

Soybean: Genetic Gain \times Fertilizer Nitrogen Interaction

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Summary

The United States (US) and Argentina (ARG) account for more than 50% of the global soybean production. Soybean yields are determined by the genotype, environment and management practices ($G \times E \times M$) interaction. On overall, 50-60% of soybean nitrogen (N) demand is usually met by the Biological Nitrogen Fixation (BNF) process. An unanswered scientific knowledge gap is still related to the ability of BNF process to satisfy soybean N demand at varying yield levels. The overall objective of this project was to study the contribution of N via utilization of different N strategies evaluating soybean genotypes released in different eras. Four field experiments were conducted during the 2016 season: Ottawa (East Central KS, US), Ashland Bottoms (Central KS, US), Rossville (Central KS, US), and Oliveros (Santa Fe province, Argentina). A wide-variety of historical and modern soybean genotypes were utilized (from the 1980's, 1990's, 2000's and 2010's release decades) in US and ARG, all tested under three N management strategies (S1: non-N applied but inoculated, S2: all N provided by fertilizer, and S3: Late-N applied) and all seeds inoculated. At Ottawa, the study was planted in an area without previous soybean history with yields ranging from 21 to 30 bu ac⁻¹. Modern genotype (2010) increased 15% yields relative to the other varieties. As related to the N management approach, higher yields occurred when the N nutrition was based on S2 (overall 10% increase). At Ashland Bottoms, yields ranged from 47 to 65 bu ac⁻¹, the 1990's variety out yielded by 13% the rest of materials. There was not statistical significance for N management at this location. At Rossville, yields ranged from 37 to 85 bu ac⁻¹, with higher yields observed for the modern genotype (released after 2010). Regarding N strategies, S2 increased yields by 18% compared to S1. At ARG, yield ranged from 40 to 74 bu ac⁻¹, with modern soybean varieties (released after 2010) yielding 34% over the rest of materials. Nitrogen application S2 increased 5% yields when compared to the S1 strategy. Relative to yield potential, in Argentina yield levels were similar to that in Central KS (Ashland and Rossville).

Keywords: soybean, nitrogen, inoculation, fertilization, genotypes.

Introduction

The United States (US) and Argentina (ARG) account for more than 50% of the global soybean production (USDA, 2016). At US, more than 85% of the soybean land area is located in the “Corn-Belt” region, where two-year corn-soybean rotation (>60%) is the main system. In Argentina, soybeans are planted in the Pampas and Chaco regions, under rainfed conditions, as monoculture or in rotations with maize and wheat.

Soybean yield potential is genetically determined. Yield potential (Y_P) can be attained under “ideal” conditions (genotype \times environment \times management practices, $G \times E \times M$), assuming no limitations of water and nutrient supply and absence of biotic and abiotic yield limiting factors (e.g., insects, diseases, etc.). Yield gaps between Y_P and actual on-farm yield (Y_A) is primarily defined by crop management practices (e.g., row spacing, planting date, fungicide and nutrient application, among others) and the interactions of those with the E

(weather factor). Maximum soybean yields are dependent on a balanced nutrition, with N nutrition as the main nutrient limiting soybean yields and seed quality (Ciampitti et al., 2016).

Interaction between soybean genotypes and fertilizer N response is not yet well understood. Rountree et al. (2013) documented an annual genetic U.S. soybean yield gain of approximately 0.37 bu ac⁻¹ for maturity group (MG) III released from 1920's to 2000's when planted around May. Yield gain for high yielding soybean was achieved in detriment of the protein concentration (Rountree et al., 2013). Thus, it is valid to hypothesize that high-yielding soybean will need higher nutrient demand to sustain protein levels – which represents the bio fortification issue.

Soybean plants have the capacity of fixing nitrogen (N) from the atmosphere through the symbiosis process of the plant with the bacteria *Bradyrhizobium* that needs to be present in the soil or added as inoculant. Nitrogen fixation is the result of the conversion of atmospheric N₂ into ammonia (NH₃), and later on into N-containing organic components that will become available to the plant (Wright & Lenssen, 2013). However, it had been documented that Biological Nitrogen Fixation (BNF) process is not able to supply the total requirement of the plant. On overall, only 50-60% of soybean nitrogen (N) demand is usually met by the BNF process (Salvagiotti et al., 2008). An unanswered scientific knowledge is still related to the ability of the BNF process to satisfy soybean N demand at varying yield levels.

