



# Soybean shows an attenuated nitrogen dilution curve irrespective of maturity group and sowing date



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## ABSTRACT

It is generally accepted that the nitrogen (N) dilution curves relating shoot nitrogen concentration (%N) and shoot biomass ( $W$ ) are similar among genotypes from the same species. However, differences in the pattern of biomass ratio between lamina and stem ( $W_{\text{lam}}:W_{\text{ste}}$ ) with increasing biomass would change the curve. Shoot biomass at flowering also affect biomass and N allocation in legumes and would affect the N dilution. In soybean, maturity groups (MG) or sowing dates can lead to differences in  $W_{\text{lam}}:W_{\text{ste}}$  and in time from sown to flowering and hence the parameters of N dilution curves could be affected. The aims of this study were: (i) to determine the growth and allocation patterns of soybean as affected by maturity group (MG IV vs II) and sowing date and (ii) to establish allometric relations between growth and nitrogen, including N dilution curves. Our working hypothesis is that the reference N dilution curve in soybean is similar to the critical N dilution curve of non-legume C3 crops.

Four experiments were performed at Balcarce, Argentina during three growing seasons with the aim to generate a wide range of growing conditions. Two soybean cultivars were evaluated: DM2200 (MG II) and DM4970 (MG IV), sown in optimum dates for the region (mid-November). DM2200 was also sown late (early January). We sampled crops between  $W \approx 1 \text{ Mg ha}^{-1}$  and R5. Shoots were separated in three compartments: lamina ( $W_{\text{lam}}$ ), stem and petiole ( $W_{\text{ste}}$ ) and pod ( $W_{\text{pod}}$ ).

Despite the marked decrease in  $W_{\text{lam}}:W_{\text{ste}}$  with increasing biomass, the N dilution curve was attenuated in soybean, with no differences among cultivars of maturity groups II and IV, and sowing dates from November to January. We propose that early pod onset and the constant %N in lamina and stem between R1 and R5 attenuated the N dilution curve in all MG and sowing date. Timely-sown MGIV showed a greater  $W_{\text{lam}}:W_{\text{ste}}$  ratio at  $W = 1 \text{ Mg ha}^{-1}$  and a more pronounced depletion of the ratio with increasing biomass. However, the N dilution curves were similar between maturity groups II and IV, and sowing dates from November to January. This could be attributed to the lower %N in lamina at V6 in comparison to R1 in timely-sown MGIV.

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## 1. Introduction

Nitrogen concentration (%N) in shoots decreases during the growth cycle in dense canopies as the result of two processes: (i) the remobilization of N from shaded leaves at the bottom of the canopy to leaves at the top, assuming that N uptake rate is lower than crop growth rate (Field, 1983; Hirose and Werger, 1987; Pons and Percy, 1994; Sinclair and Horie, 1989) and (ii) the increase in the proportion of plant structural tissues with low nitrogen concentration in comparison with metabolic tissues with higher nitrogen

concentration (Caloin and Yu, 1984). Stem ( $W_{\text{ste}}$ ) and lamina ( $W_{\text{lam}}$ ) are mainly composed by structural and metabolic tissues, respectively (Gastal et al., 2015).

Nitrogen dilution curves have been evaluated for several major crops. Comparing a range of cultivated species, Greenwood et al. (1990) concluded that the plant metabolic type, C3 or C4, is the main factor that discriminates the N dilution curves. At a given shoot mass ( $W$ ), C4 crops have a lower %N than C3 species, presumably due to a lower content of photosynthetic proteins. Legumes including lucerne (Lemaire et al., 1985), french beans (Greenwood et al., 1990) and pea (Ney et al., 1997) have N dilution curves similar to other C3 species. In these crops, however, growth of pods and seeds with a high %N could partially or totally compensate the decline in shoot %N

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caused by the decrease in  $W_{\text{lam}}:W_{\text{ste}}$  ratio and the remobilization of N (Ney et al., 1997).

Worldwide, soybean [*Glycine max* (L.) Merrill] is the most important grain legume crop, providing more than half of the world's vegetable oils and two-thirds of the world's protein meal. Soybean plants absorb inorganic N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) from the soil and can obtain reduced N from the symbiosis with *Bradyrhizobium japonicum* bacteria. Under a range of agronomic conditions, soybean crops derive between 25 and 75% of their total nitrogen from  $\text{N}_2$  fixation (Deibert et al., 1979; Salvagiotti et al., 2008; Collino et al., 2015).

Combining maturity types and sowing dates growers manipulate the timing of critical periods and accommodate crops in cropping sequences. Soybean cultivars span thirteen maturity groups (MG), from the earliest "000" adapted to long days and short summers to the latest "X" adapted to short days (Poehlman, 1987). In the south-eastern Argentinean Pampas ( $38^\circ\text{S}$ ) cultivars from MG II to IV are commonly sown. Sowing dates that maximize soybean yield in this area range from early to mid-November (Andrade, 1995). In double crops with cereals or oilseed rape, soybean is sown during December or in early January.

Variations in MG and sowing date modify plant size and architecture. Late maturity groups have an extended crop cycle in relation to early ones, mainly during the vegetative stages (Egli, 1993). This increases the number of nodes per plant, shoot mass and leaf area index (Bastidas et al., 2008). By contrast, delaying sowing with respect to the optimum reduces the duration of the vegetative period (Ball et al., 2000; Kantolic and Slafer, 2001), which reduces shoot mass, node number and modifies plant architecture (Calviño et al., 2003a,b). These differences in crop phenology and morphology caused by variations in MG or sowing date also influence the accumulation of shoot biomass from crop emergence to flowering.

It is generally accepted that the N dilution curves are similar among genotypes from the same species. However, we hypothesized that the extended vegetative period of late MG or timely sowing date would affect the parameters of nitrogen dilution curves by affecting the pattern of  $W_{\text{lam}}:W_{\text{ste}}$  ratio and the shoot biomass at flowering.

The aims of this study were: (i) to determine the growth and allocation patterns of soybeans as affected by maturity group (MG IV vs II) and sowing date and (ii) to establish allometric relations between growth and nitrogen, including reference N dilution curves. Our working hypothesis is that the N dilution curve in soybean is similar to the critical N dilution curve of non-legume C3 crops on the assumption that, in our environment, the combined supply of soil mineral N and symbiotic N fixation fully satisfy crop N requirement.

## 2. Materials and methods

### 2.1. Crop husbandry, treatments and experimental design

The study was carried out at INTA Research Station, Balcarce, Argentina ( $37.5^\circ\text{S}$ ,  $58.2^\circ\text{W}$ , 130 m above sea level). Sources of variation include seasons, water regimes, varieties (maturity group) and sowing dates. The aim was to generate a wide range of growing conditions rather than assess the effects of individual sources of variation and their interactions, which would have required a full factorial experiment with some agronomically meaningless treatments, e.g. late sowing of MG IV.

Crops were grown during three growing seasons: 2010/11 (S1), 2011/12 (S2) and 2012/13 (S3). During S1 and S2 crops were rainfed and in 2012/13 one experiment was rainfed (S3-RF) and another received complementary irrigation (S3-IR). In the irrigated experiment, water was supplemented to maintain the soil water content

(SWC) above 80% of the plant available water (PAW) during all the growing season. The rainfed experiments were under no-tillage and the irrigated experiment was under conventional tillage, which comprised moldboard plowing, disking and field cultivation with the least tillage operations necessary to get an appropriate seedbed.

Crops were grown on a fine, mixed, thermic Typic Argiudoll with a minimum effective depth of 1.5 m. Top-soil (0–0.2 m) pH was  $6.2 \pm 0.7$  and soil organic matter was  $51.0 \pm 0.6 \text{ mg kg}^{-1}$ . Soil  $\text{N-NO}_3^-$  in the 0–60 cm depth was 12.4, 40.3, 56.0 and  $124.9 \text{ kg ha}^{-1}$  for S1, S2, S3-RF and S3-IR, respectively. Inter-row spacing was 0.35 m and the plant density was about  $45 \text{ pl m}^{-2}$  for all treatments and seasons. Soybean was inoculated with *B. japonicum* before sowing. Phosphorus and sulfur were applied at sowing as triple superphosphate (0–46–0) and gypsum ( $\text{SO}_4\text{Ca}_2\text{H}_2\text{O}$ , 16% S, 20% Ca), respectively. The application rates were  $40 \text{ kg ha}^{-1}$  for both nutrients. Weeds were controlled with two applications of glyphosate ( $1.4 \text{ kg a.i. ha}^{-1}$ ) at  $\approx\text{V1}$  and V4. Diseases and insects were chemically controlled when required.

