



Article

The Impact of Polymer on the Productivity and Photosynthesis of Soybean under Different Water Levels

Lucas Felisberto Pereira ¹, Walter Quadros Ribeiro Júnior ^{2,*}, Maria Lucrecia Gerosa Ramos ^{3,*},
Guilherme Filgueiras Soares ³, Cristiane Andréa de Lima Guimarães ³, Sebastião Pedro da Silva Neto ² ,
Onno Muller ⁴ , Christina Cleo Vinson ³, André Ferreira Pereira ² and Thomas Christopher Rhys Williams ⁵

¹ Federal Institute Goiano, Faculty of Agronomy, Campus of Posse-Fazenda Vereda do Canto, Posse 73900-000, GO, Brazil

² Brazilian Agricultural Research Corporation, EMBRAPA Cerrados, Rodovia BR 020, Planaltina 73310-970, DF, Brazil

³ Faculty of Agronomy and Veterinary Medicine, University of Brasília, Campus Darcy Ribeiro ICC Sul, Brasília 70910-900, DF, Brazil

⁴ Institute for Bio- and Geosciences, IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

⁵ Department of Botany, Institute of Biological Science, University of Brasilia, Brasília 70297-400, DF, Brazil

* Correspondence: walter.quadros@embrapa.br (W.Q.R.J.); lucrecia@unb.br (M.L.G.R.)

Abstract: In order to practice sustainable and resource-efficient agriculture, the use of new technologies such as water-retaining polymers is essential. The objective of this study was to evaluate the effect of a polymer incorporated into the soil on gas exchange and yield under different water regimes (WR) in three soybean cultivars. The experiment was conducted at Embrapa Cerrados under field conditions in 2016 and 2017, using three different cultivars (BRS 5980IPRO, NA 5909RG and BRS 7280RR). Soybean cultivars were submitted to four water regimes (representing 30%, 50%, 83% and 100% of evapotranspiration replacement, namely WR1, WR2, WR3 and WR4). No beneficial results were observed in 2016 with Polymer. Most of the reductions in photosynthesis and transpiration by adding the polymer can be attributed to stomatal control, but such reductions did not influence productivity. In 2017, the yield was higher using Polymer in WR4 and WR3 by 40 to 20%, depending on the cultivar. Under severe stress (WR2 and WR1), reduced gas exchange was obtained with Polymer, but the yield was not reduced. These results indicate that Polymer contributed to the prolongation of photosynthetic activity during the reproductive phase of soybean and may represent a potential strategy for increasing yield under moderate drought stress.

Keywords: *Glycine max*; hidrorretentor; drought; phenology; abiotic stress



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1. Introduction

Soybean occupies over 28 million ha of the Brazilian national territory and contributes 3.4% to the national gross domestic product (GDP) [1]. However, spreading its cultivation in the Brazilian Cerrado has brought some challenges, principally those associated with climate inconstancy [2]. In Brazil, although soybean is grown during the rainy season, abiotic stresses such as drought may affect any of the phenological phases of the crop, resulting in impacts on physiology and plant development [3]. Moreover, the frequency and severity of drought events in this region are predicted to increase over the next years, according to Cunha et al. [4], reinforcing the importance of understanding how key crop species respond to this stress and developing strategies for mitigation the impacts of water deficit.

Lack of water affects cell expansion and plant growth [5]. Moreover, water deficit also leads to stomatal closure [6], thereby affecting photoassimilate production and resulting in perturbations to source/sink relations, as well as reducing transpiration and the capacity

of the plant to dissipate excess energy. Drought may also influence the total duration of the crop cycle, shortening its vegetative and reproductive phases, thereby reducing the time available for photosynthate accumulation and transporting to the developing embryos and directly affecting productivity [7]. In cases where drought lasts for a long period, its effects may become irreversible, resulting in lower plant growth and decreased yield [8,9].

One alternative to minimize plant water stress is using superabsorbent polymers. Oladosu et al. [10], in a recent review, indicated that water stored by superabsorbent polymer slowly returns water to the soil close to roots. Moreover, polymers increased water content in dry areas, increasing water use efficiency and irrigation intervals, consequently reducing production costs. The incorporation of superabsorbent polymers into the soil represents a potential strategy for ameliorating drought stress, already being used in perennial crops to facilitate their proper establishment [11,12], and there is also potential for their use with annual crops. However, perennial crops usually have lower relative growth rates than annual crops, which must necessarily complete their cycle in a period of months [12,13]. Thus, annual crops may demand more water over a shorter period, requiring greater efficiency to absorb water, and in this sense, water-retaining polymers could potentially avoid losses and supply water at times of greatest demand, especially under water stress. Therefore, to evaluate the potential of such polymers for soybean, an investigation of the impacts of their addition to the soil on photosynthesis in field conditions under different degrees of drought stress and over several years is required.

The objective of this study was to evaluate the effect of a polymer incorporated into the soil on gas exchange and yield under different water regimes in three soybean cultivars. It was hypothesized that the addition of polymer would permit the maintenance of photosynthesis and growth under reduced water availability and result in improved yield.

2. Materials and Methods

2.1. Experimental Design and Water Levels

The experiments were conducted under field conditions at the Brazilian Agricultural Research Corporation (Embrapa Cerrados), in Planaltina (15°35'30" S, 47°42'30" W), Brazil, between July and November 2016 and 2017, a period of extremely low precipitation, ideal for the evaluation of experiments under water deficit. The region has an average annual rainfall of 1500 mm $\pm$ 500 mm and the climate of the region is Aw, according to the Köppen classification [14]. Temperature and precipitation in the experimental area during the years 2016 and 2017 are presented in Figure 1. The soil is classified as typical Oxisol [15]. Before the installation of the experiment, soil analysis were performed at depths of 0–20 cm and 20–40 cm (Table 1), because the toxicity of aluminium is normally corrected by liming, where roots are expected to be found due to aluminum correction which were similar in both depths.

The experimental design in 2016 and 2017 was in randomized blocks with three replications in a split-plot scheme. The plots were composed of soybean cultivars (BRS 5980IPRO, NA 5909RG and BRS 7280RR), and the subplots were four different water regimes (WR). The line spacing was 50 cm and the planting density was 25 plants per linear meter. Each plot consisted of two central lines, eliminating 1.5 m of edge.

Table 1. Soil analysis in the experimental area at depths of 0–20 cm and 20–40 cm.

Depth (cm)	pH (H ₂ O)	Ca cmolc dm ^{−3}	Mg cmolc dm ^{−3}	P mg L ^{−1}	K cmolc dm ^{−3}	Al cmolc dm ^{−3}	OM (%)
0–20	5.74	2.317	1.146	9.868	118	0.069	2.246
20–40	5.62	1.51	0.931	2.725	53	0.089	1.823

OM—organic matter.

