Growth and Yield of Irrigated and Rainfed Soybean with Late Nitrogen Fertilization

Ana B. Wingeyer,* Hernán Echeverría, and Hernán Sainz Rozas

ABSTRACT

Nitrogen fertilization and supplemental irrigation during soybean [*Glycine max* (L.) Merr.] reproductive stages have gained interest to increase soybean yields. We assessed the effect of N fertilizer (0 and 60 kg N ha^{-1}) applied at the beginning of bloom (R1) and full pod (R4) combined with rainfed (NIrr) and irrigated (Irr) conditions during reproductive stages on crop growth and yield in the southeast of Buenos Aires Province, Argentina. The NIrr treatments experienced a severe drought (30 d) in 2002/2003, and a moderate but longer drought (46 d) in 2003/2004. At the beginning of seed filling (R5), aboveground biomass and plant N accumulation were unaffected by the addition of N fertilizer. Aboveground biomass at R5 was 16% greater under Irr as compared to NIrr. Average soybean yields were 4.24 and 3.39 Mg ha^{-1} for Irr and NIrr treatments, respectively, and were not affected by N fertilization. Application of N fertilizer neither reduced the anticipated plant senescence nor increased plant N accumulation under water stress conditions. Our results suggest addition of N fertilizer during soybean reproductive stages was not an effective management practice to increase yields of irrigated or rainfed soybean plants. Current rainfed soybean yields in the region can be increased significantly by maintaining soil water level at or above 60% plant available water during beginning of pod (R3.5) to full seed (R6) period.

Nitrogen fertilization of soybean during reproductive stages has gained interest as a management practice to increase soybean yields, given the dependence of soybean yields on plant N accumulation (Salvagiotti et al., 2008) and the high N requirements of this crop during later reproductive stages (Sinclair and de Wit, 1976). Nitrogen sources for soybean crops consist of mineral soil N at sowing, symbiotically nitrogen fixation (SNF), and indigenous soil N supply by mineralization during the cropping season. In the southeast of Buenos Aires Province, measured SNF in well-nodulated soybean plants yielding 4.5 to 5.5 Mg ha^{-1} represented only 25 to 40% of total soybean N uptake (González et al., 1997). Thus, alternative sources of N (e.g., N mineralization, fertilizers) should be available in soils for plant uptake to sustain those high yields. It has been suggested that application of relatively low N fertilizer rates (i.e., 20–60 kg N ha^{-1}) during reproductive soybean stages can increase plant N accumulation and soybean yields (Wesley et al., 1998; Gan et al., 2003; Purcell et al., 2004).

A.B. Wingeyer, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata. Current address: Dep. of Agronomy and Horticulture, 279 Plant Sciences, Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0915; H. Echeverría, and H. Sainz Rozas, Unidad Integrada Balcarce INTA-FCA, Ruta Nacional 226 Km 73.5, C.C. 276, B7620, Balcarce, Buenos Aires, Argentina. Received 23 May 2013. *Corresponding author (anitawingeyer@yahoo.com.ar).

Published in Agron. J. 106:567–576 (2014)
doi:10.2134/agronj2013.0255

Contradictory results regarding in-season soybean N fertilization are found in the literature in areas with similar climate conditions, soybean maturity groups and management practices to the southeast of Buenos Aires Province. In Kansas, Wesley et al. (1998) found an increase in yield between 0.44 and 0.47 Mg ha^{-1} with only 20 and 40 kg N ha^{-1} at the R3 stage on irrigated soybean. They concluded that yield response occurred in the high yielding sites (where control treatments yielded above 3.4 Mg ha^{-1}). Similarly, Melgar and Lupi (2002) in the north of Buenos Aires Province (Argentina) obtained greater N accumulation and yields when 30 kg N ha^{-1} were applied at V4 or R3 stages on well-nodulated soybean. Gan et al. (2003) in China, indicated yield increases of 9 to 26% across three soybean genotypes when 50 kg N ha^{-1} were applied at R1 compared to non-fertilized soybean but no response if N was applied at R3 or R5. No response to N fertilization strategies are reported by Schmitt et al. (2001; Minnesota), Freeborn et al. (2001; Virginia), and Gutiérrez-Boem et al. (2004; North of Buenos Aires Province) in well-nodulated high yielding soybean crops (>3.5 Mg ha^{-1}).

Soybean yields in the southeast of Buenos Aires Province can attain 5.5 Mg ha^{-1} (González et al., 1997). However, common yields in the region are limited to <3 Mg ha^{-1} (Calviño and Sadras, 1999; Calviño and Monzón, 2009) due to water stress during the seed filling period (R4–R6 stages). Reduced soil water availability (SWA) during soybean reproductive stages

Abbreviations: CGR, crop growth rate; DM, dry matter; Irr, irrigated; LI, light interception; NIrr, rainfed; P1000, weight of 1000 seeds; SNF, symbiotic nitrogen fixation; SWA, soil water availability.

increases the yield gap by reducing the photosynthetic rate and the N assimilation rate (lower SNF and reduced soil N mineralization), and by increasing remobilization of plant C and N to the seeds with shortening of the reproductive stages and premature leaf senescence (De Souza et al., 1997; Freeborn et al., 2001; Purcell et al., 2004). Earlier studies in the southeast of Buenos Aires Province determined that if SWA during R4 to R6 stages drops below 60%, yield losses of 30% or more can be expected (Andriani et al., 1991; Calviño and Monzón, 2009).

The main objective of this study was to evaluate the application of N fertilizer rates at the beginning of bloom (R1) and at R4 on yield under two SWA regimes during the R3.5 to R6 soybean stages: NIrr conditions (which represent more than 90% of the soybean cultivating area in the region) and supplementary Irr to maintain plant available water at 60% (i.e., 60% of field capacity minus wilting point). A secondary objective was to evaluate the effect of N fertilizer rates during reproductive stages of soybean on biomass and plant N accumulation, and crop growth rate (CGR) under NIrr and Irr conditions.

MATERIALS AND METHODS

Site Description and Treatments

The experiment was conducted at the Instituto Nacional de Tecnología Agropecuaria (INTA) Balcarce Experimental Station (37°45′ S and 58°18′ W, 130 m elevation) over a 2-yr period (2002/2003 and 2003/2004). Soybean plants were grown in a deep Typical Argiudoll, a shallow Petrocalcic Paleudoll soil complex with more than 20 yr of continuous agriculture. Soil organic C content (Walkley and Black, 1934), pH, and P availability (Bray and Kurtz, 1945) were determined before sowing for the 0- to 0.2-m depth, while residual soil nitrate content was determined before R1 soybean stage (Table 1) to a depth of 0.4 m. Soybean varieties used in this study are adapted to the region. Soybean varieties, planting densities, row spacings, and planting/pre-planting operations were chosen by the farm manager according to planting dates and the best management practices and are presented in Table 1. Conventional tillage involved two disks and a rototiller before sowing. No-till involved chemical burndown with glyphosate at 65 and 20 d before planting. Soybean seeds were inoculated with *Bradyrhizobium japonicum* (Laboratorio Arbo, Buenos Aires, Argentina) before sowing to ensure nodulation would not be a limiting factor (González et al., 1997). At sowing, 60 kg ha^{-1} triple superphosphate (12 kg P ha^{-1}) was applied 2 cm below the seed. The experiment was performed as a split-plot randomized complete block design (RCBD) with SWA as the main plot factor and N fertilization as the subplot factor. The experimental units (subplots) were 6 m long by 2 m wide.

