, retarding the shift to reproductive development, consequently, more nodes were produced in the main stems and branches of plants exposed to extended photoperiod. However, this direct photoperiodic effect on node production was accompanied by the retardation of the crop's reproductive development. As the post-flowering phase was extended, more iPAR was captured by the crop; therefore, part of the photoperiod extension effects on nodes per m^2 was a result of its indirect effect through PAR_{VE-R6}. Our results also suggest that the magnitude

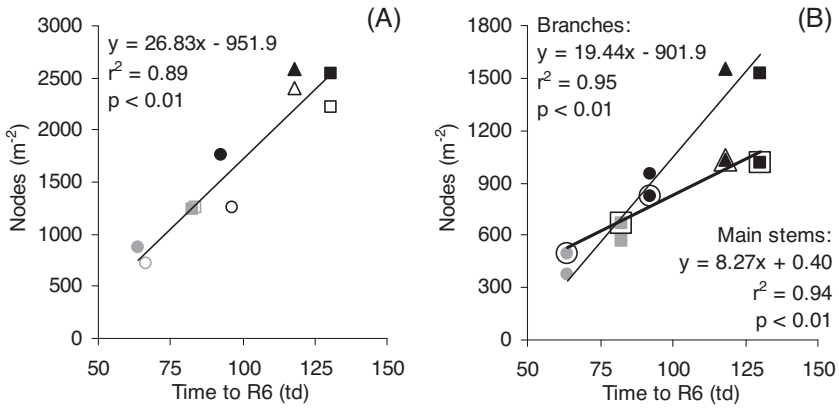

Fig. 3. Relationship between nodes per m^2 and time to full seed (R6) for whole plants (A) and main stems and branches separately (B) in Exp1 (grey) and Exp2 (black) for control photoperiod (circles), extended 1.5 h (triangles) or 3 h (squares). In A, closed and open symbols correspond to unshaded and shaded treatments respectively and the full line and equation represent the fitted model for both experiments and all treatments. (B) only contains data from the unshaded treatments; the framed symbols, thick line and lower equation represent the main stem data and linear regression; and the unframed symbols, thin line and upper equation represent data from branches and their linear regression.

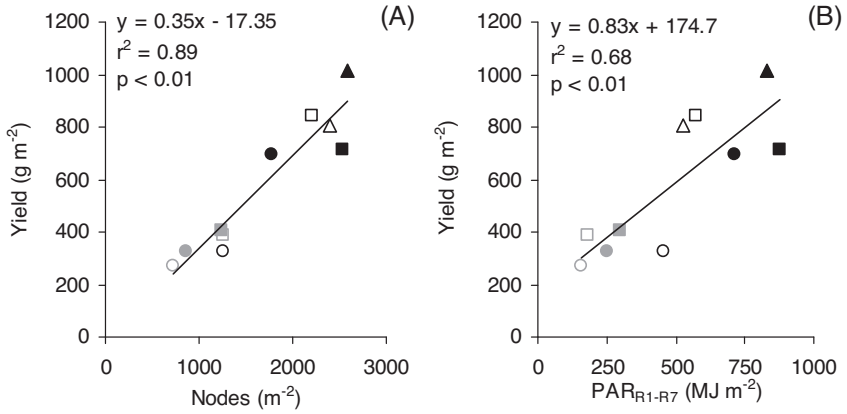

Fig. 4. Relationship between yield and nodes (A) or cumulative photosynthetically active radiation intercepted during post-flowering (PAR_{R1-R7}) (B) in Exp1 (grey) and Exp2 (black). Closed and open symbols correspond to unshaded and shaded treatments, respectively, for control photoperiod (circles), extended 1.5 h (triangles) or 3 h (squares). The full line and equation in (A) and (B) represent the fitted model for both experiments and all treatments.

of this latter effect may depend on the environmental conditions. Due to the different sowing dates, mean iPAR during VE-R6 was 37% lower on Exp1 compared with Exp2 (5.94 vs. 9.45 MJ m^{-2}). Therefore, increments in PAR_{VE-R6} when time to R6 was enhanced by photoperiod were lower in Exp1 compared with Exp2. Nevertheless, within each experiment and shading treatment, there was a linear relationship between time to R6 and PAR_{VE-R6} driven by the photoperiodic effect on time to R6.