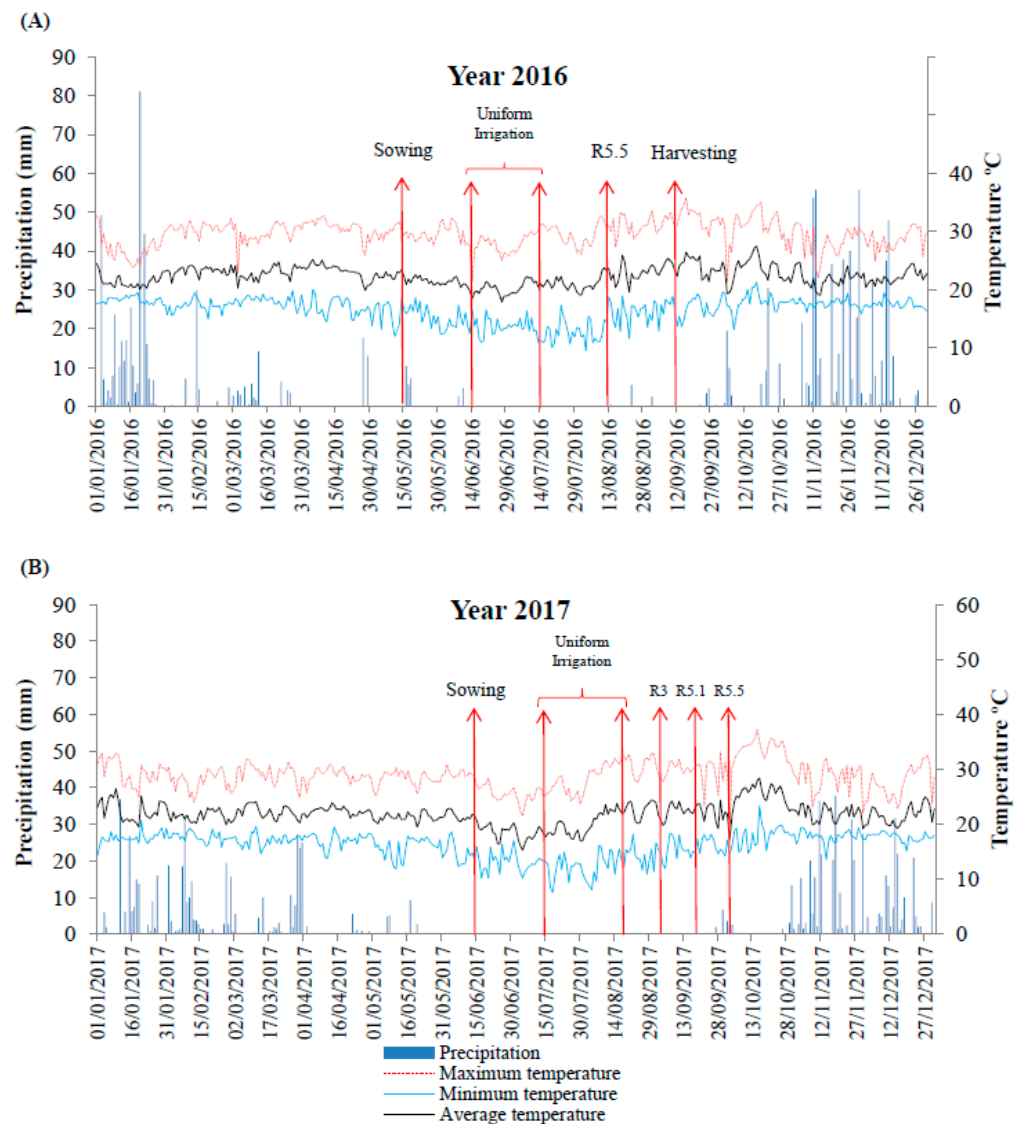


Figure 1. (A) Precipitation, maximum, minimum and average temperatures, rainfall and the sowing, evaluation and harvesting dates of soybean plants in 2016 and (B) 2017.

Irrigation of all cultivars was uniform for about 35 days after emergence (DAE) in 2016 and 2017. During the uniform irrigation phase (14 June to 14 July 2016 and 27 July to 27 August 2017) approximately 134 mm of water was supplied. After this period, the line source methodology was adopted [16], with the modification by using a 20 m wide irrigation bar (IrrigaBrasil model 36/42), connected to a self-propelled TurboMaq 75/GB, with adjustable speed according to the water level to be applied. This irrigation system uses overlapping sprinklers with different flows to create decreasing water levels from the central area to the end of the bar (Figure 2).

Along the bar, four levels of irrigation were delimited, representing the different WRs. The accumulated level of water applied over the 110 days of the crop cycle in 2016 from the outermost to the innermost sprinklers was 157 mm (16 m), 263 mm (13 m), 432 mm (10 m) and 630 mm (4 m), referred to as WR1, WR2, WR3 and WR4, respectively, and over the 118 days of the crop cycle in 2017 was 167 mm (16 m), 237 mm (13 m), 341 mm (10 m) and 534 mm (4 m), also referred to as WR1, WR2, WR3 and WR4, respectively (Figure 2). The numbers in brackets indicate the distance of the plots in relation to the center of the irrigation bar. Irrigation was performed on average every five days, depending on evapotranspiration and climate data, and the highest water level was applied according to the monitoring program for the replacement of evapotranspiration of the crop [17]. The

effects of the application of a superabsorbent polymer were tested in 2016 and 2017. This polymer is an artificial compound similar to anionic particles of acrylamide and potassium acrylates, an inert compound that can store water in its matrix, becoming a gel. It is able to store up to four hundred times its weight in deionized water in its matrix and 150 times its weight in water when it is in the soil. It has an apparent density of 0.85, specific weight of 1.10 g cm^{-3} and pH of 8.1. When dry, it has the appearance of a white powder and when hydrated it is a gel. It has a cation exchange capacity (CEC) of 4.6 meq. g^{-1} , lifetime after application of 5 years and has no toxicity under normal conditions of use. It can be applied to the soil by placing close to the seeds during planting fertilization.

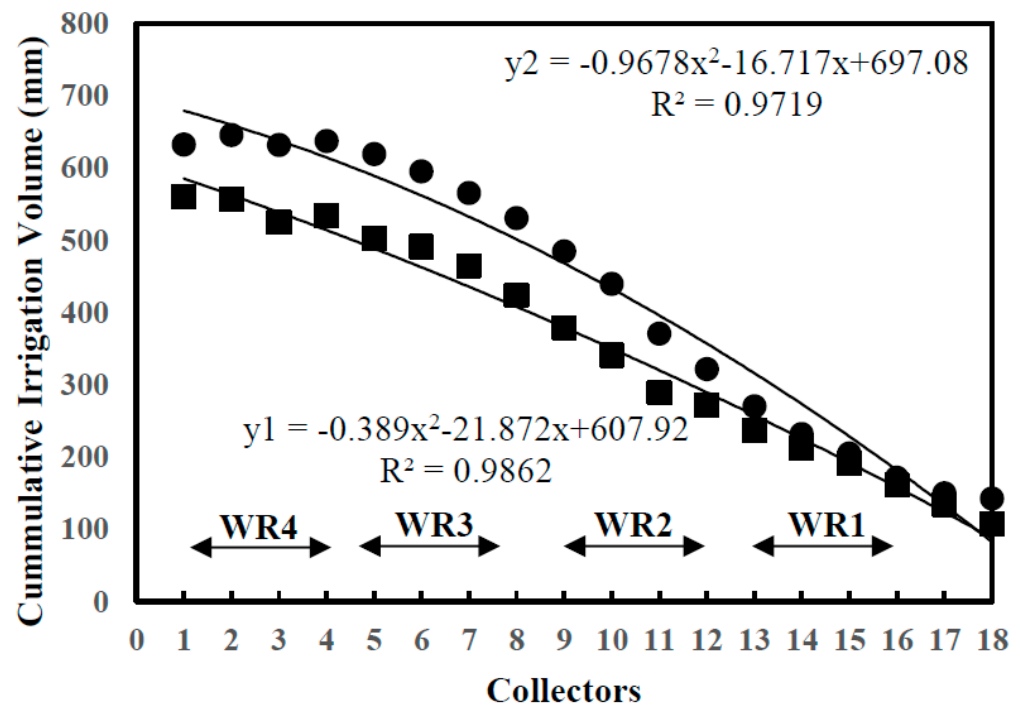


Figure 2. Cumulative irrigation volume applied in soybean crop during the dry season in 2016 (y1) and 2017 (y2).

Planting fertilization was 300 kg ha^{-1} of formulated 02-20-15 applied as maintenance fertilization. In the plots with polymer addition, 30 kg ha^{-1} was applied in both 2016 and 2017 in the sowing furrow and the same plots were used for polymer treatment in both years. Soybean seeds were inoculated with *Bradyrhizobium japonicum* ($1 \times 10^9 \text{ CFU g}^{-1}$ inoculant) at 200 g per 50 kg seeds in both years.

2.2. Plant Analysis

In 2016, the evaluations were performed at 75 DAE, when the crop approached phase R5.5 (Figure 1). In 2017, evaluations were carried out at 52, 62 and 76 DAE, when the crop was in the R3, R5.1 and R5.5 phases, respectively. The gas exchange variables: photosynthesis (A), stomatal conductance (g_s) and transpiration rate (E) were estimated in soybean cultivars from 9:00 to 13:00 h (solar time), under $1200 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ irradiance and an external CO_2 (C_a) concentration of $400 \mu\text{mol mol}^{-1}$ air. The Effective Quantum Efficiency of Photosystem II (ΦPSII) [18,19] was calculated using the formula: $\Phi \text{PSII} = (F_m' - F_s')/F_m'$ and Electron Transport Ratio (ETR) [19] was calculated using the formula: $\text{ETR} = \Phi \text{PSII} \times \text{DFF} \times (0.84) \times (0.5)$, where DFF is the Photon Flow Density, or the amount of light absorbed ($\mu\text{m photons m}^{-2} \text{ s}^{-1}$) [20]. All measurements in both years were made using a portable open flow gas exchange system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). Yield was evaluated by harvesting four linear meters of the planted area in 2016 and 2017, disregarding 1.5 m from the ends of each harvested plot. The

harvested grains were weighed and, after their humidity was corrected to 13%, the yield was measured.

To evaluate the influence of the polymer, the analysis of groups of experiments was performed, and thus, for each group (Control and Polymer), the response variables were evaluated. Each experimental year (2016 and 2017) was analyzed separately. In addition, the influence of WR on the Control and Polymer treatments and the response of each cultivar were tested separately.