Two levels of SWA were imposed during soybean reproductive stages (R1–R6): Irr where soil water content was kept above critical SWA threshold using a sprinkler irrigation system, and NIrr. We used the model calibrated by Della Maggiora et al. (2003) in soils of the region to simulate SWA, the critical SWA threshold during soybean growing season, and to determine the timing and amount of irrigation for Irr treatments. In this model, maximum SWA (100% plant available water) is the difference between soil water content at field capacity and that at wilting point, while critical SWA threshold is 40% of SWA during emergence to R3.5 stage, 60% of SWA from R3.5 to R6 stages, and 40% from R5.5 to physiological maturity (Dardanelli et al., 1991). For the model parameterization, volumetric water content at field capacity was set at 0.360 m3 m^{-3} and the volumetric water content for permanent wilting point was set at 0.192 m^{3} m^{-3} based on the soils used in the experiment (Della Maggiora et al., 2003). The SWA at the end of a 10-d interval was calculated as the balance between previous SWA, rain, and/or irrigation events and crop evapotranspiration (ET) (from Penman–Monteith method and adjusted crop factor by crop phenology). Meteorological data for reference ET calculation and model SWA simulation were collected at the weather station located in the experimental station (800 m away from the experiment plots). The simulation of SWA was started 2 mo before sowing for the 0- to 0.8-m depth soil profile. Irrigation events were conducted at an average rate of 4 mm h^{-1} during the evening and night to reduce evaporation. Six rain gauges distributed across the irrigated main plots were used to measure amount of each irrigation event. Two fertilization times: R1 and R4 stages were combined with three N rates (0, 30, and 60 kg N ha^{-1}). In 2003/2004, the 30 kg N ha^{-1} plots received 60 kg N ha^{-1}. The two reps per application time within each block were then averaged in 2003/2004. For the analysis, only three N fertilizer treatments were compared: 60 kg N ha^{-1} applied at R1, 60 kg N ha^{-1} applied at R4, and an unfertilized control. Urea (46–0–0) was manually broadcast over the crop canopy. Urea was used since it constitutes the most common N source in the region.

Crop Growth, Development, and Yield

Soybean developmental stages were weekly assessed from emergence to maturity on five plants in the central row of each experimental unit (Fehr and Caviness, 1977). Length of seed-filling period was estimated as the sum of calendar days between R3 and R6. Canopy light interception (LI) during reproductive stages was determined with the Li-Cor 191 SB

Table 1. Site characteristics, soybean varieties, Soil pH, P (Bray and Kurtz), and organic C content before sowing and mineral N content before flowering (R1) in 2002/2003 and 2003/2004 growing seasons.

Year	Tillage	Previous crop	Variety Growth type Maturity group	Planting date	Row width	Seed rate	Organic carbon	pH	Soil P	Soil NO_3–N 0–20 cm	 20–40 cm
					m	seeds m^{-1}	mg kg^{-1}		mg kg^{-1}	—— mg N kg^{-1} ——	
2002	Conventional	Wheat	Don Mario 4800 Indeterminate 4.5	10 Nov.	0.38	18	29.0	6.1	15.5	8.2	3.7
2003	No till	Maize	Don Mario 3700 Indeterminate 3.8	10 Dec.	0.19	21	31.3	6.3	20.4	6.8	3.3

sensor (Li-Cor, Lincoln, NE). For each experimental unit, one reading was made above the canopy and three readings were made below the last green layer of leaves across the central rows. Measurements were conducted around noon on days with no cloud obstruction. The LI of each plot was calculated as the average of the ratio of below and above readings. At R2 (full flowering) and R5 stages, aboveground dry matter (DM) was determined. Plants from a 1-m row were harvested at the soil surface and dried at 60°C in a chamber with forced air circulation until constant weight. Rows chosen for DM measurements were independent of rows reserved for yield harvest. Crop growth rate was estimated as linear extrapolations of DM measurements for the emergence to R2 and R2 to R5 periods and reported in kg ha^{-1} d^{-1}. At R5, nodules in soybean roots were collected by sampling roots and soil around the row (0.5 m row by 0.2 m wide by 0.2 m deep). Plant roots were removed from soil and washed with tap water. Soil was passed through a 0.5-mm sieve to collect free nodules. All nodules were rinsed with tap water and dried at 40°C until constant weight.

Soybean grain yield was determined by harvesting a 1 m wide by 5 m row area with an automated small plot combine after physiological maturity. Soybean yields are reported at commercial moisture content (135 g H_2O kg^{-1}). The weight of 1000 seeds (P1000) was measured and used to estimate the number of seed m^{-2}. Soybean plant tissue and grain were analyzed for N content (Nelson and Sommers, 1973) to estimate plant N accumulation (kg N ha^{-1}) as the product of aboveground biomass and tissue N concentration.

Data Analyses

A split-plot RCBD with three blocks was used with SWA as the main plot factor and the factorial combination of fertilization time and N rate as subplot factor. Water levels were randomly assigned to the main plots and the fertilization treatments were randomly assigned to the subplots. All data were examined for normality by Shapiro–Wilk test and homogeneity of variance assumption was tested in each case by plotting of the residuals against the estimates. All data met the normality and homogeneity of variance assumptions. Analysis of variance (Table 2) was conducted using PROC MIXED (SAS Institute, Cary, NC) with water level and fertilization treatment as fixed effects and year and block as random effects. Both, the block effect and the main plot random error where nested within year. The significance of year as a random effect was tested with a χ^2 with 1 df and $P < 0.05$ by comparing the difference in log-likelihood of the full and reduced models. The amount of the total random variation in the data associated with the random effects was determined from the covariance parameter estimates. Separation of means for significant variables ($P < 0.10$) was conducted using LSMEANS. The relationships between plant biomass, CGR, nutrient concentrations, nodule biomass, and yield components were analyzed using PROC CORR and PROC REG (SAS Institute, Cary, NC). Pearson correlation coefficients, regression equations and P values are reported. Given water level had a significant impact on the majority of the variables, correlation analyses were conducted separately for Irr and NIrr treatments.

RESULTS AND DISCUSSION

Rainfall and Soil Water Availability

The rainfall and irrigation events, simulated SWA, and the critical SWA threshold in millimeters of water are shown in Fig. 1. Rainfall events during the months before crop sowing allowed soil profile to reach 100% SWA in both years. October to March monthly precipitation was 261, 169, 39, 124, 91, and 167 mm in 2002/2003 and 69, 142, 136, 77, 48, and 47 mm for the 2003/2004 season. The historic (1971–2001) monthly precipitation for the same months is 99, 86, 129, 112, 88, and 89 mm. The reduced rainfall during soybean reproductive

Table 2. Analysis of variance for fixed (water, N) and random (year) effects on dry matter (DM), plant N content (N cont.), plant N accumulation (N mass), crop growth rate (CGR), light interception (LI), nodule biomass (Nodules), plant height (Height), yield, seed weight (P1000), seed number, seed N content (seed N cont.), and seed N accumulation (seed N mass).