Two indeterminate soybean cultivars were evaluated: DM2200 (MG II) and DM4970 (MG IV). These cultivars represent the phenological extremes for the region. Both cultivars were sown in optimum dates for the area (November 20th, 24th and 10th for S1, S2 and S3, respectively). DM2200 was also sown on January 10th in all three seasons. This combination allows the study of the effect of MG and sowing date on the patterns of biomass and N accumulation and partitioning. Hereafter, DM4970 and DM2200 sown in November are called  $G_{\text{IV-Nov}}$  and  $G_{\text{II-Nov}}$ , respectively and DM2200 sown in January  $G_{\text{II-Jan}}$ . Both cultivars are genetically modified (glyphosate-tolerant).

The experimental design was a randomized complete blocks with three replicates. The experimental unit was  $12 \text{ m} \times 5 \text{ m}$ .

### 2.2. Measurements

#### 2.2.1. Phenology, growth and nitrogen

Crop phenology was monitored every three days using the scale of Fehr and Caviness (1977).

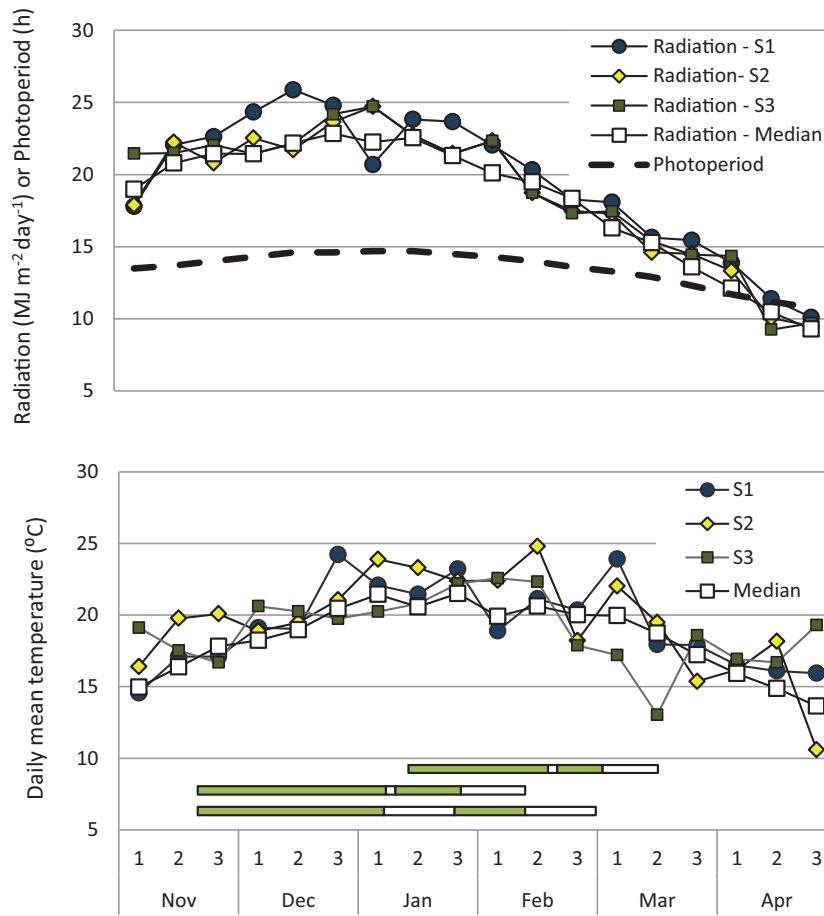
The theory underlying the N dilution curve is restricted to the window between  $W \approx 1 \text{ Mg ha}^{-1}$  because crops behave as isolated plants at lower biomass (Lemaire et al., 2007) and the end of the vegetative period (Greenwood et al., 1990; Lemaire and Gastal, 1997) because during reproductive growth, seeds conform an extra compartment in addition to  $W_{\text{lam}}$  and  $W_{\text{ste}}$  and translocation processes take place (Sadras and Lemaire, 2014). We thus sampled crops between  $W \approx 1 \text{ Mg ha}^{-1}$  and R5. At R5 grain weight was negligible. Initial sampling coincided with the V6 stage for  $G_{\text{IV-Nov}}$  and R1 for  $G_{\text{II-Nov}}$  and  $G_{\text{II-Jan}}$ . Samples were also taken at R1, R3 and R5 for  $G_{\text{IV-Nov}}$  and at R3 and R5 for  $G_{\text{II-Nov}}$  and  $G_{\text{II-Jan}}$ .

At each sampling, plants were collected from central rows in a  $0.7 \text{ m}^2$  area. Plant height, growth and development stages were determined from a sub-sample of ten plants randomly collected from the sampled section. These plants were separated into stem and petiole ( $W_{\text{ste}}$ ), lamina ( $W_{\text{lam}}$ ) and pod ( $W_{\text{pod}}$ ). Few if any senescent leaves were lost during the sampling period, and were not considered in the analysis.

Plant samples were oven dried at  $65^\circ\text{C}$  until a constant weight was reached, weighed, and ground (0.5 mm mesh). Nitrogen content in the samples was determined by dry combustion at  $950^\circ\text{C}$  and N thermo-conductivity detection with a TruSpec CN analyzer (LECO, St. Joseph, MI, USA).

#### 2.2.2. Complementary measurements: PAR interception, LAI and soil water content

Canopy closure and leaf shadowing have implications for the vertical distribution of N in the canopy (Lemaire et al., 2007). To capture these effects, we measured the intercepted photosynthet-



**Fig. 1.** Solar radiation and photoperiod (top) and daily mean temperature (bottom). Colored bars at the bottom of the lower panel indicate the periods between sowing-V6, V6-R1, R1-R3 and R3-R5 respectively (average of four experiments). Data from Season 1 (S1), Season 2(S2), Season 3 (S3) and median (1989/2009).

ically active radiation (iPAR) and LAI. iPAR was measured in clear days around noon. For each plot and stage, five pairs of readings above the canopy ( $I_0$ ) and just below the lowest layer of leaves ( $I_t$ ) were made. The percentage radiation interception (iPAR) was calculated as  $[1-(I_t/I_0)]/100$ . Measurements were made with Li-Cor 191 SB sensor (Licor Inc., Lincoln, Nebraska, USA).

Soil available water is the main source of interannual variation in soybean growth and yield in the region. So, soil water content was estimated using the locally-calibrated model by Della Maggiora et al. (2002). For the model parameterization, volumetric water content at field capacity was set at  $0.360 \text{ m}^3 \text{ m}^{-3}$  and the volumetric water content for permanent wilting point was set at  $0.192 \text{ m}^3 \text{ m}^{-3}$  (Della Maggiora et al., 2002). PAW was calculated as the difference between field capacity and permanent wilting point. Crop evapotranspiration (ET) was determined as the product between potential evapotranspiration ( $ET_0$ ) and crop coefficient (Kc). The  $ET_0$  was calculated according to Penman (1948) and the Kc ( $ET/ET_0$ ) values were those reported for the region by Della Maggiora et al. (2000). Actual ET was assumed to be equal to  $ET_0$  when  $SWC > 0.6$  PAW, and to decline linearly with PAW between 0 and 0.6 (Sadras and Milroy, 1996). Weather data were obtained from INTA's weather station, located 500 m from the experimental site.

### 2.3. Calculations

Allometric increase in lamina and stem mass was represented by the functions proposed by Gastal et al. (2015):

$$W_{(\text{lam or ste})} = cW^d \quad (1)$$

where  $W$  represents shoot biomass ( $\text{Mg ha}^{-1}$ ),  $c$  is  $W_{\text{lam}}$  or  $W_{\text{ste}}$  at  $W = 1 \text{ Mg ha}^{-1}$  and  $d$  the ratio between the relative increase in these tissues and the relative crop growth rate.

The decrease of the  $W_{\text{lam}}:W_{\text{ste}}$  ratio was represented by:

$$W_{\text{lam}} : W_{\text{ste}} = eW^{-f} \quad (2)$$

where  $e$  is the  $W_{\text{lam}}:W_{\text{ste}}$  at  $W = 1 \text{ Mg ha}^{-1}$  and  $-f$  the proportion between the relative decrease in the ratio and the relative crop growth rate.