2.3. Statistical Analysis

The data obtained were submitted to the Shapiro–Wilk normality test. Furthermore, Hartley's F test ($QMres1/QMres \leq 7$) was performed, to verify that all variables of the two groups could be compared. Data were subjected to analysis of variance (ANOVA), and means were compared using the Tukey test at 5% probability. The sources of variation were cultivars (plots), WR (subplots) and phenological phases within each group of experiments with and without polymer.

3. Results

3.1. Effect of Water Regime and Polymer Supply on Photosynthetic Parameters (Year 1—2016)

Decreased supply of water, from optimal irrigation (WR4) to stress (WR3–WR1), led to reductions in photosynthesis (A), stomatal conductance (g_s) and transpiration (E) both in the presence and absence of polymer (Table 2). In WR4, photosynthesis (A) decreased by 11–18% in control plants and 39–52% in polymer-treated plants compared to WR3. Under WR1 and WR2, decreases in A were even greater, reaching up to 88%. Stomatal conductance was also affected with reductions of 26 to 41% in control and 48 to 64% in polymer-treated plants at WR3. In WR1 decreases of photosynthesis reached 82% in control and 85% in polymer-treated plants. Decreases in E were similar to those in g_s , at 16 to 29% in control and 39 to 55% in polymer-treated plants under WR3 and 72% in control and 77% in polymer-treated plants under the lowest water regimes. In absolute values, the F_v/F_m also decreased from 0.79–0.82 under WR4 to between 0.71 and 0.76 under WR1.

In 2016, the addition of polymer generally had a negative effect on gas exchange, the extent of which depended on the water regime (Table 2). Under the greatest level of irrigation (WR4), polymer treatment led to a reduction in A in one of the cultivars (BRS 7280RR, 15.5% reduction); however, under WR3, reductions were 40, 52 and 37% for BRS 5980IPRO, NA 5909RG and BRS 7280RR, respectively (Table 2). For WR2, polymer treatment reduced A by 31% in BRS 7280RR, while under WR1, no significant differences were detected (Table 2). The impacts of polymer on g_s and E were even clearer, with reductions for all cultivars under all water regimes (Figure 3). Impacts on fluorescence parameters were modest, with a small increase in F_v/F_m in the presence of polymer for BRS 5980IPRO in WR1 and WR2. F_v'/F_m' was also slightly greater with polymer for all soybean cultivars at WR3 (Table 2).

Table 2. Photosynthesis (A), stomatal conductance (g_s), transpiration (E), maximum quantum yield of photosystem II (F_v/F_m) and effective quantum yield of photosystem II (F_v'/F_m') of three cultivars of soybean, with and without added polymer to the soil in 2016.

V	S	BRS 5980IPRO				NA 5909RG				BRS 7280RR			
		WR1	WR2	WR3	WR4	WR1	WR2	WR3	WR4	WR1	WR2	WR3	WR4
A	C	2.09	4.99	15.10	18.52	2.89	6.90	16.83	19.10	3.57	6.68 Ac	17.87	21.26
		Ad	Ac	Ab	Aa	Ac	Ac	Ab	Aa	Ac		Ab	Aa
	P	3.2	5.64	9.09	15.65	3.87	4.52	10.65	17.52	3.05		8.58	17.96
		Ad	Ac	Bb	Aa	Ac	Ac	Bb	Aa	Ac	4.53 Bc	Bb	Ba

Table 2. Cont.

V	S	BRS 5980IPRO				NA 5909RG				BRS 7280RR			
		WR1	WR2	WR3	WR4	WR1	WR2	WR3	WR4	WR1	WR2	WR3	WR4
g_s	C	0.114 Ab	0.119 Ab	0.243 Ab	0.366 Aa	0.059 Ac	0.119 Ac	0.251 Ab	0.340 Aa	0.069 Ad	0.085 Ac	0.201 Ab	0.341 Aa
	P	0.043 Bb	0.069 Bb	0.082 Bb	0.196 Ba	0.037 Bc	0.052 Bc	0.123 Bb	0.239 Ba	0.050 Bd	0.054 Bc	0.085 Bb	0.238 Ba
E	C	2.72 Ac	2.84 Abc	5.16 Ab	6.82 Aa	1.82 Ac	2.93 Ac	5.54 Ab	6.61 Aa	2.04 Ac	2.4 Ac	4.86 Ab	6.86 Aa
	P	1.23 Bc	1.88 Bbc	2.13 Bb	4.21 Ba	1.16 Bc	1.58 Bc	3.07 Bb	5.05 Ba	1.55 Bc	1.65 Bc	2.34 Bb	5.24 Ba
F_v/F_m	C	0.71 Bb	0.73 Bab	0.81 Aa	0.79 Aa	0.73 Ab	0.77 Aa	0.80 Aa	0.79 Aa	0.75 Ab	0.80 Aab	0.82 Aa	0.80 Aa
	P	0.76 Ab	0.80 Aab	0.81 Aa	0.79 Aa	0.74 Ab	0.80 Aa	0.81 Aa	0.81 Aa	0.76 Ab	0.79 Aab	0.82 Aa	0.82 Aa
F_v'/F_m'	C	0.44 Ab	0.45 Ab	0.53 Ba	0.52 Aa	0.42 Ab	0.45 Aab	0.44 Bab	0.54 Aa	0.29 Ab	0.45 Aab	0.47 Ba	0.52 Aa
	P	0.46 Ab	0.49 Ab	0.57 Aa	0.57 Aa	0.44 Ab	0.47 Aab	0.53 Aab	0.55 Ba	0.41 Ab	0.44 Aab	0.53 Aa	0.53 Aa

For each soybean cultivar, uppercase letters compare control (without polymer) and polymer treatment and lowercase letters compare between water regimes by Tukey's test ($p < 0.05$). WR1 157 mm, WR2 263 mm, WR3 432 mm, WR4 630 mm. V—variable analyzed; S—Polymer treatments. C (control—without polymer), P (with polymer).

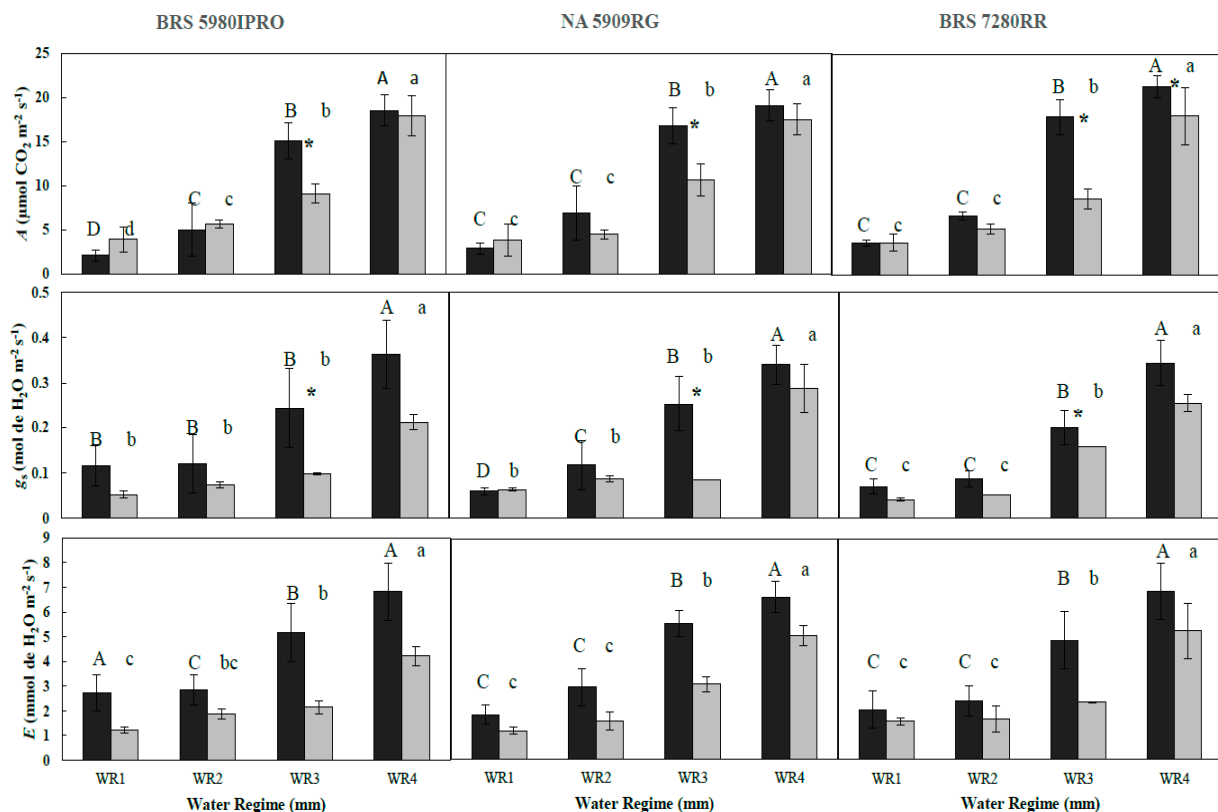


Figure 3. Photosynthesis (A), stomatal conductance (g_s) and transpiration (E) in three soybean cultivars submitted to four water regimes (WR) for three cultivars (BRS 5980IPRO, NA 5909RG and BRS 7280RR) with and without polymer supply (WR1 157 mm, WR2 263 mm, WR3 432 mm and WR4 630 mm) in 2016. Uppercase letters indicate differences between the WR for control (without polymer) and lowercase letters indicate differences between the WR for polymer treatment and “*” indicates significant differences between the treatments with and without polymer supply by Tukey's test ($p < 0.05$).