In summary, for the genotype × N interaction, the main question is: "Do high yielding soybeans need to be fertilized with nitrogen?". The understanding of genetic gain × N in conditions for expressing high yield potential is a critical factor for advancing soybean yield improvement. For instance, does genetic improvement (genetic gain) was accompanied by changes in N uptake (and partition) in soybean?

The objectives of this study were to 1) study the contribution of N in soybean under different N nutrition scenarios: (i) soybean planted under normal production conditions, (ii) All N requirement met by N fertilizer, (iii) under normal production conditions + late additional on soybean yields and plant N content, and to 2) Evaluate the yield performance of historical and modern soybean genotypes released from 1980's to 2010's.

Procedures

Four locations were evaluated: three of them were located in Kansas, US (Ottawa, Ashland, and Rossville) and one location in Santa Fe, Argentina (Oliveros) (Figure 1).

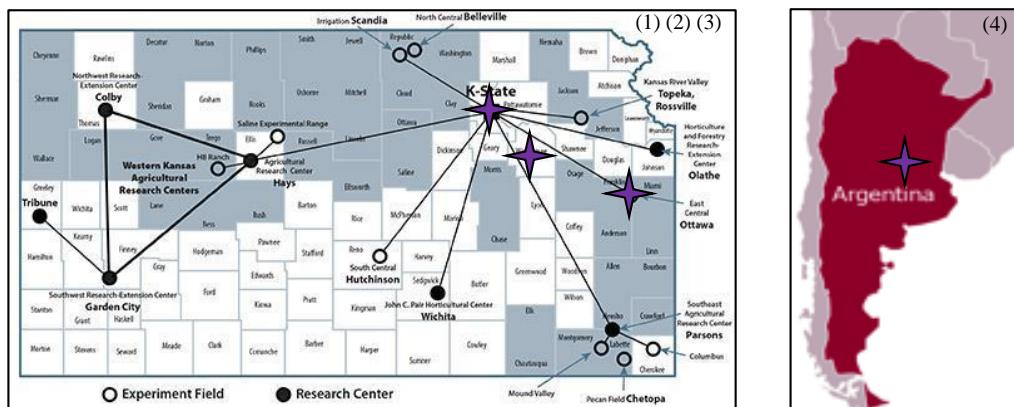


Figure 1. Map of the state of Kansas and Argentina identifying the four studies conducted during the 2016 season: Ottawa (1), Ashland (2), Rossville (3) -US and Oliveros (4) –ARG.

Experimental design

The study was conducted in experimental plots with 10-ft wide and 50-ft long at Ottawa and Ashland (US); 10-ft wide by 30-ft long at Rossville (US). Target seeding rate was 140,000 seeds per acre at Ottawa, 180,000 seeds per acre at Ashland, and 103,000 seeds per acre at Rossville. At three US locations it was used row spacing of 30 inches. At Ottawa and Ashland each treatment was replicated 6 times in a split-plot layout with a complete block arrangement (soybean variety as the main plot). Nine treatment combinations were evaluated for the genotype by N approach interaction at Ottawa and Ashland (Table 1). Nitrogen fertilizer application (expressed in lbs N ac⁻¹) per treatment is also presented in Table 2. Rossville experiment had 3 replications. At this location, the experiment was structured for 13 genotypes and 3 N strategies for a total of 39 treatment combinations.

At Argentina, experimental plots were 8.5-ft wide and 23-ft long. Each treatment was replicated 4 times. Eight varieties and 3 N strategies were evaluated for a total of 24 treatments combinations.

Table 1. Treatment description for Ottawa and Ashland sites (US), 2016 growing season.

Treatment	Release Decades	Varieties	N application
1			non-N applied
2	1990's	non-RR	All N provided by fertilizer (600 lbs ac ⁻¹)
3			late-season N (50 lbs ac ⁻¹)
4			non-N applied
5	2000's	RR-1	All N provided by fertilizer (600 lbs ac ⁻¹)
6			late-season N (50 lbs ac ⁻¹)
7			non-N applied
8	2010's	RR-2	All N provided by fertilizer (600 lbs ac ⁻¹)
9			late-season N (50 lbs ac ⁻¹)

Herbicides and hand weeding were implemented to maintain no weed interference for the entire growing season, and soil nutrient concentrations (other than N) were maintained above the recommended critical levels (through inorganic P/K applications).