The photoperiod extension effect on nodes per m^2 associated with increases in cumulative radiation due to extended duration of the phase was considered an indirect photoperiodic effect. To estimate how much of the photoperiod extension increase in nodes per m^2 corresponded to the increase in PAR_{VE-R6} (indirect photoperiodic effect on nodes per m^2), we used a regression line between nodes per m^2 and PAR_{VE-R6} fitted for the control photoperiod treatments (unshaded and shaded of both experiments; dashed line Fig. 5). The difference between the observed nodes per m^2 and the estimated indirect photoperiodic effect on nodes per m^2 was considered the direct photoperiodic effect on nodes per m^2. As an example, the arrows in Fig. 5 illustrate the photoperiodic effect for the unshaded 3 h photoperiod extension treatment of Exp2. The direction of the empty arrow illustrates the indirect photoperiodic effect (the increase in nodes per m^2 due to the increase in PAR_{VE-R6}), starting at the appropriate control treatment and

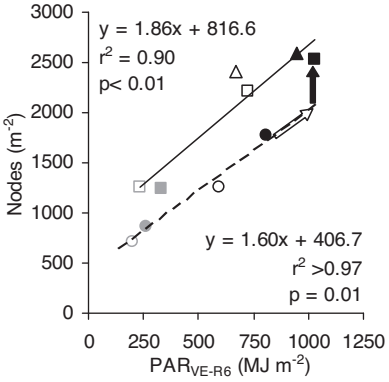

Fig. 5. Relationship between nodes per m^2 and cumulative photosynthetically active radiation intercepted from emergence to full seed stage (PAR_{VE-R6}) in Exp1 (grey) and Exp2 (black). Closed and open symbols correspond to unshaded and shaded treatments, respectively, for control photoperiod (circles), extended 1.5 h (triangles) or 3 h (squares). The dashed line and lower equation represent the fitted model for the photoperiod controls. The full line and upper equation represent the fitted model for the photoperiod extension treatments (1.5 and 3 h). As an example, the empty and full arrows indicate the estimated indirect and direct photoperiodic effect on node number, respectively, for the unshaded 3 h photoperiod extension treatment of Exp2.

Table 4
Mean ± standard deviation indirect and direct photoperiodic extension effects on nodes per m^2. The total (indirect + direct) photoperiod extension effect on nodes per m^2 was calculated as the number of nodes per m^2 under control photoperiod minus the number of nodes per m^2 under extended photoperiod. The indirect photoperiodic effect on nodes per m^2 (caused by the increased cumulative radiation) was estimated using a linear model adjusted to the control photoperiod treatments: Δ nodes per $m^2 = 1.60 \times \Delta\ PAR_{VE\text{-}R6} + 406.7$ (see Fig. 5). Direct photoperiodic effects were considered those independent of the cumulative radiation intercepted during VE-R6 and were calculated as the difference between total and indirect photoperiodic effects.

	Photoperiod extension effect on nodes per m^2	
	Indirect effect (nodes per m^2)	Direct effect (nodes per m^2)
Exp1		
Unshaded		
3 h	115.2 ± 10.3	301.5 ± 189.8
Shaded		
3 h	54.2 ± 5.8	476.7 ± 166.4
Exp2		
Unshaded		
1.5 h	220.3 ± 15.7	675.1 ± 88.9
3 h	353.3 ± 12.6	481.2 ± 224.5
Shaded		
1.5 h	116.9 ± 16.4	925.2 ± 217.5
3 h	205.7 ± 11.4	609.7 ± 284.7

ending at the value of nodes per m^2 estimated with the regression line. The full arrow illustrates the direct photoperiodic effect on nodes per m^2. The estimated values for each treatment are detailed in Table 4. The magnitude of the direct photoperiodic effect seems to interact with the radiation background, as the direct effect seems larger in shaded treatments with respect to their unshaded control.

4. Discussion

As expected, photoperiod extension during the post-flowering phase prolonged its duration with respect to the control (Han et al., 2006; Kantolic et al., 2013; Kantolic and Slafer, 2001; Kumudini et al., 2007), with the concomitant increases in cumulative photosynthetically active radiation and above-ground biomass production (Kantolic et al., 2013; Kantolic and Slafer, 2001). This indivisible increment in cumulative radiation and biomass due to photoperiod extensions generally overshadows possible direct photoperiodic effects on yield determination that are independent of the extra cumulative radiation due to the extension of the post-flowering phase. The simultaneous photoperiod extension and shading treatments used in this work allowed us to analyse soybean yield determination in response to photoperiod extension somewhat independently of the increased cumulative radiation.