Shoot%N was calculated as:

$$\%N = \frac{(\%N_{\text{lam}}W_{\text{lam}} + \%N_{\text{ste}}W_{\text{ste}} + \%N_{\text{pod}}W_{\text{pod}}) 1}{(W_{\text{lam}} + W_{\text{ste}} + W_{\text{pod}})} \quad (3)$$

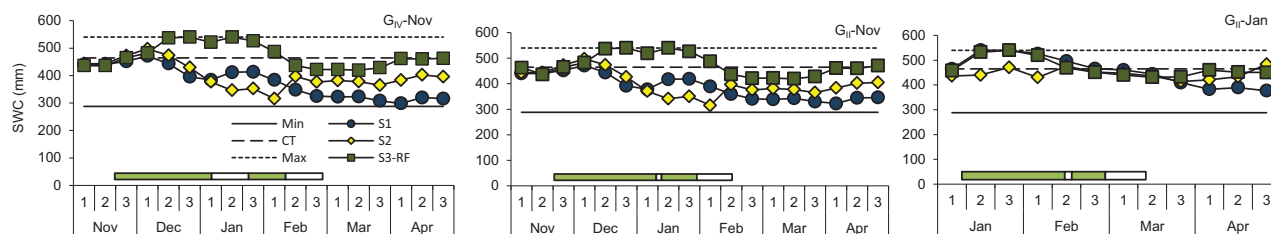
where  $\%N_{\text{lam}}$ ,  $\%N_{\text{ste}}$  and  $\%N_{\text{pod}}$  is the N concentration (%) in lamina, stem and pod, respectively.

The decline in plant%N was described empirically by a negative power function relating shoot%N to  $W$  (Lemaire and Salette, 1984):

$$\%N = aW^{-b} \quad (4)$$

coefficient  $a$  represents the%N at  $W = 1 \text{ Mg ha}^{-1}$ . Coefficient  $b$  is dimensionless and represents the ratio between the relative decline in%N and the relative crop growth rate.

The critical N dilution curve indicates the minimum nitrogen concentration required at a given crop  $W$  for maximum shoot growth. It allows the distinction between crops under either supra-optimal or sub-optimal nitrogen supply (Greenwood et al., 1990; Sadras and Lemaire, 2014). In legumes it is necessary to perform experiments with non-fixing plants under a gradient of N availability. Thus, in this work we described the "reference" N dilution curve



**Fig. 2.** Simulated soil water content (SWC) (Della Maggiora et al., 2002) for cultivar DM4970 (MG IV) sown in November ( $G_{IV}$ -Nov), DM2200 (MG II) sown in November ( $G_{II}$ -Nov) and DM2200 sown in January ( $G_{IJ}$ -Jan) in Season 1 (S1), Season 2 (S2) and Season 3 rainfed (S3-RF). Max and Min indicate soil water-holding upper and lower limits according with Della Maggiora et al. (2003). CT indicates the limit for physiological water stress (Sadras and Milroy, 1996). Colored bars at the bottom of each Figure indicate the periods between sowing-V6, V6-R1, R1-R3 and R3-R5 respectively (average of four experiments).

**Table 1**  
Phenological development, plant height, shoot biomass ( $W$ ) and intercepted photosynthetically active radiation (iPAR) for cultivar DM4970 (MG IV) sown in November ( $G_{IV}$ -Nov), DM2200 (MG II) sown in November ( $G_{II}$ -Nov) and DM2200 sown in January ( $G_{IJ}$ -Jan) in Season 1 (S1), Season 2 (S2), Season 3 rainfed (S3-RF) and Season 3 irrigated (SR-IR). Days after emergence (DAE) show the timing of key stages from V6 to R5. For each season, different letters indicate differences between phenological stages (capital letters) and variety-sowing date (lower case letters) at  $P < 0.05$  using Tukey test.

Season	Variety-sowing date	Stage	DAE	Height (cm)	$W$ (Mg ha <sup>-1</sup> )	iPAR (%)
S1	$G_{IV}$ -Nov	V6	43	26 D -	1.2 D -	75.0 B -
		R1	56	55 C a	3.0 C a	98.1 A a
		R3	71	73 B a	4.9 B a	99.0 A a
	$G_{II}$ -Nov	R5	85	86 A a	6.7 A a	99.1 A a
		R1	46	27 B b	1.1 C b	75.1 B c
		R3	63	65 A b	3.7 B a	98.5 A a
	$G_{IJ}$ -Jan	R5	73	71 A b	5.4 A b	98.7 A a
		R1	41	38 B c	2.2 B b	88.1 B b
		R3	52	43 A c	2.8 B b	90.3 A b
S2	$G_{IV}$ -Nov	R5	59	47 A c	3.8 A c	98.5 A a
		V6	48	32 D -	1.3 D -	84.8 C -
		R1	58	51 C a	3.1 C a	90.3 B a
	$G_{II}$ -Nov	R3	68	56 B a	3.5 B a	98.6 A a
		R5	80	60 A a	5.2 A a	98.4 A a
		R1	45	28 C b	1.0 C b	85.2 B b
	$G_{IJ}$ -Jan	R3	57	39 B b	2.0 B b	96.3 A a
		R5	70	45 A b	3.4 A b	98.3 A a
		R1	36	31 C b	1.2 C b	72.5 C c
S3-RF	$G_{IV}$ -Nov	R3	45	38 B b	2.2 B b	91.6 B b
		R5	56	44 A b	3.9 A b	98.3 A a
		V6	47	30 C -	1.6 D -	60.4 C -
	$G_{II}$ -Nov	R1	61	63 B a	3.2 C a	87.0 B a
		R3	75	65 B a	4.6 B a	97.3 A a
		R5	88	95 A a	7.1 A a	96.5 A
	$G_{IJ}$ -Jan	R1	51	38 C b	1.5 C b	72.1 B b
		R3	65	65 B a	3.7 B b	94.7 A ab
		R5	76	79 A b	4.8 A b	97.3 A a
S3-IR	$G_{IV}$ -Nov	R1	40	31 B c	1.9 C c	75.3 C b
		R3	49	42 A b	3.0 B c	91.0 B b
		R5	63	42 A c	3.9 A c	97.3 A a
	$G_{II}$ -Nov	V6	47	36 D -	1.6 D -	90.8 B -
		R1	61	65 C a	3.8 C a	96.1 A a
		R3	75	95 B a	5.9 B a	96.9 A a
	$G_{IJ}$ -Jan	R5	88	110 A a	8.2 A a	97.9 A a
		R1	51	51 C b	1.9 C b	87.6 B b
		R3	65	61 B b	3.7 B b	97.3 A a
$G_{IJ}$ -Jan	R5	77	85 A b	5.3 A b	97.4 A a	
	R1	40	50 B b	2.5 C c	85.2 B c	
	R3	49	62 A b	4.1 B c	98.2 A a	
		R5	63	63 A c	5.8 A c	97.6 A a
Season* Variety-sowing date* Stage				<0.01	<0.01	<0.01
S1	Variety-sowing date* Stage		<0.01	<0.01	<0.01	
	Variety-sowing date		<0.01	<0.01	<0.01	
	Stage		<0.01	<0.01	<0.01	
S2	Variety-sowing date* Stage		0.15	0.78	<0.01	
	Variety-sowing date		<0.01	<0.01	<0.01	
	Stage		<0.01	<0.01	<0.01	
S3-RF	Variety-sowing date* Stage		<0.01	0.07	<0.01	
	Variety-sowing date		<0.01	<0.01	<0.01	
	Stage		<0.01	<0.01	<0.01	
S3-IR	Variety-sowing date* Stage		<0.01	0.38	<0.01	
	Variety-sowing date		<0.01	<0.01	<0.01	
	Stage		<0.01	<0.01	<0.01	



for soybean on the assumption that the combined supply of soil mineral N and symbiotic N fixation fully satisfy crop N requirement.

#### 2.4. Statistical analysis

ANOVA was used to assess the effect of experimental sources of variation, i.e. season, variety, sowing date, and phenological stage on response variables using the Proc Mixed procedure

in SAS (Littell et al., 1996). Tukey tests were performed when treatment effects were significant ( $P$  value <0.05).