Stage	Variable	Water	N	Water × N	Year
		$P > F$			$P > \chi^2$
R2	DM	0.016	0.660	0.174	<0.001
	N cont., g kg^{-1}	0.872	0.062	0.180	0.516
	N mass	0.021	0.392	0.342	<0.001
	CGR E-R2	0.017	0.721	0.157	<0.001
	LI	0.107	0.984	0.984	<0.001
R3	LI	<0.001	0.774	0.979	0.999
R4	LI	0.003	0.357	0.662	0.072
R5	DM	0.002	0.248	0.221	0.242
	N cont., g kg^{-1}	0.057	0.627	0.144	<0.001
	N mass	<0.001	0.311	0.685	0.019
	Nodules	0.020	0.988	0.114	<0.001
	CGR R2-R5	0.087	0.190	0.133	0.010
R6	LI	0.009	0.299	0.169	<0.001
R7	Height	0.001	0.866	0.981	<0.001
R8	Yield	<0.001	0.662	0.676	0.999
	P1000	0.015	0.308	0.592	<0.001
	Seed number	<0.001	0.887	0.831	<0.001
	Seed N cont., g kg^{-1}	0.742	0.521	0.811	<0.001
	Seed N mass	<0.001	0.594	0.578	0.167

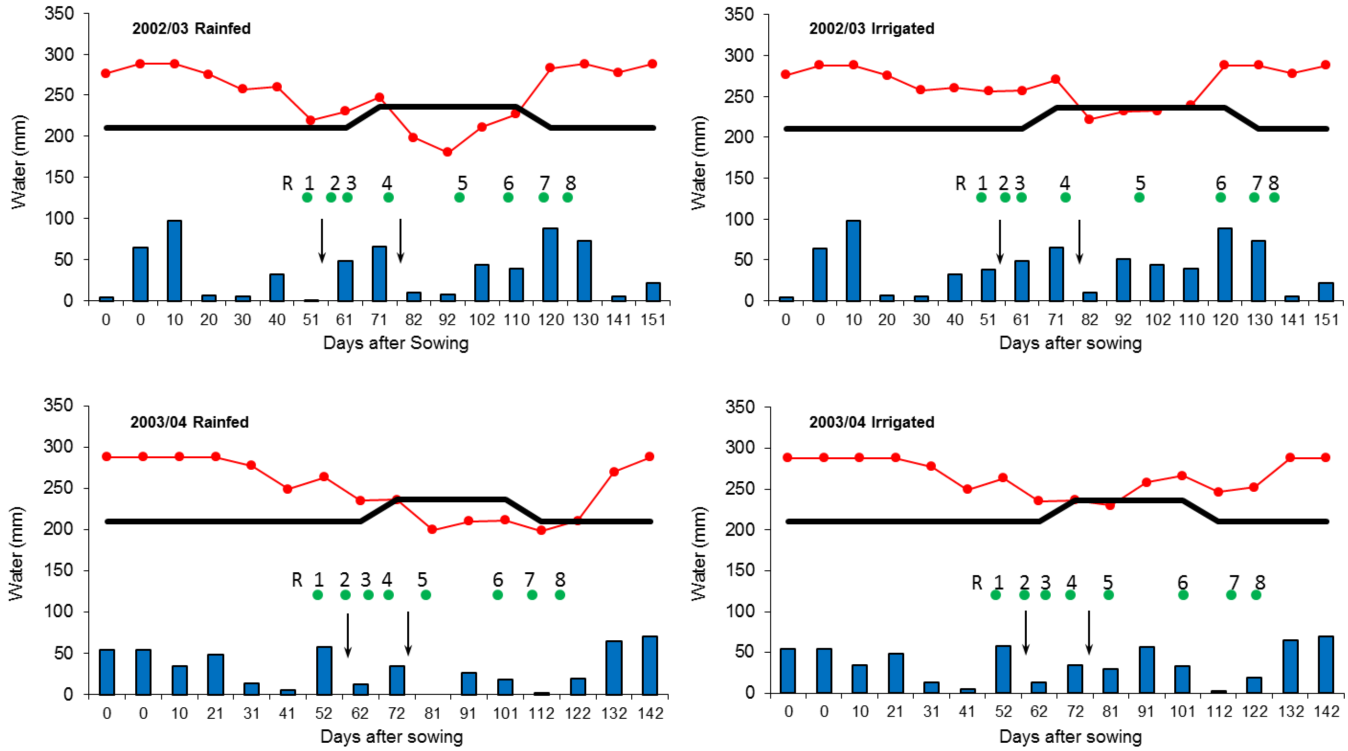

Fig. 1. Water balance for soybean crop in 2002/2003 and 2003/2004: Simulated soil water content (red), rainfall and irrigation (blue), and critical threshold of available soil water content for soybean growth (black) (Della Maggiora et al., 2003). Arrows show fertilization times at R1 and R4. Green circles show reproductive stages.

stages (December–March) constitutes the most important soybean yield limiting factor in the region (Calviño and Sadras, 1999). In our study, the rainfall amount and distribution resulted in contrasting SWA scenarios between Irr and NIrr treatments (Fig. 1). In the 2002/2003 growing season, NIrr soybean plants experienced a short drop in SWA at R1 (40% SWA) and a greater drop in SWA (SWA <35%) during the R3.5 to R6 period. In the same season, rainfall events in late February (105 d after sowing) allowed the recharge of soil profile (Fig. 1). In the 2003/2004 growing season, NIrr soybeans experienced a SWA reduction after R3, which was less severe (SWA ≥40%), but lasted longer (~45 d) than the 2002/2003 season (Fig. 1) due to reduced precipitation in February and March. The estimated total water deficit under NIrr conditions was 86 mm in 2002/2003 and 39 mm in 2003/2004.

Soybean Growth

The main effect of year accounted for 91, 9, 91, 34, and 81% of the random variability in DM at R2, DM at R5, CGR at R2, CGR at R5 and height, respectively (Table 2). In addition, the main effect of year accounted for 55, 0, 9, and 70% of the random variability in LI at R2, R3, R4 and R6, respectively. Thus, with the exception of DM at R5 and LI at R3 and R4, the random variation in crop growth was primarily due to differences among years (Table 2). The delay in sowing date in the second growing season resulted in higher temperatures during vegetative stages. The average daily mean temperature from sowing to R1 was 18.6°C in 2002/2003 and 20.2°C in 2003/2004. The higher temperatures in 2003/2004 along with narrow rows, favored a faster closure of the canopy and greater biomass accumulation compared to the 2002/2003 growing season (not shown), similar to the findings reported by Board (2000) and Ball et al. (2000).