Surprisingly, when photoperiod was extended, biomass reduction caused by shading was of a much lower magnitude, or even negligible, compared to the reduction observed under control photoperiod. Although the photoperiod × shading interaction was significant only in Exp2, the same tendency was observed in both experiments. As far as we are aware, this interaction between shade and photoperiod in soybean biomass production has not been reported before and suggests that a longer reproductive phase might confer tolerance to stress as proposed by Egli (2010b) and better adaptation to regions with poor incident radiation during post-flowering. However, attention should be paid when comparing these results, obtained with mild shades (i.e. reducing 35% of incident radiation) during the whole reproductive stage, with those in which shading treatments were of higher magnitude (i.e. 60–80% reduction of incident radiation) or during shorter periods of time (Egli, 2010a; Egli et al., 1985; Jiang and Egli, 1993).

Together with the delay in reproductive development, partitioning to reproductive structures was also delayed under extended photoperiod at crop level. In agreement with our results, but at fruit level, Zheng et al. (2003) found that fruit elongation was anticipated under short photoperiod. In the present study, partitioning to reproductive organs started shortly earlier than R3, being the R1–R3 phase the most sensitive to photoperiod. Photoperiod extension delayed the onset of partitioning but did not affect biomass accumulation or the partitioning rate. As a result, when partitioning to reproductive organs began, plants under extended photoperiod had more nodes and a larger biomass and therefore partitioned more assimilates in absolute terms. This is an interesting novel finding that clears up post-flowering photoperiodic effects on biomass partitioning and yield forming processes.

Photoperiod extension tended to increase yield and seed number (as shown by Guiamet and Nakayama, 1984a; Kantolic and Slafer, 2001, 2007) while shading tended to reduce it (as reported by Egli, 2010a; Egli and Yu, 1991; Schou et al., 1978). In Exp2, the unshaded 3 h photoperiod extension treatment raised yield potential (as shown by its number of nodes per m^2) but did not increase yield, possibly due to the worse environmental conditions explored during pod set and seed filling phases (however these conditions did not impair yield fulfilment in the shaded 3 h photoperiod extension treatment). Again, the effect of shading was stronger under control photoperiod, disclosing an interaction between photoperiod and shading treatments in soybean yield determination. Variations in yield were explained by changes in seeds, pods and nodes per unit area in both photoperiod and shading treatments. Nodes per unit area were associated with time to seed filling when photoperiod was extended as observed when comparing cultivars with different cycles (Egli, 1993). Different from previous works, no significant effects on individual seed weight were observed in response to photoperiod (Kantolic, 2006; Morandi et al., 1990; Thomas and Raper, 1976) or shading treatments (Egli, 1997), probably because in the present study treatments were initiated prior to the beginning of grain filling producing re-adjustments in seed number determination instead of seed weight. Moreover, at the onset of the full-seed stage, the source/sink relationship was the same for all the treatments within each experiment, reinforcing the idea that plants under extended photoperiod did not experience higher source limitations in spite of their greater number of seeds.

Shade and photoperiod effects on yield and its components were similar between experiments, but were generally of a lower magnitude and significance in Exp1 compared to Exp2. This difference could be attributed to the late sowing of Exp1, which exposed the crop to shorter photoperiods and lower temperature and radiation levels during the reproductive phases with respect to Exp2. Kantolic and Slafer (2001), Kantolic et al. (2013) reported interaction between the photoperiod extension response and sowing dates, and found that extended photoperiod promoted more yield gains when crops were sown in optimal than in late sowing dates. In the late sown Exp1, crop cycle was shorter than that of Exp2, and pods were mainly located on the main stem while in Exp2 yield was more evenly distributed among main stems and branches, augmenting the contribution of pods from branches to yield. This could explain why photoperiod extensions increased pods per node in main stems in Exp1 and on branches in Exp2, and suggests that the response was more evident in the sector of the plant that contributed more to yield. The lack of photoperiod extension effects on pods per node in main stem nodes observed in Exp2 differed from that observed in Exp1 and contrasts with previous findings observed by our group (Kantolic et al., 2013). A possible explanation is that pods per node were calculated from the totality of the nodes produced, and not exclusively from reproductive nodes, and in Exp2 there were several non-reproductive nodes in photoperiod extended treatments, either because they bore a branch or they remained empty until maturity. A more detailed analysis of

photoperiod effects at node level could shed some light into the photoperiodic role on pods per node determination.