Parameters of the equations were calculated by non-linear equations using NLIN procedure of SAS computer software. Comparisons among variety-sowing date treatments of growth and %N with increasing  $W$  were performed from linearized models (log-transformation) (Niklas, 2006). Intercept and parallelism tests were performed by using ANOVA with SAS PROC MIXED. All analysis considered a repeated measure model to incorporate the correlations for the errors arising from measurements on the same experimental unit through sampling dates.

### 3. Results

#### 3.1. Growing conditions

The long-term median for solar radiation in Balcarce increased 8% from November to December, when it peaked at  $22.4 \text{ MJ m}^{-2} \text{ day}^{-1}$ . From December, radiation fell 2% in January, 12% in February, 33% in March and 52% in April (Fig. 1A). Mean seasonal (November to April) solar radiation followed a similar pattern to and was 6.7%, 2.1%, and 3.6% higher than the long-term median for S1, S2 and S3, respectively. Photoperiod reaches a maximum in the summer solstice, and falls 0.1, 0.6, 1.8 and 3.5 h in January, February, March and April, respectively (Fig. 1A).

Long-term daily mean temperature increased from November, peaked on January ( $20.8^\circ\text{C}$ ) and diminished on February (4%), March (13%) and April (30%). Mean seasonal (November to April) temperatures were slightly higher than historical in the three experimental seasons. During S3, however, temperatures of the last decade of February and the first and second decades of March were cooler than the long-term median.

The evolution of SWC in  $G_{IV}$ -Nov and  $G_{II}$ -Nov was similar in seasons 1 and 2. SWC was above the critical threshold of  $\text{PAW} = 0.6$  from crop emergence to V4 but below the threshold from V4 onwards (Fig. 2). However, water deficit was more severe from V5 to R3 during S2. In the rain-fed crops in season 3, SWC was slightly below threshold from R3 for  $G_{IV}$ -Nov and from R5 for  $G_{II}$ -Nov.  $G_{II}$ -Jan did not suffer severe water deficit during the period under study, with exception of S3, where SWC was slightly under the critical threshold during the vegetative period (Fig. 2). In S3-IR, SWC was above 80% PAW in all treatments and during all season (data not shown).

#### 3.2. Development, growth and dry matter allocation

The duration of the emergence-R1 period ranked  $G_{IV}$ -Nov >  $G_{II}$ -Nov >  $G_{II}$ -Jan (Table 1). The length of the R1-R5 period was similar between  $G_{IV}$ -Nov and  $G_{II}$ -Nov but it was shorter in  $G_{II}$ -Jan (Table 1).

The longer duration of the emergence-R1 period in  $G_{IV}$ -Nov compared to  $G_{II}$ -Nov and  $G_{II}$ -Jan resulted in higher plants, more shoot biomass and higher iPAR when comparisons were done at the same phenological stage (Table 1). Differences in those variables between  $G_{II}$ -Nov and  $G_{II}$ -Jan were lower. Particularly,  $G_{IV}$ -Nov and  $G_{II}$ -Nov accumulated less biomass during S2 in comparison with S1 and S3 ( $P < 0.01$ ). On average,  $G_{IV}$ -Nov and  $G_{II}$ -Nov reached 95% iPAR at  $\approx R3$  but  $G_{II}$ -Jan reached this interception at  $\approx R5$  (Table 1).

**Table 2**

Probability values for the comparison of variety-sowing date for intercept (Inte) and parallelism (Para) of the ln–ln regression lines. Comparisons are total aboveground mass ( $W$ ) vs lamina ( $W_{\text{lam}}$ ), stem ( $W_{\text{ste}}$ ) and lamina:stem ( $W_{\text{lam}}:W_{\text{ste}}$ ). Variety-sowing date are cultivar DM4970 (MG IV) sown in November ( $G_{IV}$ -Nov), DM2200 (MG II) sown in November ( $G_{II}$ -Nov) and DM2200 sown in January ( $G_{II}$ -Jan).

	$W$ vs $W_{\text{lam}}$		$W$ vs $W_{\text{ste}}$		$W$ vs $W_{\text{lam}}:W_{\text{ste}}$	
	Inte	Para	Inte	Para	Inte	Para
$G_{IV}$ -Nov vs $G_{II}$ -Nov	0.04	0.04	0.05	0.11	0.04	0.01
$G_{IV}$ -Nov vs $G_{II}$ -Jan	0.10	0.03	0.03	0.001	0.05	<0.01
$G_{II}$ -Nov vs $G_{II}$ -Jan	0.60	0.59	0.59	0.33	0.95	0.38

Lamina represented the majority of shoot biomass at  $W = 1 \text{ Mg ha}^{-1}$  (Fig. 3) (0.64, 0.59 and  $0.58 \text{ Mg ha}^{-1}$  for  $G_{IV}$ -Nov,  $G_{II}$ -Nov and  $G_{II}$ -Jan, respectively). The proportion was higher in  $G_{IV}$ -Nov than  $G_{II}$ -Nov ( $P < 0.05$ ) (Table 2). The rate of lamina accumulation with  $W$  was lower in  $G_{IV}$ -Nov than the other treatment (Table 2). Contrarily, stem accumulation at  $W = 1 \text{ Mg ha}^{-1}$  was lower in  $G_{IV}$ -Nov and the rate of stem accumulation as  $W$  increased was higher in this treatment than  $G_{II}$ -Jan (Table 2). Pod onset was at  $W \approx 5.0, 3.5$  and  $3.0$  for  $G_{IV}$ -Nov,  $G_{II}$ -Nov and  $G_{II}$ -Jan, respectively. No allometric functions were proposed for pod accumulation because only two samples were taken for this compartment (R3 and R5) (Fig. 3).

As  $W$  increased, the pattern of  $W_{\text{lam}}:W_{\text{ste}}$  ratio (Fig. 4) was similar between  $G_{II}$ -Nov and  $G_{II}$ -Jan (Table 2). However, this ratio was higher for  $G_{IV}$ -Nov than for  $G_{II}$ -Nov and  $G_{II}$ -Jan at  $W = 1 \text{ Mg ha}^{-1}$  (Table 2). In addition,  $G_{IV}$ -Nov showed a more pronounced depletion of the ratio  $W_{\text{lam}}:W_{\text{ste}}$  with increasing  $W$  than the other treatments (Table 2).

#### 3.3. Nitrogen concentration, accumulation and allocation

N concentration in lamina was constant as soybean developed in 8 out of 12 combinations of growing conditions, varieties and sowing dates (Table 3). Interestingly,  $G_{IV}$ -Nov had a lower %N in lamina at V6 than at R1, R3 and R5 in 3 out of 4 experiments (Table 3). %N in stems was constant with growing crops in 6 combinations and decreased in the others (Table 3). Finally, %N in pods was higher at R5 (4.2% on average) than at R3 (3.3% on average) (Table 3).

Nitrogen dilution curves (Fig. 5) were similar among varieties and sowing dates ( $P > 0.3$  and  $P > 0.6$  for intercept and parallelism, respectively). Thus, we propose a common empirical model for all combinations of cultivar and sowing date:

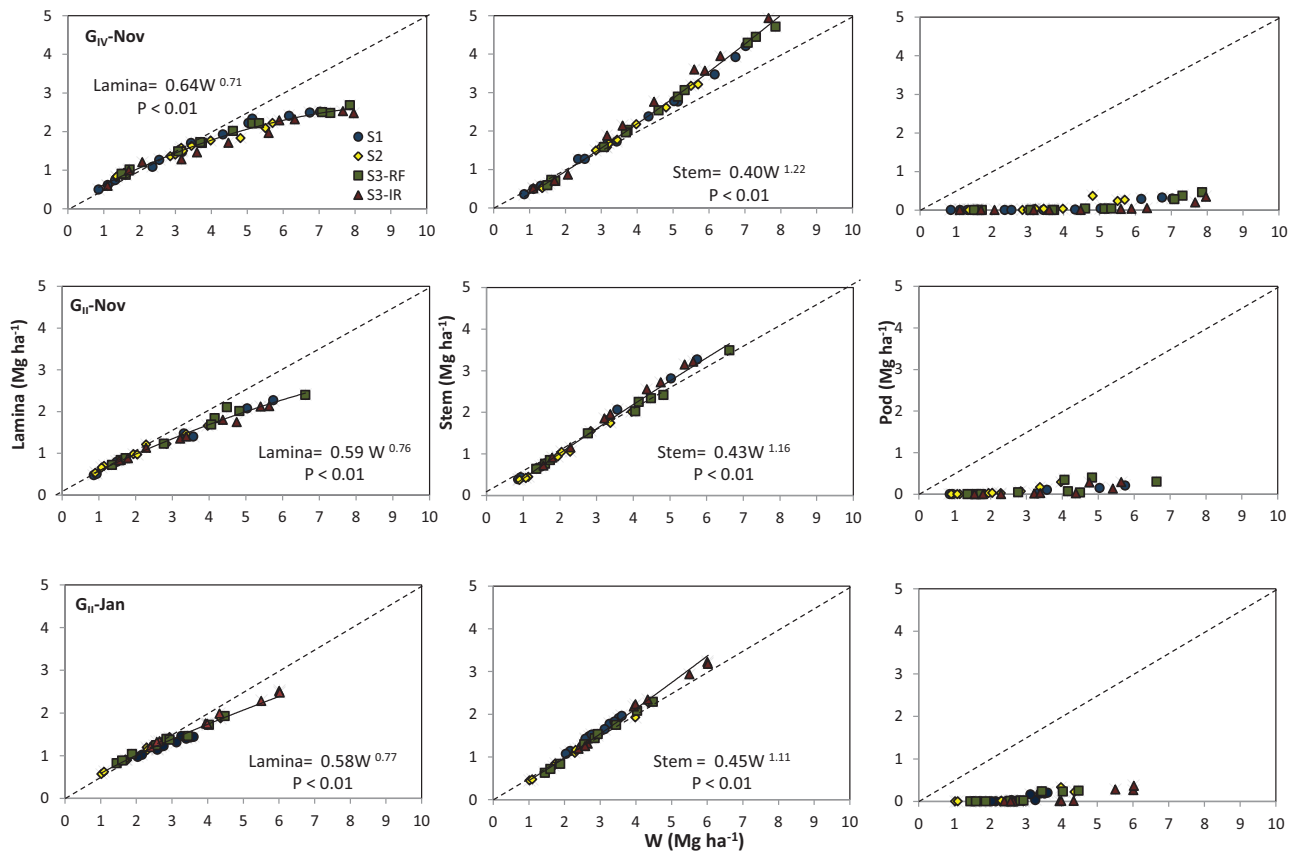
$$\%N = 3.7W^{-0.08} \quad (5)$$

### 4. Discussion

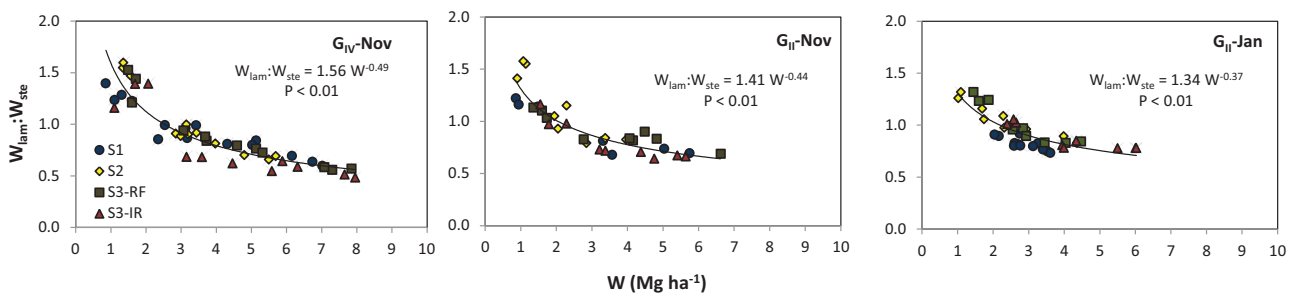
#### 4.1. Growth and development

Fig. 1 shows the marked reductions in radiation, photoperiod and temperature after the summer solstice that constrain soybean production, especially in late-sown crops (Calviño and Sadras, 1999). Particularly, the cooler temperatures of the last decade of February and the first and second decades of March during S3 reduced crop growth rate and delayed crop development, especially for  $G_{II}$ -Jan. Despite some differences in radiation and temperature among seasons, soil water content was the main environmental factor that constrained soybean growth and development across experiments (Fig. 2). Accordingly, Calviño and Sadras (1999) reported for the south-eastern Argentinean Pampas that rainfall was the main source of interannual variation in soybean yield in farmer's fields.

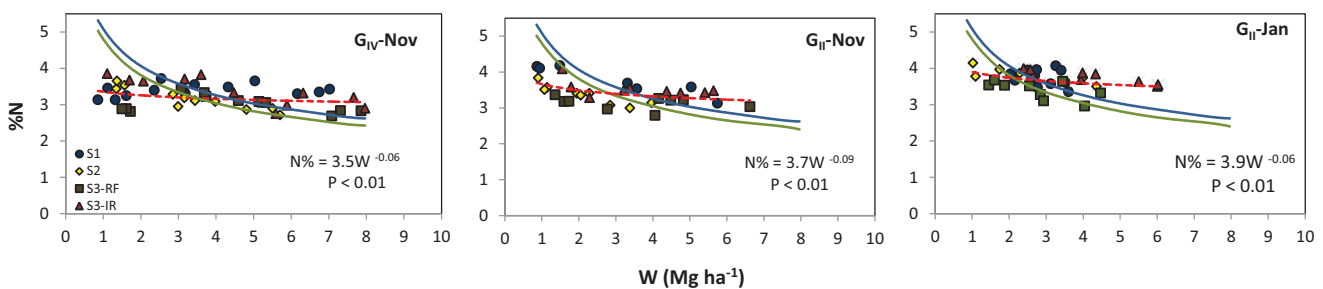
The longer duration of the emergence-R1 period in  $G_{IV}$ -Nov compared to  $G_{II}$ -Nov was related to the greater developmental



**Fig. 3.** Lamina (left), stem (middle) and pod (right) mass as a function of shoot mass ( $W$ ) for cultivar DM4970 (MG IV) sown in November (G<sub>IV</sub>-Nov), DM2200 (MG II) sown in November (G<sub>II</sub>-Nov) and DM2200 sown in January (G<sub>II</sub>-Jan). Data from Season 1 (S1), Season 2 (S2), Season 3 rainfed (S3-RF) and Season 3 irrigated (SR-IR). Dotted line represents 0.5 of  $W$ .



**Fig. 4.** Lamina:stem ratio ( $W_{\text{lam}}:W_{\text{ste}}$ ) as a function of shoot mass ( $W$ ) for cultivar DM4970 (MG IV) sown in November (G<sub>IV</sub>-Nov), DM2200 (MG II) sown in November (G<sub>II</sub>-Nov) and DM2200 sown in January (G<sub>II</sub>-Jan). Data from Season 1 (S1), Season 2 (S2), Season 3 rainfed (S3-RF) and Season 3 irrigated (SR-IR).  $P$  indicates significance of regression.



**Fig. 5.** Shoot nitrogen concentration (%N) as a function of shoot biomass ( $W$ ) for cultivar DM4970 (MG IV) sown in November (G<sub>IV</sub>-Nov), DM2200 (MG II) sown in November (G<sub>II</sub>-Nov) and DM2200 sown in January (G<sub>II</sub>-Jan). Data from Season 1 (S1), Season 2 (S2), Season 3 rainfed (S3-RF) and Season 3 irrigated (SR-IR). Red dashed line represents the empirical model for each cultivar and sowing date. Blue full line represents the N dilution curve for lucerne ( $\%N = 5.1W^{-0.32}$ ) (Lemaire et al., 1985). Green full line represents the N dilution curve for pea ( $\%N = 4.8W^{-0.33}$ ); Ney et al. (1997).

**Table 3**

Nitrogen concentration (%) in lamina, stem and pod for cultivar DM4970 (MG IV) sown in November (G<sub>IV</sub>-Nov), DM2200 (MG II) sown in November (G<sub>II</sub>-Nov) and DM2200 sown in January (G<sub>II</sub>-Jan) in Season 1 (S1), Season 2 (S2), Season 3 rainfed (S3-RF) and Season 3 irrigated (S3-IR). For each experiment, different letters indicate differences between phenological stages (capital letters) and variety-sowing date (lower case letters) at  $P < 0.05$  using Tukey.