Height; DM at R2 and R5; LI at R3, R4, and R6; and CGR at R2 were all affected by the main effect of water level, but not by N treatment or the interaction between water level and N treatment (Table 2). Soybean plants under Irr were 19% taller, and had 18 and 16% greater DM at R2 and R5 than the NIrr plants (Tables 3 and 4). Canopy LI values were greater from R3 to R6 under Irr compared to NIrr treatments (Table 2, Fig. 2). Soybean plants under NIrr had LI <95% between R4 to R6, and showed an accelerated leaf senescence and shortening of reproductive stages (Fig. 1). An enhanced remobilization of plant C and N to the seeds and decreased photosynthetic rate can be expected in soybean plants under NIrr conditions (De Souza et al., 1997) which may lead to increased leaf senescence and reduced light interception during late reproductive stages (Andriani et al., 1991; Freeborn et al., 2001; Brevedan and Egli, 2003). Similar to Andriani et al. (1991) and Freeborn et al. (2001), the length of reproductive stages was reduced under NIrr conditions in the present study. Compared to Irr soybean, the reproductive period of NIrr soybean in this study was 10 and 12 d shorter in 2002/2003 and 2003/2004 seasons, respectively (Fig. 1). In 2002/2003, R1 to full maturity (R8) stages occurred at 51, 58, 64, 76, 99, 121, 131, and 136 d after planting under Irr and at 51, 58, 64, 74, 97, 109, 120, and 126 d after planting under NIrr, respectively. In 2003/2004, R1 to R8 stages occurred at 51, 59, 65, 72, 84, 103, 117, and 128 d after planting under Irr and at 51, 59, 65, 71, 79, 100, 110, and 116 d after planting under NIrr, respectively. The values of CGR between R2 and R5 in our study are within the range reported by Bodrero et al. (1997) and Andrade (1995) for the region. Crop growth rates were 18 and 12% higher

Table 3. Crop growth and N accumulation of irrigated and rainfed soybean by N treatment at R2 and R5 stages.

Soybean stage	R2		R5	
N treatment†	Irrigated	Rainfed	Irrigated	Rainfed
		Dry matter, kg ha^{-1}		
Control	3350	2600	7130	5710
60 (R1)	3170	2940	7150	6890
60 (R4)	nd‡	nd	7480	6160
Average§	3260A	2770B	7250A	6260B
		N content, g kg^{-1}		
Control	3.32	3.43	3.28	3.18
60 (R1)	3.57	3.48	3.42	3.02
60 (R4)	nd	nd	3.25	3.08
Average	3.44A	3.46A	3.31A	3.09B
		N mass, kg ha^{-1}		
Control	113	89	232	180
60 (R1)	112	102	243	208
60 (R4)	nd	nd	243	188
Average	112A	96B	240A	192B
		Nodule mass, g m^{-2}		
Control	nd	nd	19.6	12.1
60 (R1)	nd	nd	15.2	15.9
60 (R4)	nd	nd	20.0	10.8
Average			18.3A	13.0B
		Crop growth rate, kg ha^{-1} d^{-1}		
	Emergence to R2		R2 to R5	
Control	55	42	153	120
60 (R1)	51	48	141	152
60 (R4)	nd	nd	173	144
Average	53A	45B	156A	139B

† Treatments: Control: 0 kg N ha^{-1}; 60 (R1): 60 kg N ha^{-1} applied at beginning of bloom stage; 60 (R4): 60 kg N ha^{-1} applied at full pod stage.

‡ nd: no data.

§ Averages followed by same letter across water levels for each soybean stage are not significantly different (α = 0.05).

for Irr compared to NIrr, for the emergence to R2 and R2 to R5 periods, respectively (Table 3). Andriani et al. (1991) determined reductions of up to 30% in soybean CGR when the water stress occurred between R3 to R5 stages compared to irrigated plants. Reductions in both LI and radiation use efficiency can explain the reduction observed in CGR between R2 and R5 stages under NIrr compared to Irr conditions (Andriani et al., 1991; Bodrero et al., 1997).

The lack of response of soybean growth to N supply (Tables 2, 3, and 4) is in agreement with the reports of field trials by Santos (2001) and Schmitt et al. (2001), suggesting N supply from soil and SNF was adequate to meet crop N requirements of Irr soybean. In addition, the application of N fertilizer to NIrr soybean did not result in higher LI values or longer seed-filling period compared to the control. Thus any reduction in N supply from SNF (Purcell et al., 2004; Mastrodomenico et al., 2013) and soil (Kim et al., 2008) under NIrr conditions was not compensated by addition of N fertilizer during reproductive stages.

Soybean Nitrogen

The indirect impact of SWA and N treatments on N supply from SNF was determined from the biomass of root nodules at R5 (Table 3). The mineral soil N availability determined before R1 in the first 40 cm soil depth was below 30 kg N ha^{-1} in both years (Table 1), indicating mineral soil N did not constitute a limitation to the establishment of SNF system in this study (Taylor et al., 2005; Hungria et al., 2006). The main effect of year on nodule biomass at R5 was significant (Table 2) and accounted for 77% of the random variability. Approximately three times greater biomass of nodules was recovered in the 2003/2004 (24.6 g m^{-2}) season compared to 2002/03 (8.0 g m^{-2}). Although lower soil mineral N content and better SWA in 2003/2004 could have contributed to higher nodule biomass in that season (Di Ciocco et al., 2008), different nodulation ability between the soybean varieties used in this study could have also contributed to the difference in nodule biomass between years (Gan et al., 2003). Neither application of N fertilizer nor the interaction between water levels and N treatments influenced nodule biomass (Tables 2 and 3), suggesting N fertilization in this study did not affect the established SNF system. Our results do not agree with Gan et al. (2003), who reported a reduction in nodule biomass during reproductive stages when 50 kg N ha^{-1} was applied at R1 or R3 stages. The main effect of water had a significant effect on nodule biomass. Average nodule mass of Irr soybean was 41% greater compared to NIrr soybean (Table 3). These results are in agreement with Cicore et al. (2005) and suggest that the water deficit during reproductive stages compromised the N supply from SNF source under NIrr. Nodule biomass was associated to DM at R2 for both Irr (r = 0.76; P = 0.011) and NIrr soybean (r = 0.83; P = 0.003), indicating soybean growth earlier in the season had a significant effect on the establishment of SNF system (data not shown).

Table 4. Yield, yield components, and grain N accumulation of irrigated and rainfed soybean by N treatment.

N treatment†	Irrigated	Rainfed
	Yield, Mg ha^{-1}	
Control	4.18	3.36
60 (R1)	4.32	3.37
60 (R4)	4.23	3.44
Average†	4.24A	3.39B
	Seed weight, g 1000 seeds^{-1}	
Control	151	141
60 (R1)	154	142
60 (R4)	151	143
Average	152A	142B
	Seed number, seeds m^{-2}	
Control	2735	2375
60 (R1)	2780	2355
60 (R4)	2766	2403
Average	2760A	2378B
	Seed N content, g kg^{-1}	
Control	5.95	5.91
60 (R1)	5.94	5.89
60 (R4)	5.96	5.98
Average	5.95A	5.92A
	Seed N mass, kg ha^{-1}	
Control	248	198
60 (R1)	257	199
60 (R4)	252	206
Average	253A	201B
	Plant height at R7, m	
Control	0.92	0.76
60 (R1)	0.92	0.77
60 (R4)	0.93	0.78
Average	0.92A	0.77B

† Averages followed by same letter across water levels are not significantly different (α = 0.05).