Even though changes in the spatial distribution of pods can be expected in response to shade and photoperiod extension (Kantolic et al., 2013) as was also observed in our experiments (data not shown), a common relationship was found between pods and nodes per unit area for all treatments and both experiments, indicating that node production was the main driving force for variation in pods per unit area. As all the plots exposed to photoperiod and shading treatments reached complete radiation interception before treatments initiation (at R1), the positive relationship between pods and nodes per unit area does not reflect differences in radiation capture. Numerous works reported a positive relationship between pods and nodes per unit area (Ball et al., 2001; Board and Tan, 1995; Egli and Bruening, 2000; Kahlon et al., 2011). Quijano and Morandi (2011) reported that the number of initiated pods increased even when nodes per unit area augmented as a result of leaflet defoliation treatments, although the final number of pods at maturity diminished if light interception decreased below 95%. The range of nodes per square meter explored in the present work (from 720 to 2584 per m^2) greatly exceeds those explored in previous works. However, we found that pods increased linearly with increasing nodes per unit area, different to the curvilinear response found by Egli (2013) when different cultivars or plant populations were included as the source of variation of nodes per unit area.

As was recently addressed by Egli (2013), it is difficult to determine if the increase in pods per unit area is a result of the high crop growth rate or the accompanying production of more nodes. We propose an approximation to distinguish between indirect and direct photoperiodic effects on node production. The relationship presented in Fig. 5 provides a graphical quantification of the indirect photoperiodic effect (i.e. resultant of the increased PAR offer consequent of the prolonged cycle) and the direct effect on node production (those nodes coming from the continued vegetative development).

In previous published works, when photoperiod was extended from R3 to maturity, post-flowering photoperiodic effects on seed number were exclusively considered as "indirect effects" (Kantolic et al., 2013; Kantolic and Slafer, 2001, 2005). Differently, we found that direct photoperiodic effects were consistently higher than indirect effects, especially under shade conditions, when photoperiod was extended from R1 to maturity. The differences between our work and previous literature can be attributed to the moment when photoperiod treatment was applied, as in the present work photoperiod extension treatments were applied at R1 while in previous works photoperiod extension treatments were applied at R3 stage. The direct photoperiodic effects were related to the delayed initiation of reproductive partitioning and the continued production of nodes. These processes occurred during the R1–R3 phase that was markedly prolonged when we began the photoperiod extension at R1, while when the photoperiod extension is initiated at R3 these effects might not be observed.

The direct effect of photoperiod extension on node number could be interpreted as a result of the delay of reproductive development that allows continuation of vegetative growth in the stem apex (Guiamet and Nakayama, 1984a; Han et al., 2006). Recently, two *phyA* genes (phytochrome A), *E3* and *E4*, were linked to persistent vegetative activity of the shoot apical meristem during long days in indeterminate soybeans (Xu et al., 2013). This finding shows that the perception of photoperiod is essential for determining the final number of nodes, making evident the direct nature of the response.

How photoperiod extension enhanced yield through node production beyond its effect on cumulative intercepted radiation has not been solved yet. The direct photoperiodic effect on node production can be explained by physiological and molecular processes, but how those nodes translate into more yield without further increases in radiation has no clear explanation and probably encloses direct photoperiodic effects on pod establishment.

It could be hypothesised that photoperiod extension increased pod number by enhancing flower production, as variation in flowers per plant often accounts for much of the variation in pods per plant (Egli, 2005; Jiang and Egli, 1993). The reasons associated with this speculation are: first, there is a close relationship between the number of nodes and flowers produced at plant (Egli, 2005) and crop levels (Egli, 2013), and second, flowers produced per node depend on the duration of the flowering period (Egli and Bruening, 2000) rather than the assimilate availability. The duration of the flowering period is, in turn, prolonged as development is delayed under long photoperiod (Guiamet and Nakayama, 1984b; Summerfield et al., 1998). However, in our experiments plant pod number is unlikely to be limited by the number of flowers, as considerable levels of flower and fruit abortion were observed in the field (data not shown). Nevertheless, it cannot be discarded that photoperiod extension might have altered the temporal distribution of flower and pod production affecting pod set (Egli, 2005; Egli and Bruening, 2006a).

Given that interferences among pods mainly occur within a node (Bruening and Egli, 1999, 2000), photoperiod extension might have increased pod number relieving intra-nodal interactions through the delay of pod growth. Charles-Edwards et al. (1986) proposed that the number of potential grain sites per unit area is a function of daily canopy photosynthesis partitioned to reproductive growth and the minimum flux of assimilate required to avoid abortion of a potential grain site. As individual pod elongation was initially delayed under extended photoperiod (Zheng et al., 2003), it can be speculated that their daily demand for assimilates was diminished, alleviating temporal intra-nodal interactions. Our results suggest that photoperiod may alleviate this source limitation through direct effects on the timing of pod growth beginning. However, these suggestions deserve further analysis.