Fertilizer applications

The fertilizer N applications were performed utilizing liquid urea-ammonium-nitrate (UAN at 32-0-0) as needed per each treatment combination. The three N strategies were the same at all 4 locations. Strategy 1 (S1) was a control with non N applied but seeds were inoculated; strategy 2 (S2) was all N provided by fertilizer at a rate of 600 lbs ac⁻¹ which was split in 3 timings: planting, R1, and R3; and finally strategy 3 (S3) with a late season application (at R3) of 50 lbs N ac⁻¹. Nitrogen applications at planting and R1 were performed using an All-Terrain Vehicle (ATV) equipped with Teejet spraying technology. The last N applications (at R3) were performed using a CO₂ Back Pack sprayer with drop tubes attached to the spraying boom in order to place the liquid fertilizer direct to the soil.

Site characteristics

Soil samples were taken before planting at 6 and 24 inches' depth for US locations (Ottawa, Ashland, and Rossville). Parameters analyzed from this samples were pH; Mehlich P;

Cation Exchange Capacity (CEC); organic matter (OM); Ca, Mg, and K availability; and nitrate concentration (N-NO₃) (Table 2).

At Argentina, soil samples were taken at 8 inches' depth. Parameters analyzed were pH; Bray 1 P; organic matter (OM); and nitrate concentration (N-NO₃) (Table 2).

Table 2. Pre-plant soil characterization at 6 and 24-inch depth for US sites (Ottawa, Ashland, and Rossville) and 8-inch depth for Argentina (Oliveros).

Soil parameters	Location			
	Ashland 6 inch depth	Ottawa 6 inch depth	Rossville 6 inch depth	Argentina 8 inch depth
pH	6.7	5.7	6.9	5.55
Mehlich P (ppm)	22	14	21	12
CEC (meq/100g)	9	18.5	11	-
OM (%)	1.5	4.3	2.17	2.14
K (ppm)	181	80	153	-
Ca (ppm)	1599	2665	2074	-
Mg (ppm)	179	393	202	-
N-NO ₃ (ppm)*	2.5	5	3	6.3

*N-NO₃ (ppm): all 3 US locations samples were taken at 24-inch depth.

In-season measurements

A variety of in-season measurements were performed at US locations. Main in-season activities are listed below:

- Stand counts (early in the season).
- Plant height (ground to the last developed leaf): At V4, R2, and R5 stages.
- Light bar interception (above and below canopy): At V4, R2, and R5 stages.
- Leaf area index (above and below canopy): At V4, R2, and R5 stages.
- Biomass sampling at V4, R2, R5, and R8 stages.

At Argentina location, biomass samples were collected at the R2, R5, and R7 stages.

Biomass determination was performed from a sample of five linear feet per plot at four growth stages: V4, R2, R5, and before harvest (R8). Each individual plant was cut at the stem base out in the field. Total fresh weight of the sample was taken and then it was sub-sampled to ten plants per plot. These 10-plants sub-samples were separated into different fractions: 1) leaves and stem (vegetative phase); and 2) pods, grain, leaves, and stems (reproductive phase). Each independent fraction was separately chopped and dried to constant weight at 140°F. When samples were dry they were grinded to fine particles that later were sent to a Lab for getting nutrient concentrations analysis.

At Ottawa and Ashland 2016, root samples were collected at the V4 stage. Ten roots per plot were sampled for root scanning and nodules count at three repetitions for each treatment. In addition, five ground pictures per plot were taken with a professional camera for calculating canopy cover with software analysis in the future. As a complement, at Ashland and Rossville (US) it was performed imagery analysis collecting information from different parameters using drones, but which main outputs focused were NDVI and canopy cover information at different stages during the season.

Results

Weather information

Seasonal precipitation distribution, expressed in inches, and maximum and minimum temperatures were documented throughout the entire growing season 2016 (Figure 2). In US similar temperatures were observed across locations but Ottawa at the end of the season experienced cooler temperatures exposed by the late planting date (June 8th). Mean temperatures were almost identical as well (Max at 70°F and Min at 48°F). Cumulative precipitation was higher at Rossville location (46 inches) while in Ottawa and Ashland was between 35 and 39 inches. Precipitation distribution was slightly different at each location. At Ottawa, rainfall was mainly distributed between June 28th and October 6th; at Ashland and Rossville precipitation was better distributed in the entire growing season (Figure 2).