With the exception of N content at R2, the random variation in soybean N content and mass was primarily due to differences among years (Table 2). The main effect of year accounted for 12 and 81% of the random variation in the N content of soybean at R2 and R5, and 90 and 32% of the random variation in N accumulation at R2 and R5, respectively. The larger N content at R5 in the 2003/2004 season (3.55 g kg^{-1}) compared to 2002/2003 (2.78 g kg^{-1}) was consistent with greater nodule biomass and smaller water deficit in that season. Soybean N content at R2 was affected by the main effect of N treatment, but not by the water level or the interaction between water level and N treatment (Table 2). The application of 60 kg N ha^{-1} at R1 (60R1) resulted in greater N content compared to the control (3.52 vs. 3.38 g N kg^{-1}, respectively). This increase in N content may suggest a better N status of the crop and potential growth, but there was no clear relationship between N content at R2 and CGR (r = –0.21, P = 0.322) or DM at R5 (r = 0.027, P = 0.899). Soybean N content at R5 and N accumulation at R2 and R5 were all affected by the main effect of water level, but not by N treatment or the interaction between water level and N treatment (Table 2). Irrigated soybean had 7% greater N content at R5 compared to NIrr ones (Table 3), suggesting water deficit affected the N status of NIrr soybean. Compared to NIrr, Irr soybean accumulated 17 and 25% more N at R2 and R5, respectively (Table 3) as the result of reduced biomass

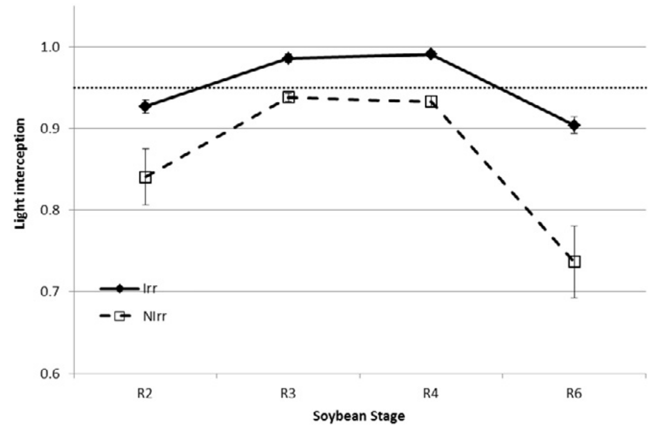

Fig. 2. Light interception (as fraction of incident light) and SE by irrigated (Irr) and rainfed (NIrr) soybean at R2, R3, R4, and R6 stages. The critical threshold of light interception (0.95) during reproductive stages is indicated by the dotted line.

at both stages and reduced N content at R5 stage in NIrr soybean. Under water stress conditions, plant N accumulation can be compromised due to reduced SNF (Purcell et al., 2004), and reduced N mineralization and mineral N uptake (Kim et al., 2008). The association between nodule biomass and plant N content at R5 was used as a rough indicator of the SNF system activity (Fig. 3). A positive relationship between plant N content at R5 and nodule biomass was determined for both Irr (r = 0.74; P = 0.001) and NIrr soybean (r = 0.92; P < 0.001) suggesting the size of SNF system had a direct contribution to soybean N status. Similar to nodule biomass, plant N content at R5 was also associated to DM accumulation at R2 for both Irr (r = 0.89; P < 0.001) and NIrr soybean (r = 0.71; P = 0.010), suggesting greater soybean growth earlier in the season in our study contributed to greater N content through increased nodulation (Fig. 3).

Soybean Yield and Yield Components

The main effect of year was not significant for yield and accounted for <1% of the random variation (Table 2). However 82 and 77% of the random variation in P1000 and seed number, were associated to year effect. The larger P1000 in the 2002/2003 season (157.5 g 1000 seeds^{-1}) compared to 2003/2004 (136.4 g 1000 seeds^{-1}) was compensated by smaller seed number in 2002/2003 (2362 seeds m^{-2}) compared to 2003/2004 (2775 seeds m^{-2}) resulting in similar yield averages across seasons. Difference in soybean varieties could have contributed to the different P1000 between years. Soybean yields, P1000 and number of seeds were all affected by the main effect of water level, but not by N treatment or the interaction between water level and N treatment (Table 2). Soybean yields ranged from 3.36 to 4.32 Mg ha^{-1} (Table 4). In the present study, irrigation during reproductive stages to maintain a minimum SWA threshold resulted in 25% higher yields compared to NIrr conditions. These results were in agreement with the reports of Andriani et al. (1991) and Calviño and Sadras (1999). Soybean yields have been found dependent on plant N accumulation (Salvagiotti et al., 2008). In our study, soybean yields were associated to DM at R5 under NIrr conditions (r = 0.53, P = 0.024), but did not show significant association with N accumulation at R5