3.2. The Effects of Polymer Addition on Photosynthetic Parameters (Year 2—2017)

Given the negative effects of polymer on photosynthetic parameters in R5.5 in 2016, the experiment was repeated in 2017 on the same experimental site, and analyses were performed in R3, R5.1 and R5.5. In 2017, there were significant differences between control and polymer treatments for A , E and g_s in all three soybean cultivars that depended both on the WR and phenological phases (Table 3). Under WR4, the greatest level of irrigation, the presence of polymer in the soil increased A by 16, 31 and 23% for BRS 5980IPRO, NA 5909RG and BRS 7280RR cultivars, respectively, during the R5.5 phase (Table 3). For E , increments of 10, 26 and 14% occurred for the cultivars BRS 5980IPRO, NA 5909RG and BRS 7280RR, respectively, under polymer treatment, while no significant effects on g_s were detected (Table 3). While $\Phi PSII$ was unaffected for NA5909RG in all phenological phases, the addition of polymer increased this parameter for BRS 5980IPRO and BRS 7280RR cultivars (Table 4). The presence of polymer increased ETR at R3 and R5.5 for all cultivars and also at R5.1 for BRS7280RR (Table 4). A positive effect of polymer on $\Phi PSII$ also occurred at more than one stage for both BRS 5980IPRO and BRS7280.

Table 3. Photosynthesis (A), stomatal conductance (g_s) and transpiration (E) in three phenological stages of soybean cultivars for control and polymer treatments, in 2017.

Cultivar	Polymer	WR4								
		A			g_s			E		
		R3	R5.1	R5.5	R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	18.68 Aa	17.97 Aa	15.64 Bb	0.34 Aa	0.27 Aa	0.26 Ab	6.97 Ab	6.12 Ab	5.58 Ba
	Polymer	18.73 Aa	19.58 Aa	18.63 Aa	0.30 Aa	0.25 Aa	0.30 Aa	6.99 Ab	6.18 Ab	9.58 Aa
NA 5909RG	Control	20.71 Aa	18.56 Aa	12.65 Bb	0.30 Aa	0.28 Aa	0.19 Ab	6.89 Ab	6.77 Ab	7.46 Ba
	Polymer	16.88 Aa	18.69 Aa	18.50 Aa	0.23 Aa	0.23 Aa	0.29 Aa	5.91 Ab	6.09 Ab	10.13 Aa
BRS 7280RR	Control	18.80 Aa	18.18 Aa	13.84 Bb	0.28 Aa	0.27 Aa	0.22 Ab	6.65 Ab	6.69 Ab	8.44 Ba
	Polymer	18.20 Aa	20.70 Aa	17.99 Aa	0.27 Aa	0.24 Aa	0.27 Aa	6.88 Ab	6.46 Ab	9.85 Aa
		WR3								
		A			g_s			E		
		R3	R5.1	R5.5	R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	19.82 Aa	18.35 Aa	15.20 Bb	0.35 Aa	0.35 Aa	0.27 Ab	7.15 Aa	7.26 Aa	8.35 Ba
	Polymer	19.71 Aa	19.22 Aa	19.26 Aa	0.26 Ba	0.22 Ba	0.31 Aa	6.36 Ab	5.65 Ab	9.93 Aa
NA 5909RG	Control	19.92 Aa	17.20 Aa	11.30 Bb	0.29 Aa	0.25 Aa	0.18 Ab	6.63 Aa	6.34 Aa	7.21 Ba
	Polymer	18.73 Aa	18.26 Aa	16.35 Aa	0.25 Ba	0.22 Ba	0.23 Aa	6.46 Ab	6.10 Ab	8.94 Aa
BRS 7280RR	Control	20.64 Aa	18.84 Aa	14.19 Bb	0.31 Aa	0.27 Aa	0.20 Ab	7.37 Aa	6.78 Aa	8.04 Ba
	Polymer	18.36 Aa	18.75 Aa	17.02 Aa	0.20 Ba	0.19 Ba	0.25 Aa	5.55 Ab	5.72 Ab	9.44 Aa
		WR2								
		A			g_s			E		
		R3	R5.1	R5.5	R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	19.94 Aa	16.14 Ab	11.29 Ac	0.30 Aa	0.24 Ab	0.18 Ab	6.55 Ab	5.70 Ac	6.94 Aa
	Polymer	15.17 Ba	11.50 Bb	8.90 Bc	0.18 Ba	0.12 Bb	0.15 Bb	5.01 Bb	3.55 Bc	6.12 Ba
NA 5909RG	Control	15.64 Aa	12.39 Ab	8.62 Ac	0.20 Aa	0.16 Ab	0.16 Ab	5.17 Ab	4.76 Ac	6.80 Aa
	Polymer	15.36 Aa	8.82 Bc	9.93 Ab	0.17 Ba	0.08 Bb	0.14 Bb	5.05 Bb	2.89 Bc	5.93 Ba
BRS 7280RR	Control	18.07 Aa	14.32 Ab	14.53 Ac	0.22 Aa	0.17 Ab	0.22 Ab	5.87 Ab	4.83 Ac	8.70 Aa
	Polymer	16.19 Ba	13.94 Bb	6.85 Bc	0.18 Ba	0.14 Bb	0.10 Bb	5.10 Bb	4.55 Bc	4.36 Ba

Table 3. Cont.

		WR1								
		A			g _s			E		
		R3	R5.1	R5.5	R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	16.0 Aa	9.12 Ab	8.27 Ab	0.21 Aa	0.11 Ab	0.21 Aa	4.98 Ab	3.42 Ac	7.52 Aa
	Polymer	12.21 Aa	9.65 Ab	3.68 Bb	0.12 Aa	0.10 Aab	0.07 Bb	3.63 Aa	3.33 Aa	3.10 Ba
NA 5909RG	Control	13.18 Aa	6.70 Ab	6.78 Ab	0.14 Aa	0.08 Ab	0.15 Aa	3.99 Ab	2.62 Ac	6.15 Aa
	Polymer	11.81 Aa	9.09 Ab	4.44 Bb	0.12 Aa	0.08 Aab	0.07 Bb	3.84 Aa	2.68 Aa	3.39 Ba
BRS 7280RR	Control	12.98 Aa	6.90 Ab	10.37 Ab	0.14 Aa	0.08 Ab	0.16 Aa	4.14 Ab	2.75 Ac	6.90 Aa
	Polymer	12.28 Aa	12.02 Ab	3.63 Bb	0.13 Aa	0.11 Aab	0.07 Bb	3.82 Aa	4.08 Aa	3.26 Ba

Uppercase letters compare within each phenological stage and lowercase letters compare between phenological stage by Tukey's test ($p < 0.05$). (WR1 167 mm, WR2 237 mm, WR3 341 mm, WR4 534 mm).

Table 4. Photosystem II yield (Φ PSII) and electron transport rate (ETR) in three phenological stages of soybean cultivars and control and polymer treatments, in 2017.