Besides, it has been demonstrated that in soybean, the interferences between fruits at node level are not exclusively dependent on assimilate availability (Bruening and Egli, 1999, 2000; Heitholt et al., 1986; Huff and Dybing, 1980) since pod and seed number per node responded curvilinearly to increasing nodal carbon input (Bruening and Egli, 1999, 2000). Therefore, extra assimilates allocated to developing fruits located at the same node may not result in extra pods at maturity, decreasing the efficiency with which assimilates are used to produce pods and behaving as a sink limited situation as proposed by Egli (2013), for crops with different node number due to cultivar maturity and population treatments).

In our experiments the manipulation of photoperiod and radiation generated a range of radiation per node (PAR_{R1-R7} $node^{-1}$). As photoperiod extension increased the number of nodes per square meter in a higher proportion than PAR, this ratio diminished under extended photoperiod. Additionally, shading also reduced intercepted radiation per node, as it reduced more PAR than nodes per unit area (except in the control photoperiod treatment of Exp2). Ignoring uneven PAR distribution within canopy layers, PAR per node can be considered a gross estimator of assimilate supply at node level as assimilate distribution within the plant changes dynamically in response to sink demand (Egli et al., 1976; Fellows et al., 1979; Thorne and Koller, 1974). In these terms, extended photoperiod reduced the amount of assimilates per node.

A further analysis of our results indicates that radiation use efficiency defined in terms of seed yield ($RUE_{Y,PAR}$, estimated as the ratio between yield and PAR_{R1-R7}) was negative and linearly associated with PAR per node across all treatments and experiments (Fig. 6), this negative relationship is in accordance with the curvilinear relationship between seed number per node and increasing nodal carbon input found at isolated nodes (see Figure 6

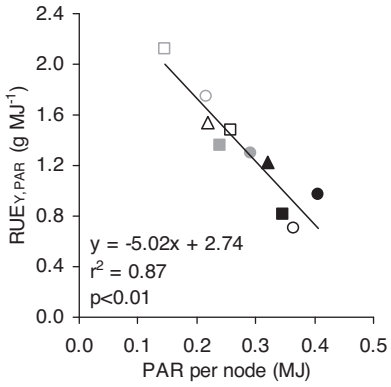

Fig. 6. Radiation use efficiency for yield during post-flowering ($RUE_{Y,PAR}$) as a function of cumulative photosynthetically active intercepted during the post-flowering phase ($PAR_{R1–R7}$) available per node, in Exp1 (grey) and Exp2 (black). Closed and open symbols correspond to unshaded and shaded treatments, respectively, for control photoperiod (circles), extended 1.5 h (triangles) or 3 h (squares). The line and equation represent the fitted model for both experiments and all treatments.

in Bruening and Egli, 1999; see Figure 1 in Bruening and Egli, 2000). Thus $RUE_{Y,PAR}$ increased when the source (assimilate supply) and the sinks (pods) are spatially distributed in more nodes. The spatial separation of developing pods in different nodes could result in a more efficient use of radiation to produce yield when photoperiod is extended, and should be integrated to assimilate-based models that predict pod number, together with dynamic aspects of flowering and pod set, as proposed by Egli (2005).

The increment in nodes per unit area caused by photoperiod extension might have also been altered the distribution of radiation and light quality within the canopy. An improvement of the light penetration – less saturated leaves – would increase radiation use efficiency (Loomis and Amthor, 1999). Increments in red light can directly enhance pod set in soybean plants (Heindl and Brun, 1983; Myers et al., 1987), but a denser canopy would reduce the proportion of red light within the canopy. Unfortunately, we did not measure these attributes, but their probable effects on growth and yield cannot be discarded.

Summarizing, shading tended to reduce yield and photoperiod extension to increase it through their effects on node number per unit area, affecting pod and seed number. Node number augmented with increasing PAR (i.e. shading effect or indirect photoperiodic effect) and also as a result of direct photoperiodic effects on crop development. As a result, more pods were established per unit of PAR under extended photoperiod. We hypothesize that photoperiod extension enhanced $RUE_{Y,PAR}$ due to relieved intra-nodal interferences, and suggest further investigations at node level to continue unveiling the complex process of pod number determination in soybean crops.

Acknowledgements

The authors gratefully acknowledge P. J. Lo Valvo, C. Guillén and L. Pedace for their excellent technical assistance and the anonymous reviewers for helpful comments on the manuscript. MN currently holds a postgraduate scholarship from FAUBA funded by Monsanto Argentina. This research was funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT Raíces 1368 and PICT 1846) and University of Buenos Aires (UBACyT20020120100282).

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