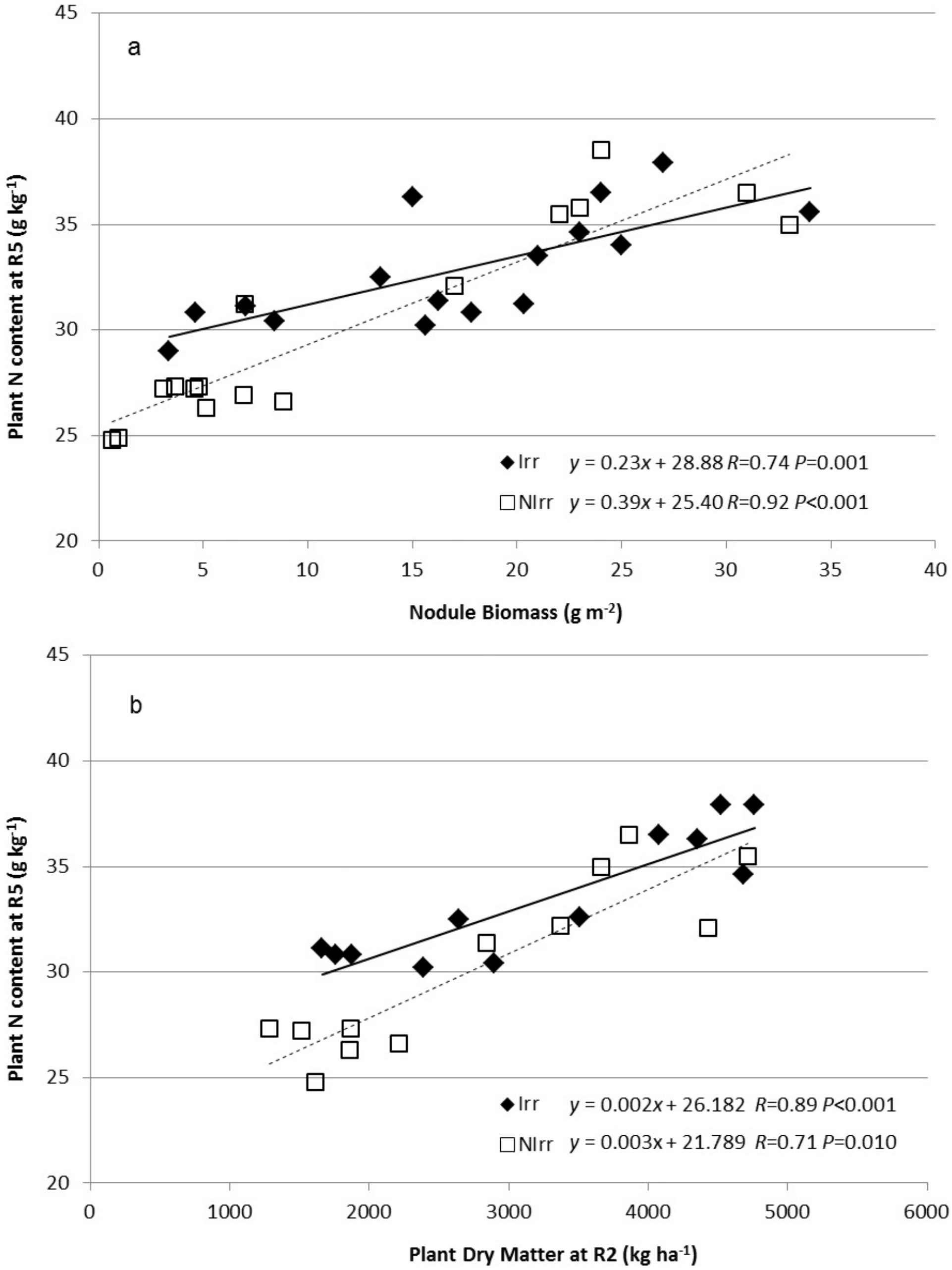

Fig. 3. Association between (a) plant N content at R5 and nodule biomass at R5 and (b) plant N content at R5 and dry matter accumulation at R2 (b) for irrigated (Irr) and rainfed (NIrr) soybean.

under Irr ($r = -0.36$, $P = 0.137$) or NIrr ($r = 0.15$, $P = 0.556$) conditions (Fig. 4), suggesting N accumulation after R5 stage may have been significant.

Reduced P1000 (–7%) and seed number (–16%) explained the lower yields under NIrr compared to Irr (Table 4). Number of seeds per unit area has been associated to the biomass accumulation during reproductive stages (Vega et al., 2001; Board and Modali, 2005), and lower seed number for water stressed soybean during reproductive stages resulted from increased flower and pod abortion (Vega et al., 2001). In our study, seed number of NIrr soybean was associated with CGR between R2 and R5 stages ($r = 0.64$; $P = 0.004$), while there was no clear relationship for Irr soybean ($r = 0.06$; $P = 0.819$). In addition, positive relationships of seed number with plant N content at R5 were determined for Irr ($r = 0.58$; $P = 0.011$) and NIrr ($r = 0.82$; $P < 0.001$) conditions. These results indicate a dependence of seed number on both C and N assimilation of the crop, suggesting N status may play a more critical role than C assimilation. As water stress is moderate or occurs later in the growing season, seed number may be less affected. However, later water deficit conditions can result in enhanced remobilization of plant C and N to the seeds (De Souza et al., 1997), premature senescence and reduced ability to compensate for shorter seed-filling duration and greater seed number leading to reduced seed weight (Ball et al., 2000; Brevedan and Egli, 2003; Mastrodomenico et al., 2013). In this study, P1000 of Irr and NIrr soybean showed negative association with plant N content at R5, nodule biomass, height, and DM at R2 (Table 5), indicating better growing condition during early reproductive stages did not necessarily lead to larger seeds as seed filling progresses likely associated to larger seed number being set.

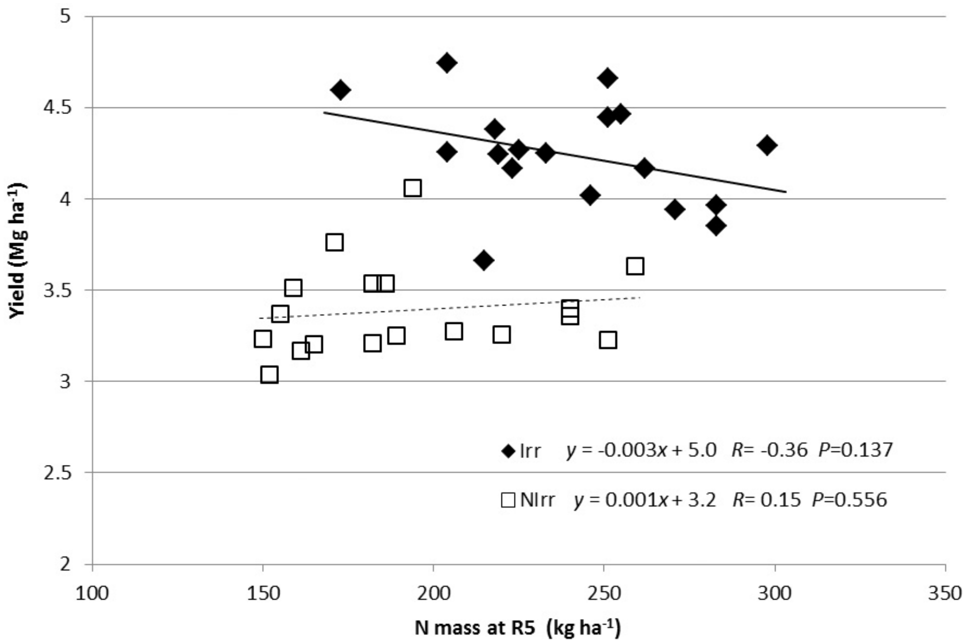

Fig. 4. Association between soybean yield and plant N accumulation at R5 for irrigated (Irr) and rainfed (NIrr) soybean.

It has been hypothesized that under moderate soil water deficit conditions, addition of N fertilizer during reproductive stages could alleviate the plant N stress given the high N demand and minimizes its effect on anticipated senescence and yield (Purcell and King, 1996). Addition of N fertilizer during reproductive stages in the present study did not affect the seed number or P1000 compared to non-fertilized treatments. Thus, unlike Wesley et al. (1998) and Melgar and Lupi (2002), a significant increase in the yield of well nodulated high-yielding soybean was not found in this study with the addition of N fertilizer during reproductive stages. Others (Freeborn et al., 2001; Virginia; Schmitt et al., 2001; Minnesota; Gutiérrez-Boem et al., 2004; Buenos Aires Province; Barker and Sawyer, 2005; Iowa) also reported no yield response to N fertilizer rates applied during reproductive stages for irrigated and rainfed soybean with grain yields in the same range as presented here. The lack of response in plant N accumulation and yield to the addition of N fertilizer in the present study may be indicative of adequate supply of N from SNF and soil (Gutiérrez-Boem et al., 2004) and substitution of the SNF by fertilizer N.

Seed Nitrogen

The main effect of year accounted for 64 and 11% of the random variation in seed N content and seed N accumulation, respectively. The larger seed N content in the 2003/2004 season (61.0 g kg^{-1}) compared to 2002/2003 (57.7 g kg^{-1}) was in agreement with greater plant N content at R5 and greater nodule biomass in 2003/2004 compared to the first season. Contrarily to plant N content at R5, seed N content was not affected by water levels (Table 2). Seed N content of Irr and NIrr soybean was associated with plant N content at R5, nodule biomass, and DM at R2, while seed N content of NIrr soybean was also associated with height (Table 5). These results suggest that increasing seed N content through management may not be related to improving water availability during R4 to R6 stages but with the growing conditions before R5, as supported by the similar slopes among water levels for the

Table 5. Seed weight, seed number, and seed N content associations with soybean growth and N variables under irrigated and rainfed conditions.