Cultivar	Polymer	WR4					
		Φ PSII			ETR		
		R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	0.27 Ba	0.27 Ba	0.25 Aa	144.63 Ba	142.56 Aa	133.21 Ba
	Polymer	0.29 Aa	0.29 Aa	0.26 Aa	152.47 Aa	152.47 Aa	138.43 Aa
NA 5909RG	Control	0.29 Aa	0.29 Aa	0.25 Aa	146.82 Ba	146.82 Aa	134.32 Ba
	Polymer	0.29 Aa	0.29 Aa	0.30 Aa	154.25 Aa	154.25 Aa	157.72 Aa
BRS 7280RR	Control	0.27 Ba	0.27 Ba	0.25 Ba	139.50 Ba	139.50 Ba	129.38 Ba
	Polymer	0.31 Aa	0.31 Aa	0.30 Aa	165.35 Aa	165.35 Aa	159.65 Aa
		WR3					
		Φ PSII			ETR		
		R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	0.27 Aa	0.27 Aa	0.22 Ab	141.57 Aa	141.57 Aa	116.96 Aa
	Polymer	0.28 Aa	0.28 Aa	0.24 Ab	145.69 Aa	145.69 Aa	126.89 Aa
NA 5909RG	Control	0.28 Aa	0.28 Aa	0.26 Aa	148.07 Aa	148.07 Aa	133.82 Ab
	Polymer	0.30 Aa	0.30 Aa	0.26 Aa	155.25 Aa	155.25 Aa	137.21 Ab
BRS 7280RR	Control	0.28 Aa	0.28 Aa	0.31 Aa	148.86 Aa	148.86 Aa	160.48 Aa
	Polymer	0.30 Aa	0.30 Aa	0.26 Aa	156.70 Aa	156.70 Aa	137.66 Aa
		WR2					
		Φ PSII			ETR		
		R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	0.21 Aa	0.21 Aa	0.17 Aa	109.24 Aa	109.24 Aa	90.55 Aa
	Polymer	0.19 Aa	0.19 Aa	0.17 Aa	97.47 Ba	97.47 Ba	87.30 Aa
NA 5909RG	Control	0.22 Aa	0.22 Aa	0.23 Aa	117.79 Aa	117.79 Aa	118.61 Aa
	Polymer	0.20 Ba	0.20 Ba	0.18 Ba	106.26 Ba	106.26 Ba	92.80 Ba
BRS 7280RR	Control	0.25 Aa	0.25 Aa	0.26 Aa	129.75 Aa	132.58 Aa	139.12 Aa
	Polymer	0.24 Ba	0.24 Ba	0.16 Ba	126.58 Ba	126.58 Ba	85.62 Ba

Table 4. Cont.

		WR1					
		Φ PSII			ETR		
		R3	R5.1	R5.5	R3	R5.1	R5.5
BRS 5980IPRO	Control	0.12 Ba	0.12 Ba	0.12 Aa	62.18 Ba	62.18 Ba	61.63 Aa
	Polymer	0.16 Aa	0.16 Aa	0.10 Aa	86.11 Aa	86.11 Aa	51.74 Ba
NA 5909RG	Control	0.14 Ba	0.14 Ba	0.15 Aa	75.00 Ba	75.00 Ba	76.96 Aa
	Polymer	0.16 Aa	0.16 Aa	0.10 Aa	84.95 Aa	84.95 Aa	53.16 Ba
BRS 7280RR	Control	0.14 Ba	0.14 Ba	0.18 Aa	71.27 Ba	71.27 Ba	92.46 Aa
	Polymer	0.21 Aa	0.21 Aa	0.11 Aa	109.46 Aa	109.46 Aa	55.51 Ba

Uppercase letters compare within each phenological stage and lowercase letters compare between phenological stage by Tukey's test ($p < 0.05$). (WR 167 mm, WR 237 mm, WR 341 mm, WR 534 mm).

The same general trend occurred under WR3 with greater A and E for all cultivars in R5.5 in the presence of the polymer (Table 3). However, under this reduced level of irrigation, the polymer negatively affected g_s with reductions in phase R3 of 25, 14 and 35%, and in R5.1 of 37, 12 and 30% for cultivars BRS 5980IPRO, NA 5909RG and BRS 7280RR, respectively (Table 3). This negative effect was not detected in phase R5.5 (Table 3). Indeed, while g_s decreased from R3 to R5.5 for all cultivars in the absence of polymer, in the presence of polymer, this parameter remained constant (Table 3).

The level of water applied in WR1 and WR2 (167 and 237 mm, respectively) was insufficient to maintain photosynthetic and transpiration rates (Table 3) as large decreases were observed at later developmental stages (R5.1 and R5.5) both in relation to WR3 and WR4 and in relation to R3. Under these conditions, the addition of polymer generally had a negative effect on A , E and g_s (Table 3; Figures 4–6). For example, under WR2 in phases R3, R5.1 and R5.5, photosynthesis was higher in control plants compared to polymer-treated plants for cultivars BRS 5980IPRO and BRS 7280RR, while for NA5090RG in R3 and R5.5, the control was similar to the polymer treatment (Table 3). Similarly, for g_s and E , the control treatment presented statistically greater averages for all developmental phases, ranging from 13 to 54% and 13 to 50%, for g_s and E , respectively (Figures 4–6). Again, for the lowest level of water availability (WR1) A , E and g_s were all higher in control plants of all cultivars at R5.5 when compared to polymer treatment (Table 3; Figures 4–6).

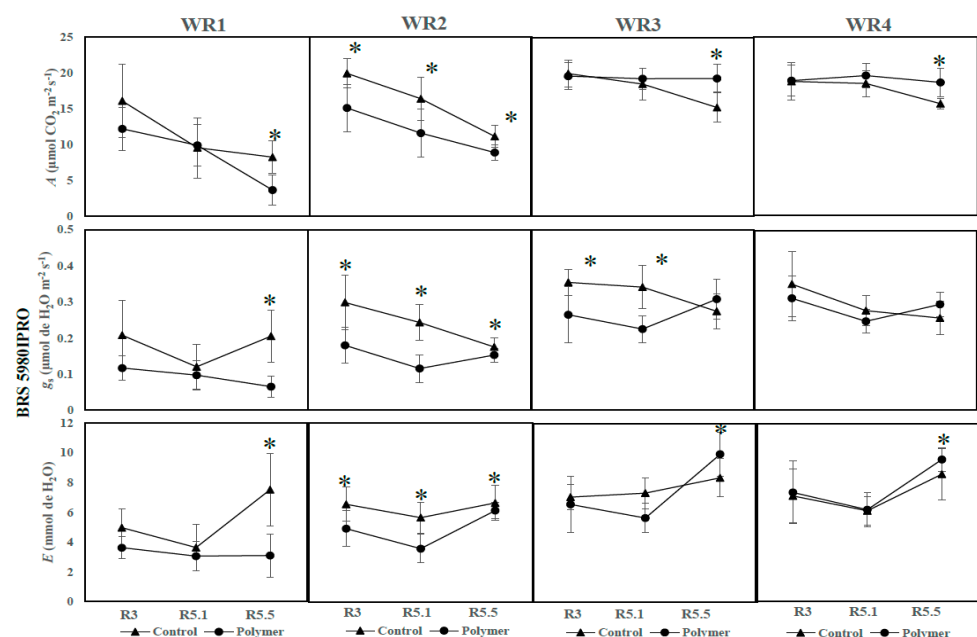


Figure 4. Photosynthesis, (A), stomatal conductance (g_s) and transpiration (E) under four water regimes (WR)

in three phenological stages of soybean with and without polymer supply (WR1 167 mm, WR2 237 mm, WR3 341 mm, WR4 534 mm) for the BRS 5980IPRO cultivar, in 2017. * indicates significant differences between the control and polymer treatments within each WR and phenological phase by Tukey's test ($p < 0.05$).

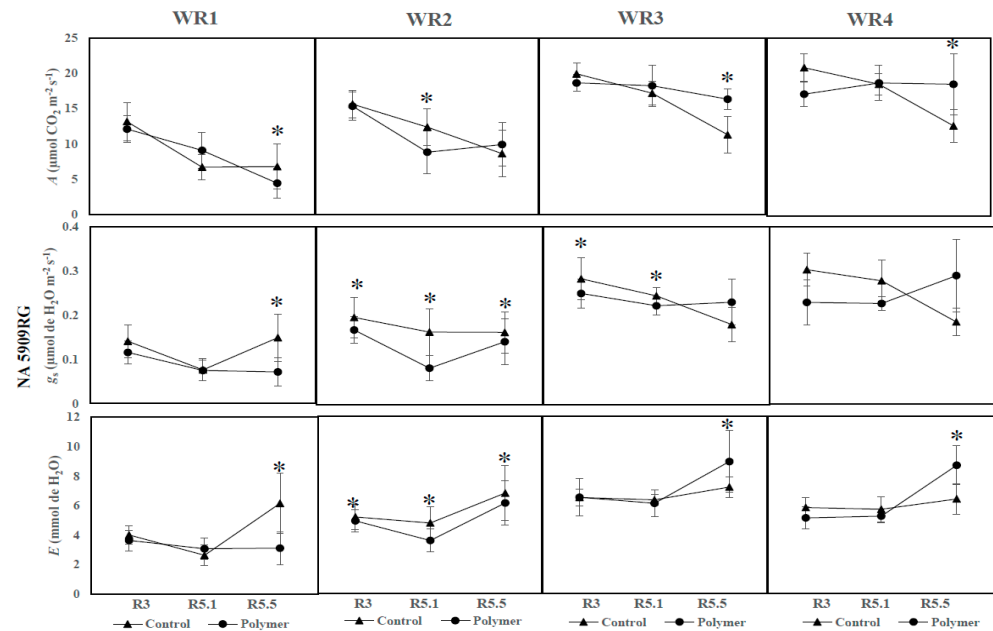


Figure 5. Photosynthesis, (A), stomatal conductance (g_s) and transpiration (E) under four water regimes (WR) in three phenological stages of soybean with and without polymer supply (WR1 167 mm, WR2 237 mm, WR3 341 mm, WR4 534 mm) for the NA 5909RG cultivar, in 2017. * indicates significant differences between the control and polymer treatments within each WR and phenological phase by Tukey's test ($p < 0.05$).