Season	Variety-sowing date	Stage	N lamina (%)	N stem (%)	N pod (%)	
S1	G <sub>IV</sub> -Nov	V6	4.3 C -	1.8 AB -	-	
		R1	5.3 B b	1.8 AB a	-	
		R3	5.6 B b	1.6 B b	3.0 B b	
		R5	5.9 A a	2.0 A a	3.9 A b	
		R1	6.0 A a	2.0 AB a	-	
	G <sub>II</sub> -Nov	R3	5.9 A a	2.2 A a	3.8 B a	
		R5	5.7 A ab	1.7 B b	4.4 A a	
		R1	5.8 AB a	2.0 A a	-	
	G <sub>II</sub> -Jan	R3	6.0 A a	2.0 A a	3.9 B a	
		R5	5.5 C b	2.1 A a	4.2 A a	
		R1	5.4 A a	2.3 A a	-	
	S2	G <sub>IV</sub> -Nov	V6	4.7 A -	1.8 A -	-
			R1	4.8 A b	1.6 A c	-
			R3	4.7 A b	1.8 A a	3.0 B c
			R5	4.8 A b	1.4 B c	3.9 A c
R1			4.8 A b	1.9 A b	-	
G <sub>II</sub> -Nov		R3	4.8 A b	1.9 A a	3.8 B b	
		R5	4.6 A b	1.7 A b	4.4 A a	
		R1	5.4 A a	2.3 A a	-	
G <sub>II</sub> -Jan		R3	5.6 A a	2.0 B a	3.9 B a	
		R5	5.6 A a	2.0 A a	4.2 A b	
		R1	4.7 A a	1.7 A b	-	
S3-RF		G <sub>IV</sub> -Nov	V6	3.8 B -	1.6 A -	-
			R1	5.1 A a	1.8 A b	-
			R3	4.9 A a	1.6 A b	3.0 B a
			R5	5.1 A a	1.5 A b	3.6 A b
	R1		4.7 A a	1.7 A b	-	
	G <sub>II</sub> -Nov	R3	4.8 A a	1.7 A b	3.1 B a	
		R5	4.9 A a	1.6 A b	3.7 A b	
		R1	4.9 A a	1.9 A a	-	
	G <sub>II</sub> -Jan	R3	4.8 A a	1.8 A a	3.0 B a	
		R5	4.7 A a	1.9 A a	4.8 A a	
		R1	5.1 A a	1.5 A b	-	
	S3-IR	G <sub>IV</sub> -Nov	V6	5.0 B -	2.1 A -	-
			R1	5.7 A a	2.2 A a	-
			R3	5.1 B b	1.8 B b	2.9 B a
			R5	5.8 A a	1.7 B b	4.1 A b
R1			5.1 A b	2.1 A a	-	
G <sub>II</sub> -Nov		R3	5.6 A a	2.0 A b	3.0 B a	
		R5	5.6 A a	1.6 B b	3.9 A c	
		R1	5.5 A a	2.3 A a	-	
G <sub>II</sub> -Jan		R3	5.8 A ab	2.2 A a	3.1 B a	
		R5	4.9 B b	2.4 A a	5.0 A a	
		R1	5.1 A b	2.1 A a	-	
Season *Variety-sowing date* Stage			<0.01	<0.01	<0.01	
S1		Variety-sowing date* Stage		<0.01	0.01	<0.01
		Variety-sowing date		<0.01	0.04	<0.01
		Stage		<0.01	0.99	<0.01
S2	Variety-sowing date* Stage		0.14	0.05	<0.01	
	Variety-sowing date		<0.01	<0.01	<0.01	
	Stage		0.83	<0.01	<0.01	
S3-RF	Variety-sowing date* Stage		0.73	0.4	<0.01	
	Variety-sowing date		0.09	<0.01	<0.01	
	Stage		<0.01	0.49	<0.01	
S3-IR	Variety-sowing date* Stage		<0.01	0.04	<0.01	
	Variety-sowing date		0.85	<0.01	<0.01	
	Stage		0.05	0.04	<0.01	

rate of earlier MG varieties when exposed to the same photoperiod (Cregan and Hartwig, 1984; Setiyono et al., 2007). The shorter period of G<sub>II</sub>-Jan was mainly associated with higher temperatures (Fig. 1) (Ball et al., 2000). In accordance with our results, Kantolic and Slafer (2001) and Calviño et al. (2003) reported that the duration of the R1-R5 period was shorter in cultivars of lower MG, and differences were less marked than the observed in the duration of the emergence-R1 period. These authors also determined that the period R1-R5 was shorter with delayed sowing in response to shorter photoperiod (Fig. 1).

Canopy closure and maximum canopy photosynthesis correspond to iPAR  $\geq 95\%$  (Westgate et al., 1999). For soybean, this

condition should be achieved during early reproductive stages to maximize grain yield (Yusuf et al., 1999; Malone et al., 2002). Remarkably, canopy closure has several implications for the patterns of N accumulation and dilution because it affects leaf shadowing,  $W_{lam}:W_{ste}$  ratio and vertical N distribution in canopy (Lemaire et al., 2007). In the present study, row spacing was constant among treatments with the objective of comparing MG and sowing date under equal conditions. However, the small plant size of G<sub>II</sub>-Jan indicates the convenience of reducing row space in late sown soybean crops (Calviño et al., 2003a,b).

#### 4.2. Dry matter allocation

Older plants with similar W could have lower  $W_{lam}:W_{ste}$  ratio (Coleman et al., 1994). Thus, the greater ratio of G<sub>IV</sub>-Nov at  $W = 1 \text{ Mg ha}^{-1}$  could be explained because this crop was at V6 while G<sub>II</sub>-Nov and G<sub>II</sub>-Jan were at R1 (Table 1). However, G<sub>IV</sub>-Nov reached the successive reproductive stages with greater biomass and taller plants than G<sub>II</sub>-Nov and G<sub>II</sub>-Jan (Table 1). Both, biomass accumulation and plant height are mentioned as responsible of decreases in the  $W_{lam}:W_{ste}$  ratio because plants invest a greater proportion of biomass in structural tissues to support the increasing leaf area (Lemaire and Gastal, 1997). Based on these results, we propose that the interaction between growth and development was the main cause of the differences between treatments in the patterns of the  $W_{lam}:W_{ste}$  ratio.

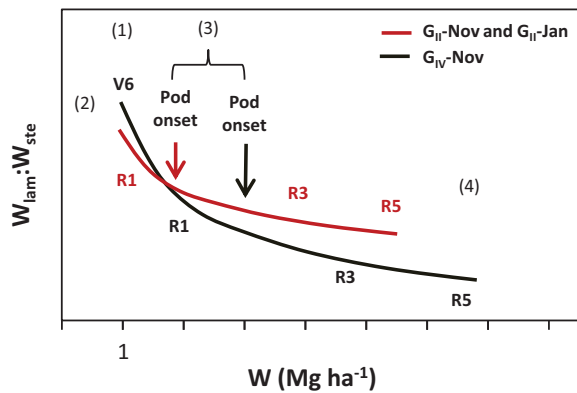
Time from crop emergence to flowering ranked G<sub>IV</sub>-Nov > G<sub>II</sub>-Nov > G<sub>II</sub>-Jan and affected shoot biomass at R1, that followed the same ranking (Table 1). Ney et al., (1997) stated that flowering and pod onset attenuated the decrease in shoot%N. So MG or sowing date would affect the N dilution curve.

#### 4.3. Nitrogen allocation and nitrogen dilution curve

Our results for %N in lamina were similar to those assumed in the model of Sinclair et al. (2003). They proposed a constant specific N content in leaves (SLN) of approximately  $2.5 \text{ g N m}^{-2}$  during vegetative stages, based on the reports of Hanway and Weber (1971) and Lugg and Sinclair (1981). This value is similar to the one obtained in our work after proper conversion ( $2.2 \pm 0.2 \text{ g N m}^{-2}$ ). Moreover, Sinclair et al. (2003) proposed a value of 2% for stem N concentration, which is also similar to our determination (Table 3).

Most of our experiments showed a constant %N in lamina with increasing W, which is associated with a low reallocation of N from shaded leaves at the bottom of the canopy to those at the top. Reallocation is associated with a lower N uptake rate than lamina growth rate which results in a reduction of %N in  $W_{lam}$  with increasing biomass (Gastal and Lemaire, 2002). Accordingly, Shiraiwa and Sinclair (1993) reported a low linear decrease of specific leaf N (SLN,  $\text{g N m}^{-2}$ ) from leaves at the top of the canopy to those at the bottom in soybean, which is different from the exponential decline described for non-legume species (Anten et al., 1995). This indicates low N reallocation in soybean.