	Seed weight		Seed number		Seed N content	
Variables	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
DM at R2	–0.75† (0.005)‡	–0.86 (<0.001)	0.66 (0.019)	0.92 (<0.001)	0.77 (0.003)	0.93 (<0.001)
DM at R5	0.37 (0.131)	0.22 (0.371)	–0.54 (0.021)	0.12 (0.646)	–0.45 (0.053)	0.01 (0.970)
CGR R2-R5	–0.32 (0.198)	–0.41 (0.090)	0.06 (0.819)	0.64 (0.004)	–0.05 (0.857)	0.47 (0.047)
Height	–0.59 (0.010)	–0.80 (<0.001)	0.47 (0.049)	0.83 (<0.001)	0.44 (0.069)	0.85 (<0.001)
Nodule biomass	–0.63 (0.008)	–0.77 (<0.001)	0.27 (0.311)	0.75 (<0.001)	0.67 (0.004)	0.77 (<0.001)
N content R2	0.16 (0.625)	–0.05 (0.888)	–0.19 (0.550)	–0.17 (0.601)	–0.19 (0.546)	–0.18 (0.581)
N content R5	–0.65 (0.004)	–0.83 (<0.001)	0.58 (0.011)	0.82 (<0.001)	0.66 (0.003)	0.72 (<0.001)

† Pearson correlation coefficients.
‡ $P > |R|$.

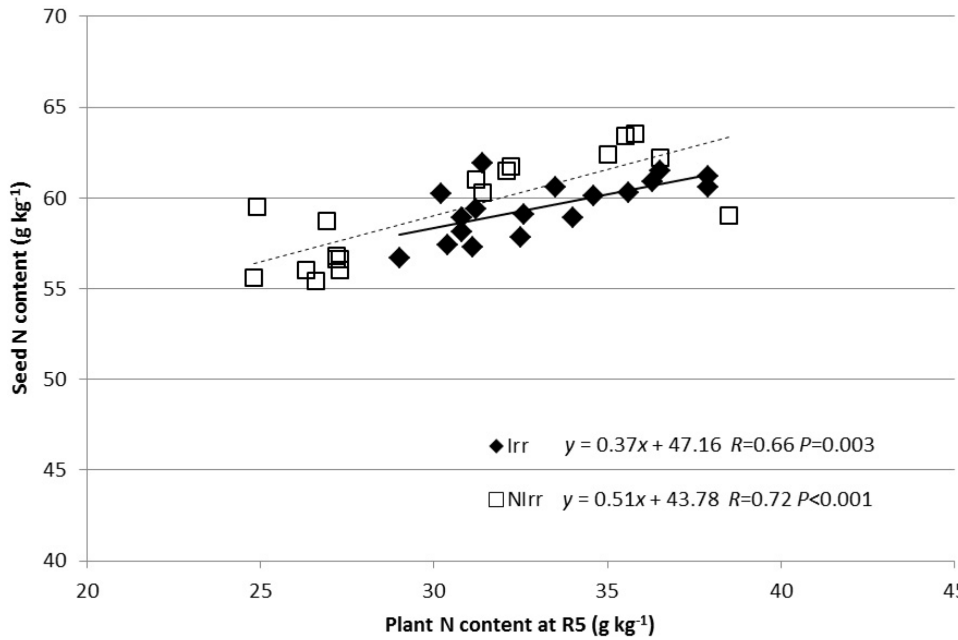

Fig. 5. Association between seed N content and plant N content at R5 for irrigated (Irr) and rainfed (NIrr) soybean. Pooled data: $y = 0.42x + 45.97$ $R = 0.70$ $P < 0.001$.

relationship between seed N content at harvest and plant N content at R5 (Fig. 5). Similar to yield, seed N accumulation was affected by the main effect of water with 26% greater N accumulation under Irr compared to NIrr conditions. The ratios between seed and plant N accumulation at R5 were >1 indicating additional N uptake after R5 in all treatments. This may partially explain the lack of associations between yield and plant N accumulation at R5 in our study (Fig. 4). Seed N content and seed N accumulation were not affected by N treatment or the interaction between water level and N treatment (Table 2). Despite a greater demand for N is expected during soybean reproductive stages, and improved plant N status can be achieved with N fertilization (Gan et al., 2003) our results indicate that addition of N fertilizer during reproductive stages may not constitute a recommended practice to improve seed N content under Irr or NIrr conditions.

CONCLUSIONS

Research evaluating the performance of management practices that can reduce the yield gap of soybean and increase resource efficiency is needed. The main objective of this study was to evaluate the application of N fertilizer at R1 and R4 stages on yield under two SWA regimes: NIrr (which represent more than 80% of the soybean crops in the region) and supplementary irrigation to maintain 60% of plant water availability (Irr). It was expected that the N applied during reproductive stages of soybean will contribute to enhance yields of high yielding (>4 Mg ha^{-1}) Irr soybean by increasing plant N accumulation and to reduce the yield gap of NIrr soybean by maintaining LI and CGR. Soybean growth, nodule biomass, and yield were strongly influenced by SWA during reproductive stages, while the addition of N fertilizer during reproductive stages, did not affect LI, CGR, plant N accumulation, or yields regardless of SWA conditions. Our results indicate that addition of 60 kg N ha^{-1} during R1 or R4 stages do not constitute a recommended practice to reduce the yield gap of Irr or NIrr soybean in this region. However, addressing water availability during reproductive stages has the potential to increase yields by 25% in the region: maintaining SWA level during reproductive stages around 60% of plant available water plays a key role to obtain >4 Mg ha^{-1}. Under scenarios where irrigation water becomes a limited resource, more research is needed to evaluate the feasibility of reduced irrigation practices to support soybean growth and yield.

ACKNOWLEDGMENTS

Appreciation is extended to Javier Crespo and all of the INTA-Soil Fertility crews for their assistance. We thank Guillermo Studdert for critical discussion and Tri Setiyono, Fernando García, Dan Walters, and anonymous reviewers for their valuable comments to this manuscript. This study was supported by grants from the Instituto Nacional de Tecnología Agropecuaria (INTA Soil Program, PICT 2011-1796) and the Universidad Nacional de Mar del Plata– Facultad de Ciencias Agrarias (FCA-UNMP AGR383/12).