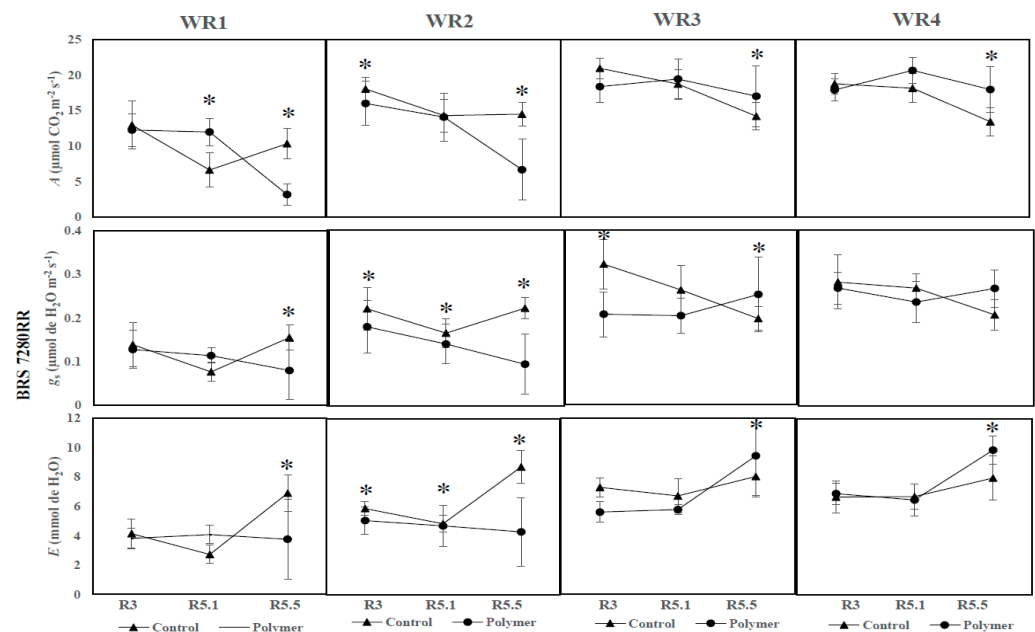


Figure 6. Photosynthesis, (A), stomatal conductance (g_s) and transpiration (E) under four water regimes (WR) in three phenological stages of soybean with and without polymer supply (WR1 167 mm, WR2 237 mm, WR3 341 mm, WR4 534 mm) for the BRS 7280RR cultivar, in 2017. * indicates significant differences between the control and polymer treatments within each WR and phenological phase by Tukey's test ($p < 0.05$).

The responses of Φ PSII and ETR generally mirrored those of gas exchange parameters. In WR4, both of these parameters increased in the presence of polymer; polymer resulted in increased ETR at developmental phases R3 and R5.5 for all cultivars and was also increased at R5.5 in BRS 7280RR. Small but significant increases in Φ PSII were detected in all phenological phases for BRS 7280RR and BRS 5980IPRO, but not in NA 5909RG. In WR2, the presence of polymer resulted in significant decreases in Φ PSII in cultivars NA 5909RG and BRS 7280RR in all phenological phases compared to control (Table 4), while ETR was lower in the polymer treatment in all soybean cultivars.

3.3. The Influence of Water Regime and Polymer on Physiological Parameters across Different Phenological Phases (Year 2—2017)

In 2017, the use of polymer not only impacted physiological parameters when comparing the same phenological phase but also affected how these parameters changed from R3 to R5.5 (Tables 3 and 4). Photosynthesis was affected by the different phenological phases in the WR4 treatment in control plants for all cultivars, with a decrease in R5.5 compared to the earlier phases (Table 3; Figure 7). Interestingly, no such decrease was observed in the polymer-treated plants. The same pattern of a lack of a decrease in R5.5 in the presence of the polymer was also observed for g_s (Table 3). Transpiration rate, on the other hand, responded differently, as it was greater in R5.5 compared to the other phenological phases for both the control and polymer treatments as well as being greater in the polymer treatment compared to the control during phase R5.5 (Table 3; Figure 7). It is worth noting that despite the alterations in gas exchange, alterations were not observed in fluorescence parameters between the phenological phases in WR4 for either control or polymer-treated plants.

Similar to WR4, in WR3, photosynthesis decreased in R5.5 compared to R3 and R5.1, while no decreases occurred for the polymer treatment, suggesting that the polymer also aids in maintaining photosynthesis during different developmental phases under conditions of moderate water stress (Figure 7). Polymer treatment led to similar g_s in all phenological phases and an increased transpiration in R5.5 compared to the other two phases, which was not observed in the controls (Table 3). Fluorescence parameters were little affected by phenological phases with only minor decreases in BRS 5980IPRO for WR3 in R5.5, and no effect of the polymer was observed.

Different from that observed for WR3 and WR4, in WR1 and WR2, the presence of the polymer did not reduce the impact of the phenological phase on the photosynthetic gas exchange parameters (Figure 7). Indeed, for all cultivars, A , g_s and E tended to decrease to a greater extent from R3 to R5.5 in polymer-treated plants compared to control. Despite these changes in gas exchange, no significant differences were detected in Φ PSII and ETR between phenological phases under WR2 or WR1 (Table 4).