The lower %N in lamina at V6 in comparison with R1 for G<sub>IV</sub>-Nov was unexpected. The use of glyphosate for weed control at early soybean stages may have caused a temporary N deficiency because the N<sub>2</sub>-fixing symbiont, *B. japonicum*, is not resistant to the herbicide (Moorman et al., 1992). Lower nodulation, N fixation activity (King et al., 2001) and soybean chlorophyll content (Reddy et al., 2000; Reddy and Zablutowicz, 2003) were reported as a consequence of glyphosate applications at early stages. The available data precluded to assert that the lower %N in lamina at V6 was consequence of a slight N deficiency. However, this hypothesis is not supported by the fact that the lower %N in lamina at V6 was observed also in the S3-IR experiment, where soil N-NO<sub>3</sub><sup>-</sup> content ( $124.9 \text{ kg ha}^{-1}$ ) was much higher than crop uptake ( $60.2 \text{ kg ha}^{-1}$ ).



**Fig. 6.** Morphological and phenological characteristics involved in the attenuated N dilution curve in soybean cultivars  $G_{IV}$ -Nov,  $G_{II}$ -Nov and  $G_{II}$ -Jan. Figure shows the pattern of the ratios lamina:stem ( $W_{lam}:W_{ste}$ ) with increasing shoot mass ( $W$ ). V6, R1, R3 and R5 are phenological stages (Fehr and Caviness, 1977).  $G_{II}$ -Nov and  $G_{II}$ -Jan treatments are represented with the same red line in order to show that phenological and morphological differences between sowing dates were lower than between MG. (1) Indicates higher  $W_{lam}:W_{ste}$  of  $G_{IV}$ -Nov at  $W = 1 \text{ Mg ha}^{-1}$ . (2) Indicates relatively low %N in lamina in  $G_{IV}$ -Nov at V6. (3) Shows the greater  $W$  at pod onset in  $G_{IV}$ -Nov in comparison with  $G_{II}$ -Nov and  $G_{II}$ -Jan. (4) Indicates that  $G_{IV}$ -Nov reach R3 and R5 at higher  $W$  and with lower  $W_{lam}:W_{ste}$ . Red arrow indicates pod onset for  $G_{II}$ -Nov and  $G_{II}$ -Jan. Blue arrow is pod onset for  $G_{IV}$ -Nov.

A common empirical model for the N dilution with increasing  $W$  is proposed for soybean cultivars of contrasting MG and sowing dates. Remarkably, the coefficients  $a$  and  $b$  of the model (Eq. (5)) were lower than those reported for other legumes such as lucerne (%N =  $5.08 W^{-0.32}$ ; Lemaire et al., 1985) and pea (%N =  $4.8 W^{-0.33}$ ; Ney et al., 1997) (Fig. 5) and also than the generic parameters proposed by Greenwood et al. (1990) for C3 species (%N =  $5.67 W^{-0.5}$ ). However, the attenuated N dilution curve is in agreement with the pattern of shoot %N with increasing  $W$  reported by Ney et al., (1997) for soybean, although no equations were proposed by these authors. Interestingly, our results also coincide with a recent report about N accumulation in modern soybean cultivars (Bender et al., 2015). Calculations we made based on their data indicate that these crops had a low depletion of %N from V7 to R5 (3.7–3.1%). Our data, therefore, do not support the working hypothesis that soybean dilution curve is similar to non-legume C3 crops.

The attenuated N dilution curve in soybean can be attributed to the early accumulation of N in pods. Ney et al., (1997) highlighted the difference among legumes crops in the pattern of %N dilution based on differences in  $W$  at flowering. Thus, when cultivated as forage, lucerne does not produce pods and seeds, and therefore shoot %N follows the general pattern described for C3 crops. Pea would show an intermediate situation since this crop flowers with higher shoot biomass than soybean.

Fig. 6 summarized our results for the phenological and morphological characteristics that are proposed to affect the pattern of %N dilution in legumes (Ney et al., 1997). As discussed previously,  $G_{IV}$ -Nov showed higher  $W_{lam}:W_{ste}$  at  $W = 1 \text{ Mg ha}^{-1}$  and reached the subsequent reproductive stages at higher  $W$  and with lower  $W_{lam}:W_{ste}$ . Additionally, pod onset occurred at higher  $W$  in  $G_{IV}$ -Nov. These patterns were similar between  $G_{II}$ -Nov and  $G_{II}$ -Jan. Despite the differences between  $G_{IV}$ -Nov and the other treatments, N dilution curves were similar among MG and sowing date. Lower %N in lamina at V6 in comparison to R1 in  $G_{IV}$ -Nov was an important cause of the similarity among the curves.

The phenological and morphological characteristics that generate differences in the pattern of %N dilution among legumes crops (Ney et al., 1997) would also cause differences among soybean crops with high contrast in sowing date or, more probably in MG. It would be interesting, therefore, to test this hypothesis in future research.

#### 4.4. Agronomic implications

The determination of the critical N dilution curves has been useful for diagnosing the N status of various species (Greenwood et al., 1990; Justes et al., 1994; Lemaire and Gastal, 1997; Lemaire et al., 2008). In soybean, this is biologically interesting and agronomically important given the interest in increasing crop yield with N fertilization (Salvagiotti et al., 2008; Wingeyer et al., 2014). However, based on our results, some considerations arise for the use of the critical curve in soybean:

- Despite the similar N dilution curve among MG or sowing date, differences among treatments in the pattern of  $W_{lam}:W_{ste}$  and the earliness of pod onset add variability to shoot %N, which negatively affect the accuracy of the method.
- Stresses that lead to flower or pod abortion, such as water deficit (Calviño et al., 2003a,b) or low temperature (Gibson and Mullen, 1996) would increase variability in shoot %N.

Therefore, diagnosing the N status of the crop by using indexes based on critical dilution curve (i.e. nitrogen nutrition index, NNI) would be less accurate for soybean than for other crops.

Finally, developing the critical N dilution curve in soybean is more complex than for non-fixing crops, because it should involve experiments with non-fixing lines under a gradient of soil N availability and a comparison with well nodulated lines.

#### 5. Concluding remarks

Despite the marked decrease in  $W_{lam}:W_{ste}$ , the N dilution curve was attenuated in soybean, with no differences among cultivars of maturity groups II and IV, and sowing dates from November to January. We propose that early pod onset and the constant %N in lamina and stem between R1 and R5 attenuated the N dilution curve in all MG and sowing date. In addition,  $G_{IV}$ -Nov showed a greater  $W_{lam}:W_{ste}$  ratio at  $W = 1 \text{ Mg ha}^{-1}$  and a more pronounced depletion of the ratio with increasing biomass. However, the N dilution curves were similar between maturity groups II and IV, and sowing dates from November to January.

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#### References

- Andrade, F.H., 1995. Analysis of growth and yield of maize, sunflower and soybean grown at Balcarce, Argentina. *Field Crops Res.* 41, 1–12.
- Anten, N.P.R., Schieving, F., Werger, M.J.A., 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C3 and C mono- and dicotyledonous species. *Oecologia* 101, 504–513.
- Ball, R.A., Purcell, L.C., Vories, E.D., 2000. Optimizing soybean plant population for a short-season system in the southern USA. *Crop Sci.* 40, 757–764.
- Bastidas, A.M., Setiyono, T.D., Dobermann, A., Cassman, K.G., Elmore, R.W., Graef, G.L., Specht, J.E., 2008. Soybean sowing date: the vegetative, reproductive and agronomic impacts. *Crop Sci.* 48, 727–740.
- Bender, R.R., Haegele, J.W., Below, F.E., 2015. Nutrient uptake, partitioning and remobilization in modern soybean varieties. *Agron. J.* 107, 563–573.
- Caloin, M., Yu, O., 1984. Analysis of the time course of change in nitrogen content in *Dactylis glomerata* L. using a model of plant growth. *Ann. Bot.* 54, 69–76.
- Calviño, P.A., Sadras, V.O., 1999. Interannual variation in soybean yield: interaction among rainfall, soil depth and crop management. *Field Crops Res.* 63, 237–246.
- Calviño, P.A., Sadras, V.O., Andrade, F.H., 2003a. Development, growth and yield of late-sown soybean in the southern Pampas. *Eur. J. Agron.* 19, 265–275.