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. doi:10.1016/0378-4290(94)00107-N

Andriani, J.M., F.H. Andrade, E. Suero, and J. Dardanelli. 1991. Water deficit during reproductive growth of soybeans. I. Their effects on dry matter accumulation, seed yield and its components. Agronomie 11:737–746. doi:10.1051/agro:19910904

Ball, R.A., L.C. Purcell, and E.D. Vories. 2000. Short-season soybean yield compensation in response to population and water regime. Crop Sci. 40:1070–1078. doi:10.2135/cropsci2000.4041070x

Barker, D.W., and J.E. Sawyer. 2005. Nitrogen application at early reproductive development. Agron. J. 97:615–619. doi:10.2134/agronj2005.0615

Board, J. 2000. Light interception efficiency and light quality affect yield compensation of soybean at low plant populations. Crop Sci. 40:1285–1294. doi:10.2135/cropsci2000.4051285x

Board, J.E., and H. Modali. 2005. Dry matter accumulation predictors for optimal yield in soybean. Crop Sci. 45:1790–1799. doi:10.2135/cropsci2004.0602

Bodrero, M.L., E.N. Morandi, R.A. Martignone, H.E. Baigorri, F.H. Andrade, S. Maira, and E. Guevara. 1997. Ecofisiología del cultivo. In: L.M. Giorda and H.E.J. Baigorri, editors, El cultivo de la soja en Argentina. SAGPyA. INTA, Argentina. p. 29–50.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39–45. doi:10.1097/00010694-194501000-00006

Brevedan, R.E., and D.B. Egli. 2003. Short periods of drought stress during seed filling, leaf senescence, and yield of soybean. Crop Sci. 43:2083–2088. doi:10.2135/cropsci2003.2083

Calviño, P.A., and J.P. Monzón. 2009. Farming systems of Argentina: Yield constraints and risk management. In: V.O. Sadras., and D.F. Calderini, editors, Crop physiology. Elsevier, Amsterdam. p. 55–70.

Calviño, P.A., and V.O. Sadras. 1999. Interannual variation in soybean yield: Interaction among rainfall, soil depth and crop management. Field Crops Res. 63:237–246. doi:10.1016/S0378-4290(99)00040-4

Cicore, P.L., H.R. Sainz Rozas, H.E. Echeverría, and P.A. Barbieri. 2005. Nodular dry matter and accumulated N in soybeans as a function of water and sulfur availability and tillage system. Ciencia del Suelo (Argentina) 23:205–210.

Dardanelli, J., E. Suero, F. Andrade, and J. Andriani. 1991. Water deficit during reproductive growth of soybeans. II. Water use and water deficiency indicators. Agronomie 11:747–756. doi:10.1051/agro:19910905

De Souza, P.I., D.B. Egli, and C. Bruening. 1997. Water stress during seed filling and leaf senescence in soybean. Agron. J. 89:807–812. doi:10.2134/agronj1997.00021962008900050015x

Della Maggiora, A.I., A.I. Irigoyen, J.M. Gardiol, O. Caviglia, and L. Echarte. 2003. Evaluation of a maize's soil water availability model. (In Spanish with English abstract.) Rev. Argentina de Agrometeorol. 2(2):167–176.

Di Ciocco, C., C. Coviella, E. Penón, M. Díaz-Zorita, and S. López. 2008. Biological fixation of nitrogen and N balance in soybean crops in the pampas region. Span. J. Agric. Res. 6:114–119. doi:10.5424/sjar/2008061-5259

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Special Rep. 80. Iowa Agric. Home Econ. Exp. Stn., Iowa State Univ. Ames.

Freeborn, J.R., D.L. Holshouser, M.M. Alley, N.L. Powell, and D.M. Orcutt. 2001. Soybean yield response to reproductive stage soil-applied nitrogen and foliar-applied boron. Agron. J. 93:1200–1209. doi:10.2134/agronj2001.1200

Gan, Y., I. Stulen, H. van Keulen, and P.J.C. Kuiper. 2003. Effect of N fertilizer top-dressing at various reproductive stages on growth, N2 fixation and yield of three soybean (*Glycine max* (L.) Merr.) genotypes. Field Crops Res. 80:147–155. doi:10.1016/S0378-4290(02)00171-5

González, N., A. Perticari, B. Stegman de Gurfinkel, and E. Rodríguez Cáceres. 1997. Nitrogen nutrition. In: L.M. Giorda and H.E.J. Baigorri, editors, The soybean in Argentina. (In Spanish.) SAGPyA INTA., AR. Editar, San Juan, Argentina. p. 188–198.

Gutiérrez-Boem, F.H., J.D. Scheiner, H. Rimski-Korsakov, and R. Lavado. 2004. Late season nitrogen fertilization of soybeans: Effects on leaf senescence, yield and environment. Nutr. Cycling Agroecosyst. 68:109–115. doi:10.1023/B:FRES.0000019040.02605.ee

Hungria, M., J.C. Franchini, R.J. Campo, C.C. Crispino, J.Z. Morales, R.N.R. Sibadelli, I.C. Mendes, and J. Arihara. 2006. Nitrogen nutrition of soybean in Brazil: Contributions of biological N_2 fixation and N fertilizer to grain yield. Can. J. Plant Sci. 86:927–939. doi:10.4141/P05-098

Kim, K., D.E. Clay, C.G. Carlson, S.A. Clay, and T. Trooien. 2008. Do synergistic relationships between nitrogen and water influence the ability of corn to use nitrogen derived from fertilizer and soil? Agron. J. 100:551–556. doi:10.2134/agronj2007.0064

Mastrodomenico, A.T., L.C. Purcell, and C.A. King. 2013. The response and recovery of nitrogen fixation activity in soybean to water deficit at different reproductive developmental stages. Environ. Exp. Bot. 85:16–21. doi:10.1016/j.envexpbot.2012.07.006

Melgar, R., and A. Lupi. 2002. Soybean with nitrogen. (In Spanish.) Fertilizar (Argentina) 28:14–17.

Nelson, D.W., and L.E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65:109–112. doi:10.2134/agronj1973.00021962006500010033x

Purcell, L.C., and C.A. King. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. J. Plant Nutr. 19:969–993. doi:10.1080/01904169609365173

Purcell, L.C., R. Serraj, T.R. Sinclair, and A. De. 2004. Soybean N2 fixation estimates, ureide concentration, and yield responses to drought. Crop Sci. 44:484–492.

Salvagiotti, F., K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, and A. Dobermann. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Res. 108:1–13. doi:10.1016/j.fcr.2008.03.001

Santos, D.J. 2001. Effect of soil mineral nitrogen availability and nitrogen fertilization at flowering on soybean growth and yield. MSc.Thesis. (In Spanish.) Natl. Univ. of Mar del Plata, Balcarce, Argentina.

Schmitt, M.A., J.A. Lamb, G.W. Randall, J.H. Orf, and G.W. Rhem. 2001. In-season fertilizer nitrogen applications for Soybean in Minnesota. Agron. J. 93:983–988. doi:10.2134/agronj2001.935983x

Sinclair, T.R., and C.T. de Wit. 1976. Analysis of the carbon and nitrogen limitations to soybean yield. Agron. J. 68:319–324. doi:10.2134/agronj1976.00021962006800020021x

Taylor, R.S., D.B. Weaver, C.W. Wood, and E. van Santen. 2005. Nitrogen application increases yield and early dry matter accumulation in late-planted soybean. Crop Sci. 45:854–858. doi:10.2135/cropsci2003.0344

Vega, C.R.C., F.H. Andrade, and V.O. Sadras. 2001. Reproductive partitioning and seed set efficiency in soybean, sunflower and maize. Field Crops Res. 72:163–175. doi:10.1016/S0378-4290(01)00172-1

Walkley, A., and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29–38. doi:10.1097/00010694-193401000-00003

Wesley, T.L., R.E. Lamond, V.L. Martin, and S.R. Duncan. 1998. Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. J. Prod. Agric. 11:331–336. doi:10.2134/jpa1998.0331