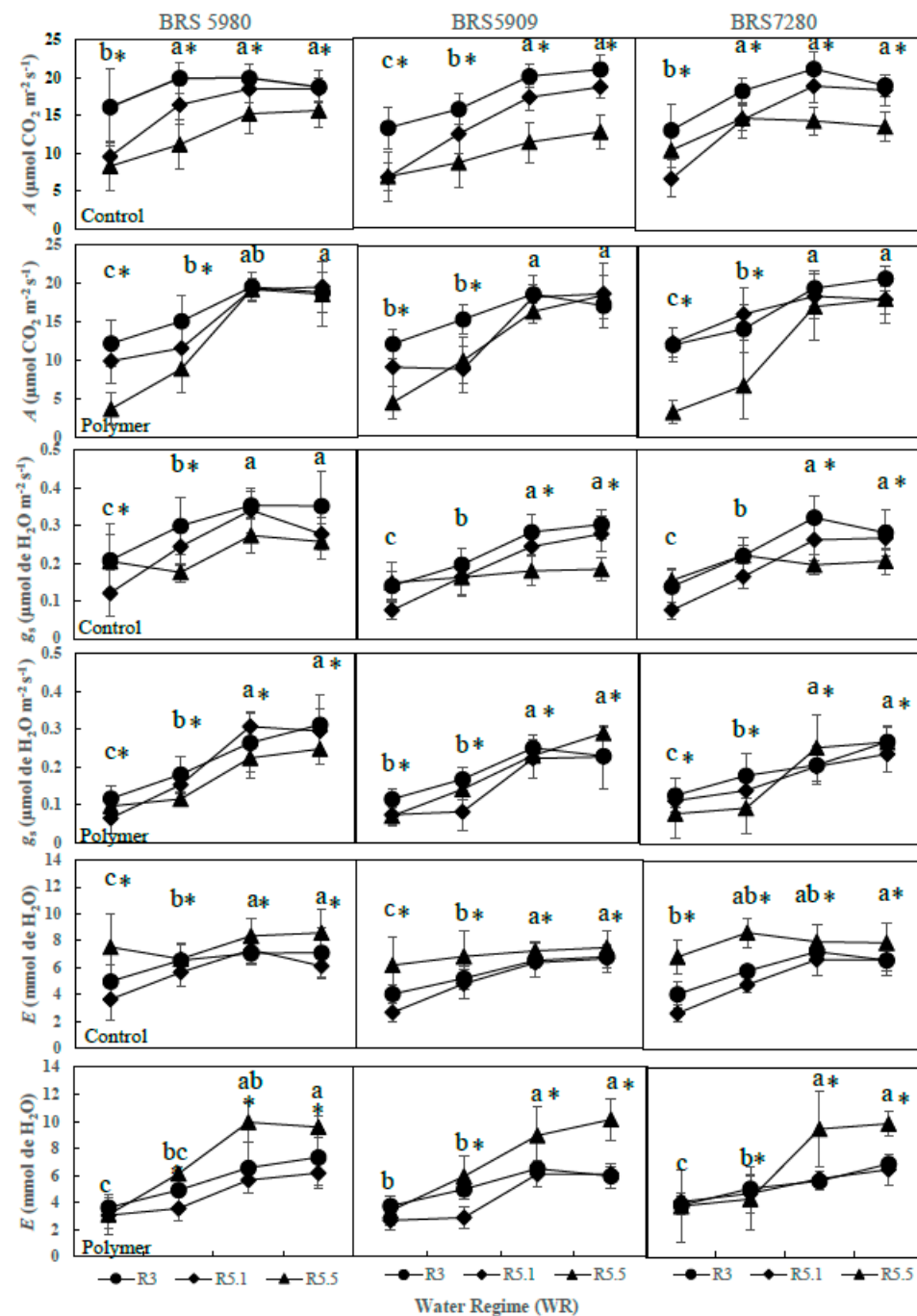


Figure 7. Photosynthesis, (A), stomatal conductance (g_s) and transpiration (E) in three soybean cultivars submitted to four water regimes (WR) in three phenological stages of soybean development with and without polymer supply (WR1 167 mm, WR2 237 mm, WR3 341 mm, WR4 534 mm), in 2017. Lowercase letters indicate differences between the WR for each cultivar and * indicates significant differences between the phenological phases within each WR by Tukey's test ($p < 0.05$).

3.4. The Impact of Polymer and Water Regime on Soybean Productivity (Years 1 and 2)

In 2016, lower water regime led to decreased productivity for all cultivars, with WR1 and WR2 being particularly affected compared to WR3 and WR4. All cultivars (BRS 5980IPRO, NA 5909RG and BRS 7280RR) had a productivity, under WR1 and WR2, of 558 and 1350 kg ha⁻¹, 522 and 1474 kg ha⁻¹ and 423 and 1425 kg ha⁻¹, respectively, in the control and 485 and 1279 kg ha⁻¹, 560 and 1524 kg ha⁻¹ and 602 and 1501 kg ha⁻¹ in the polymer treatment (Figure 8). In WR3 and WR4, the most irrigated levels, the productivity

for the such cultivars was 2452 and 2754 kg ha⁻¹, 2329 and 2974 kg ha⁻¹ and 2596 and 3454 kg ha⁻¹, respectively, for control and 2152 and 2782 kg ha⁻¹, 2449 and 2727 kg ha⁻¹ and 3077 and 3392 kg ha⁻¹, respectively, for polymer treatment (Figure 8). However, in 2016, addition of polymer had no effect on productivity under any of the water regimes.

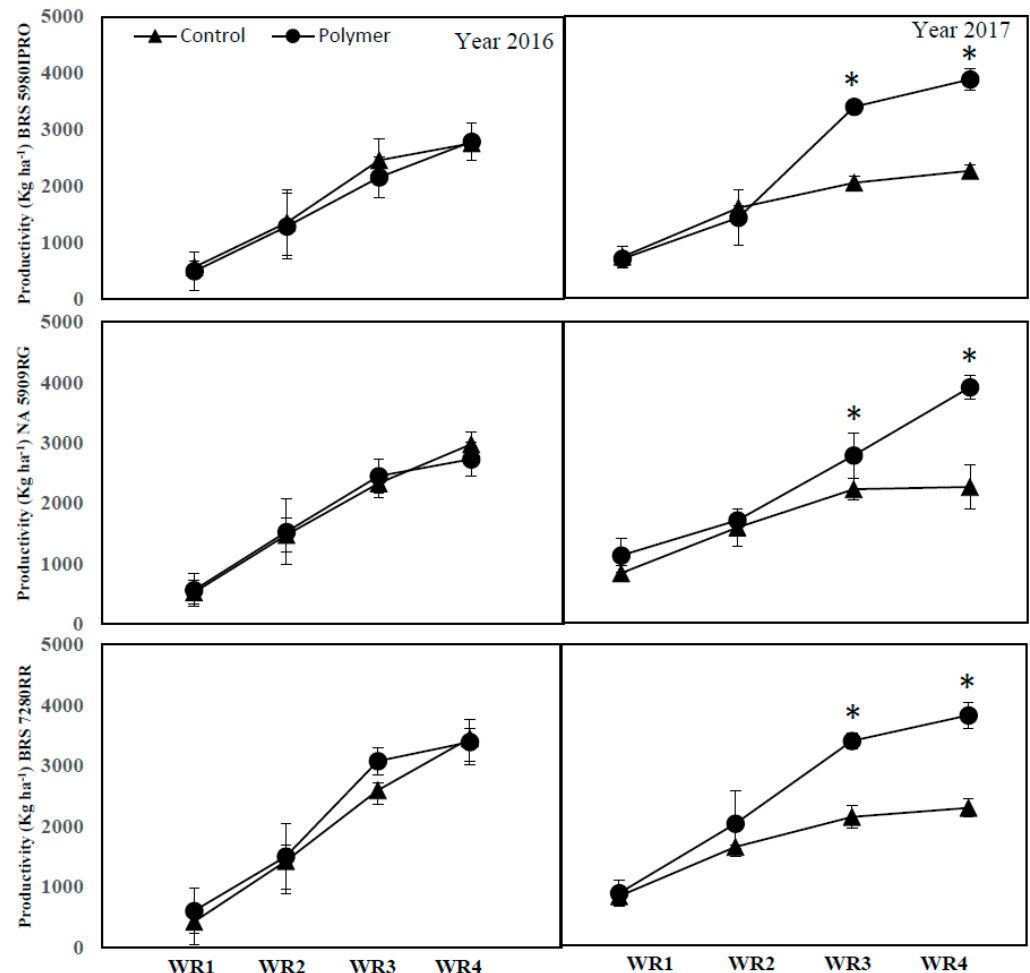


Figure 8. Productivity (Kg ha⁻¹) in three soybean cultivars submitted to four water regimes WRs. control (triangles) and polymer-treated (circles) in 2016 and 2017. * indicates significant differences between control and polymer-treated plants by Tukey's test ($p < 0.05$) (WR1 157 mm, WR2 263 mm, WR3 432 mm and WR4 630 mm in 2016; WR1 167 mm, WR2 237 mm, WR3 341 mm and WR4 534 mm in 2017).

In 2017, as in 2016, decreased water regime led to reduced productivity; all three cultivars (BRS 5980IPRO, NA 5909RG and BRS 7280RR) had a productivity, respectively, of WR1 and WR2, 725 and 1598 kg ha⁻¹, 816 and 1574 kg ha⁻¹ and 809 and 1630 kg ha⁻¹ for control and 690 and 1421 kg ha⁻¹, 1111 and 1695 kg ha⁻¹ and 859 and 2019 kg ha⁻¹ (Figure 8). In WR3 and WR4, the most irrigated levels, the productivity for the such cultivars was 2044 and 2255 kg ha⁻¹, 2214 and 2252 kg ha⁻¹ and 2130 and 2281 kg ha⁻¹, respectively, for control and 3393 and 3882 kg ha⁻¹, 2777 and 3911 kg ha⁻¹ and 3394 and 3822 kg ha⁻¹, respectively, for polymer treatment (Figure 8).

The presence of polymer resulted in increased productivity under WR3 and WR4 only in the 2017 (Figure 8). Specifically, the addition of polymer led to increases of 42, 42 and 40% in WR4 and 39, 20 and 37% in WR3 for the BRS 5980IPRO, NA 5909RG and BRS 7280RR, respectively. However, under WR2 and WR1, there was no effect of polymer on productivity. Comparing the two years, productivity under WR1 and WR2 was similar; however, productivity under WR3 and WR4 was generally greater in 2016 when comparing the control treatments. This was particularly clear for BRS 7280RR, where productivity under control conditions in 2016, WR4 was close to that under polymer treatment in 2017.

4. Discussion

Addition of absorbent polymers to the soil can increase soil field capacity [21,22]. Following treatment with polymer irrigation may, therefore, increase soil water availability and retention, which in turn will favor maintenance of transpiration, operation of the photosynthetic electron transport chain and carbon fixation via photosynthesis, ultimately permitting increased productivity. However, experiments involving polymers in crops of agronomic interest are typically carried out in a greenhouse, with tight control of environmental factors [21]. Field studies are, therefore, of great value when assessing new technologies that can impact plant physiology as variables, such as soil type, crop management and climatic conditions, which may vary between years, can alter their effectiveness [23]. This is particularly the case when the technology is currently little used, as is the case for polymers in annual crops such as soybean. Here, we aimed to determine the effects of water retaining polymer on physiological parameters and productivity of three soybean cultivars during two growing seasons [24]. As anticipated, decreased water availability led to reductions in photosynthetic gas exchange (A , g_s and E) for all cultivars during 2016. However, in 2016, application of water absorbent polymer also led to reduced photosynthetic gas exchange; this effect was mainly limited to reductions in E and g_s under WR4, WR2 and WR1, but under WR3 polymer treatment, also resulted in large decreases in A (37–52%).