- Calviño, P.A., Sadras, V.O., Andrade, F.H., 2003b. Quantification of environmental and management effects on the yield of late-sown soybean. *Field Crops Res.* 83, 67–77.
- Coleman, J.S., Mc Connaughay, K.D.M., Ackerly, D.D., 1994. Interpreting phenotypic variation in plants. *Trends Ecol. Evol.* 9, 187–191.
- Collino, D.J., Salvagioti, F., Peticari, A., Piccinetti, C., Ovando, G., Urquiaga, S., Racca, R.W., 2015. Biological nitrogen fixation in soybean in Argentina: relationships with crop, soil, and meteorological factors. *Plant Soil* XX, 1–14.
- Cregan, P.B., Hartwig, E.E., 1984. Characterization of flowering response to photoperiod in diverse soybean genotypes. *Crop Sci.* 24, 659–662.
- Deibert, E.J., Bijeriego, M., Olson, R.A., 1979. Utilization of 15N fertilizer by nodulating and non-nodulating soybean isolines. *Agron. J.* 71, 717–723.
- Della Maggiora, A.I., Gardiol, J.M., Irigoyen, A.I., 2000. Requerimientos hídricos. In: Andrade, F.H., Sadras, V.O. (Eds.), *Bases Para El Manejo Del Maíz, El Girasol Y La Soja*. INTA-Universidad de Mar del Plata, Balcarce, pp. 155–171.
- Della Maggiora, A.I., Irigoyen, A.I., Gardiol, J.M., Caviglia, O., Echarte, L., 2002. Evaluación de un balance de agua en el suelo para maíz. *Rev. Argent. Agrometeorología* 2, 167–176.
- Egli, D.B., 1993. Cultivar maturity and potential yield of soybean. *Field Crops Res.* 32, 147–158.
- Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development. *Iowa Agric. Exp. Station Spec. Rep.*, 80.
- Field, C., 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* 56, 341–347.
- Gastal, F., Lemaire, G., 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. *J. Exp. Bot.* 53, 789–799.
- Gastal, F., Lemaire, G., Durand, J.L., Louarn, G., 2015. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. In: Sadras, V.O., Calderini, D.F. (Eds.), *Crop Physiology: Applications for Breeding and Agronomy*. Elsevier Academic Press, San Diego, California, pp. 161–206.
- Gibson, L.R., Mullen, R.E., 1996. Soybean seed quality reductions by high day and night temperatures. *Crop Sci.* 36, 1615–1619.
- Greenwood, D.J., Lemaire, G., Gosse, G., Cruz, P., Draycott, A., Neeteson, J.J., 1990. Decline in percentage N of C3 and C4 crops with increasing plant mass. *Ann. Bot.* 66, 425–436.
- Hanway, J.J., Weber, C.R., 1971. N, P, and K Percentages in Soybean (*Glycine max* (L.) Merrill) Plant Parts. *Agron. J.* 63, 286–290.
- Hirose, T., Werger, M.J.A., 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* 72, 520–526.
- Justes, E., Mary, B., Meynard, J.M., Machet, J.M., Thelierhuche, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. *Ann. Bot.* 74, 397–407.
- Kantolic, A.G., Slafer, G.A., 2001. Photoperiod sensitivity after flowering and seed number determination in indeterminate soybean cultivars. *Field Crops Res.* 72, 109–118.
- King, A.C., Purcell, L.C., Vories, E.D., 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to glyphosate applications. *Agron. J.* 93, 179–186.
- Lemaire, G., Cruz, P., Gosse, G., Chartier, M., 1985. Etude des relations entre la dynamique de prélèvement d'azote et le dynamique de croissance en matière sèche d'un peuplement de luzerne (*Medicago sativa* L.). *Agronomie* 5, 685–692.
- Lemaire, G., Gastal, F., 1997. On the critical N concentration. In: *agricultural, crops, uptake, N, distribution in plant, canopies*, Lemaire, G. (Eds.), *Diagnosis of the Nitrogen Status in Crops*. Springer-Verlag, Heidelberg, pp. 3–44.
- Lemaire, G., Oosterom, E., Sheehy, J., Jeuffroy, M.H., Massignam, A., Rossato, L., 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? *Field Crops Res.* 100, 91–106.
- Lemaire, G., Jeuffroy, M.-H., Gastal, F., 2008. Diagnosis tool for plant and crop N status in vegetative stage theory and practices for crop N management. *Eur. J. Agron.* 28, 614–624.
- Lemaire, G., Salette, J., 1984. Relation entre dynamique de croissance et dynamique de prélèvement d'azote par un peuplement de graminées fourragères 1—Etude de l'effet du milieu. *Agronomie* 4, 423–430.
- Littell, R., Milliken, R., Stroup, W., Wolfinger, R., 1996. *SAS System for MIXED Models*. SAS Institute, Cary, NC.
- Lugg, D.G., Sinclair, T.R., 1981. Seasonal changes in photosynthesis of field grown soybean leaflets, 2: relations to nitrogen content. *Photosynthetica* 15, 138–144.
- Malone, S., Herbert Jr., D.A., Holshouser, D.L., 2002. The relationship between leaf area index and yield in double-crop and full- soybean systems. *J. Econ. Entomol.* 95, 945–951.
- Moorman, T.B., Becerril, J.M., Lydon, J., Duke, S.O., 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. *J. Agric. Food Chem.* 40, 289–293.
- Ney, B., Doe, T., Sagan, M., 1997. The nitrogen requirement of major agricultural crops: grains legumes. In: Lemaire, G. (Ed.), *Diagnosis of the Nitrogen Status in Crops*. Springer-Verlag, Heildeberg, pp. 107–117.
- Niklas, K.J., 2006. Plant allometry, leaf nitrogen and phosphorus stoichiometry, and interspecific trends in annual growth rates. *Ann. Bot.* 97, 155–163.
- Penman, H.L., 1948. Natural evaporation from open water, bare soil, and grass. *Proc. Roy. Soc. Lond. A* 193, 120–145.
- Poehlman, J.M., 1987. Breeding soybeans. In: Poehlman, J.M. (Ed.), *Breeding Field Crops*. Van Nostrand Reinhold, New York, pp. 421–450.
- Pons, T.L., Pearcy, R.W., 1994. Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiol. Plant* 92, 636–644.
- Reddy, K.N., Hoagland, R.E., Zablutowicz, R.M., 2000. Effect of glyphosate on growth, chlorophyll content and nodulation in glyphosate-resistant soybeans (*Glycine max*) varieties. *J. New Seeds* 2, 37–52.
- Reddy, K.N., Zablutowicz, R.M., 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Sci.* 51, 496–502.
- Sadras, V.O., Lemaire, G., 2014. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. *Field Crops Res.* 164, 54–64.
- Sadras, V.O., Milroy, S.P., 1996. Soil-water thresholds for the responses of leaf expansion and gas exchange. *Field Crops Res.* 47, 253–266.
- Salvagioti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann, A., 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: a review. *Field Crop Res.* 108, 1–13.
- Setiyono, T.D., Weiss, A., Specht, J., Bastidas, A.M., Cassman, K.G., Dobermann, A., 2007. Understanding and modeling the effect of temperature and daylength on soybean phenology under high-yield conditions. *Field Crops Res.* 100, 257–271.
- Shiraiwa, T., Sinclair, T.R., 1993. Distribution of nitrogen among leaves in soybean canopies. *Crop Sci.* 33, 804–808.
- Sinclair, T.R., Farias, J.R.B., Neumaier, N., Nepomuceno, A.L., 2003. Modeling nitrogen accumulation and use by soybean. *Field Crops Res.* 81, 149–158.
- Sinclair, T.R., Horie, T., 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: a review. *Crop Sci.* 29, 90–98.
- Westgate, M.E., Piper, E., Barchelor, W.D., Hurburgh, C., 1999. In: Drackley, J.K. (Ed.), *Effects of Cultural and Environmental Conditions During Soybean Growth on Nutritive Value of Soy Products*. Soy Animal Nutrition Symposium, Chicago, IL, pp. 75–89.
- Wingeyer, A.B., Echeverría, H.E., Sainz Rozas, H.R., 2014. Growth and yield of irrigated and rainfed soybean with late nitrogen fertilization. *Agron. J.* 106, 567–576.
- Yusuf, R.I., Siemens, J.C., Bullock, D.G., 1999. Growth analysis of soybean under no-tillage and conventional tillage systems. *Agron. J.* 91, 928–933.