The reductions in net photosynthesis in the presence of polymer, appear to result from stomatal closure, as shown by reductions in g_s , rather than damage to the photosynthetic apparatus as the effects of polymer on F_v/F_m and F_v'/F_m' were limited [25]. Stomatal closure caused by polymer can potentially be explained by the water absorbed by it being sequestered, and hence, not effectively available for uptake by the plants.

Despite causing stomatal closure, application of polymer in 2016 did have a positive effect on F_v/F_m and F_v'/F_m' . Under WR1 and WR2, F_v/F_m was slightly greater for BRS 5980IPRO in the presence of polymer, suggesting some capacity to alleviate photoinhibition under conditions of water deficit [26], though in a manner that depends on the cultivar.

The WR3 that has intermediate stress revealed the minimum level of water reduction at which the polymer was able to attenuate this negative effect, since all cultivars showed a reduction in F_v'/F_m' in the control from this stress level (Table 2). The most drastic levels could not be mitigated. The most intriguing issue is that photosynthetic rates were drastically reduced with polymer treatment, and according to Rehman et al. [27], such reductions in photosynthetic rates may not be due to damage to photosynthetic machinery but rather to stomatal closure.

Given the generally negative effects of polymer on gas exchange in R5.5 during 2016, and the fact that impacts of polymer may alter over time a second experiment spanning additional phenological phases was carried out in 2017 [28]. While decreasing water regime also led to reductions in gas exchange the effects of polymer in 2017 were different to those observed in 2016. Under WR4 and WR3, polymer generally increased A and E during R5.5. Stomatal conductance was unaffected by polymer in WR4, but was decreased during R3 and R5.1, though not during R5.5. In soybean, grain filling occurs during the R5 phase, during which period source–sink relationships can have a significant impact on productivity [29,30]. Specifically, productivity is likely to be maximized when the source is able to meet the demand of drain organs for photoassimilates, and stress factors such as drought that interfere with this relationship are likely to reduce productivity [29]. On the other hand, regulating water loss through stomatal closure can help to maintain productivity when the crop is under water stress conditions [31]. Soybean can begin production of abscisic acid following 7 days of moderate water stress [32], and when transported to the shoot, this hormone induces responses that include stomatal closure and consequent reduced gas exchange, reallocation of reserves, alterations in plant architecture and accumulation of compatible solutes [33,34]. Hence, in 2017 although 35% less water was applied in WR3 compared to WR4 the addition of the polymer to the soil appears to have led to partial stomatal closure in the plants from the polymer-treated plots during R3 and R5 (Table 3)

and allowed maintenance of plant hydration during peak grain filling, thereby leading to increased photosynthesis at R5.5 (Figure 7). In WR4, though stomatal closure was not apparent during the earlier reproductive phases, the presence of polymer again appears to have permitted greater photosynthesis during R5.5. As discussed below, these increases in photosynthesis in the presence of polymer were associated with increased yield.

While polymer led to increased photosynthesis and transpiration at R5.5 in WR3 and WR4, this was not the case during WR2 and WR1, where it led to reduced A , g_s and E . The reductions in A , g_s and E detected in plants treated with polymer under WR2 agree with the decreases we detected in Φ PSII and ETR in this WR, suggesting potential damage to the photosynthetic machinery (Tables 3 and 4). On the other hand, in WR1, the opposite pattern was observed with polymer-treated plants, showing higher values for Φ PSII and ETR; thus, the reductions in A under very low irrigation can be attributed to the expressive reduction in g_s in R5.5 and stomatal closure (Tables 3 and 4).

Measurement of physiological parameters over three phenological phases allowed us to observe the effect of polymer on these parameters over time. For both WR4 and WR3, in control plants, A and g_s were reduced in R5.5 relative to the other phenological phases. Interestingly, no such decrease was observed in the polymer-treated plants, suggesting that its presence helps to maintain higher photosynthetic rates and perhaps postpone senescence that may occur due to water deficit [35,36]. The water that was retained by the polymer in WR4 and WR3 may permit plants to maintain their stomata open during the later stages of the growth cycle, and hence, maintain higher rates of transpiration and photosynthesis [21,37,38]. However, it is possible that water absorbed by the polymer will not necessarily always be made available to the roots of soybean plants. This appears to have occurred under WR1 and WR2 as the presence of the polymer accentuated the reduction of g_s in two of the cultivars (BRS 5980IPRO and NA 5909RG), while BRS 7280RR was not affected (Table 3) and such reductions were at least partly responsible for the negative impacts on photosynthetic metabolism.

Differences between species and genotypes in the decrease in productivity due to water stress created by water gradients, with or without polymer-treated plants that mitigate the water deficit in the Cerrado of Central Brazil have been documented [39–41]. Overall, to reduce the stress, the presence of polymer in the soil impacted physiological parameters in different ways and to different extents, depending on the WR and the year of planting.

In 2016, despite the reductions in gas exchange parameters in the presence of polymer, there were no differences in productivity between these treatments under any of the water regimes (Figure 8). This is somewhat unanticipated, as the R5.5 developmental stage represents the peak of grain filling [42], where maximum demand for photoassimilates occurs, and hence, reductions in photosynthesis during this phase would be expected to impact productivity. However, it is worth noting that net photosynthesis was actually less affected by polymer than either g_s or E in 2016, perhaps explaining why productivity was not affected.

On the other hand, in 2017, the increases in photosynthetic parameters in the presence of the polymer under WR4 and WR3, and the capacity of the polymer to maintain physiological parameters throughout plant development were also associated with increased productivity with increases of up to 40% that may result from the greater presence of water in the soil available for absorption (Figure 8). Specifically, the addition of polymer led to increases of 42, 42 and 40% in WR4 and 39, 20 and 37% in WR3 for the BRS 5980IPRO, NA 5909RG and BRS 7280RR cultivars, respectively, suggesting that maintenance of photosynthesis throughout the reproductive cycle can increase productivity under field conditions. Importantly, while under WR2 and WR1 polymer treatment in 2017 reduced physiological parameters, productivity, though lower than under WR3 and WR4, was not affected by the presence of polymer (Figure 8). The number of pods, grain size and grain weight are all harmed by periods of drought [43–45]. Restrictions in the water supply that occur during the reproductive period of the crop may slow ovary expansion and, eventually, productivity due to reduced photoassimilate supply [46]. Reductions in CO_2 assimilation

that occur when the crop is under severe water deficit may also be a result of the cumulative effect of water deficit, resulting in photooxidative damage to photosynthesis-related proteins [47,48]. While a number of studies have reported a positive impact of water-retaining polymers [20], very few have demonstrated increased yield under field conditions. The positive and negative effect of the addition of water-retaining polymers depends on the physicochemical properties of the soil and the severity of the water deficit [49]. Nonetheless, polymer fertilization has been suggested as an important practice for the maintenance of appropriate soil water levels such that under situations of short and moderately intense drought, physiological processes remain functional [49]. This assessment broadly agrees with our results from 2017; polymer addition was beneficial under WR3 both in terms of physiological parameters and yield, while under 55% (WR2) and 70% (WR1) reductions in applied water, yield was unaffected and physiological parameters reduced in the presence of the polymer.

Overall, these results suggest that polymer could be used to maximize the positive impacts of irrigation, but that application over multiple years may be required to see benefits in annual crops. Though irrigation of soybean is not common in Brazil, this crop may be subjected to periods of drought during its growth cycle [50,51], and under a situation of moderate water deficit similar to WR3 polymer, the application may be advantageous. Since under severe drought conditions, the use of polymer did not lead to increases in photosynthetic parameters nor in yield, its use is likely to be more beneficial under moderate stress conditions only.

5. Conclusions

In general, water stress affected the physiological parameters and productivity, and therefore, there were consistent responses to irrigation. In the first year of application, under severe stress, the polymer negatively affected photosynthesis; however, productivity was not affected. In the second year of polymer application, positive effects on gas exchange and mainly on productivity occurred at intermediate and high water levels in all soybean cultivars and phenological phases. Therefore, polymer is not recommended for severe water stress. Due to differences obtained in the two years of evaluation, more years of research will be necessary to obtain a conclusive result about the addition of polymer in soils under water stress.

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