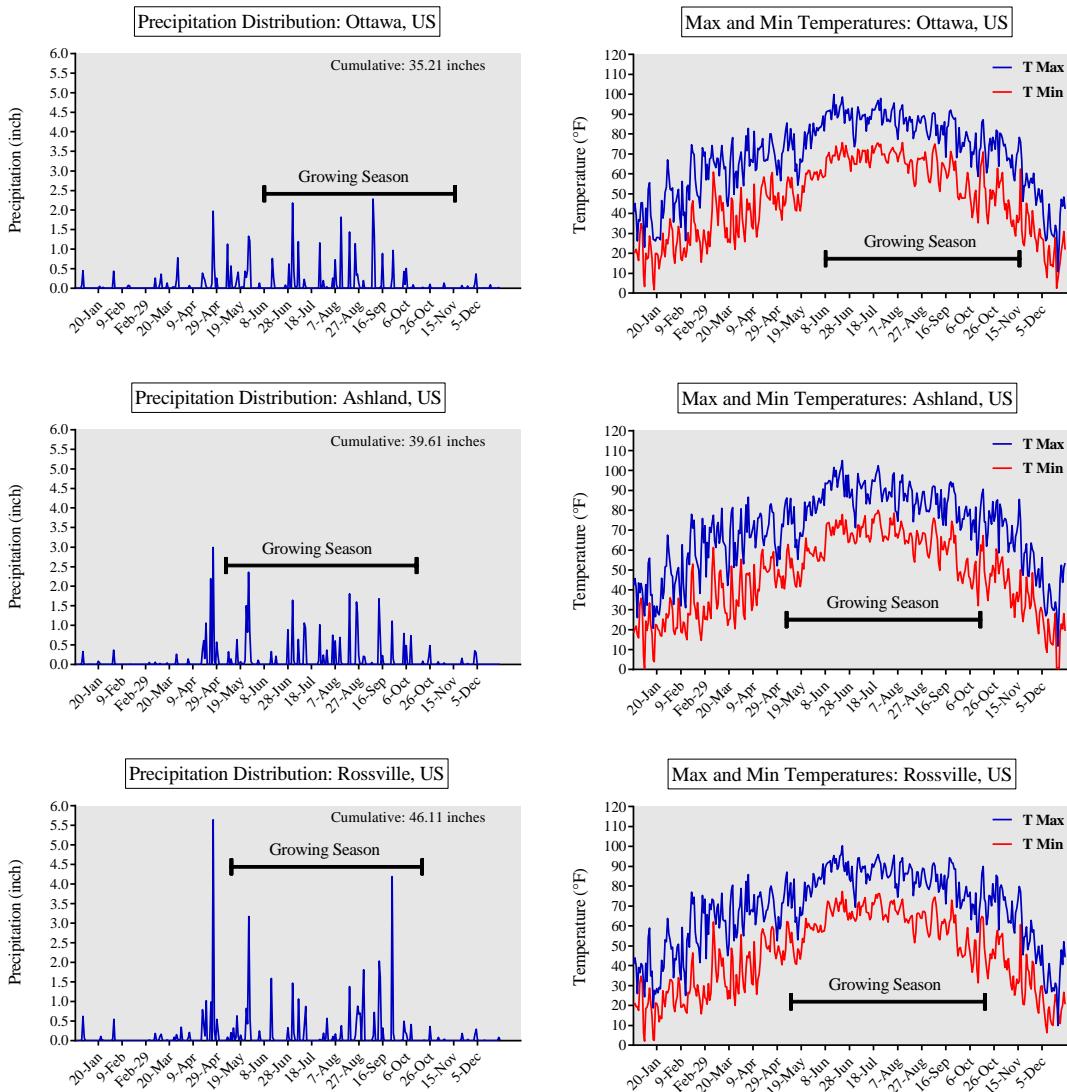


Figure 2. Daily precipitation (from January to December) (left panels) and seasonal minimum and maximum temperatures (right panels) for the 2016 growing season at Ottawa, Ashland, and Rossville (US locations).

Stand Counts

Early-season stand counts were collected in two 17.5-ft sections per plot immediately after emergence (VE) (Table 3). Stand count efficiency when compared to seeding rate at Ottawa ranged from 39% to 89% with average of 64%. At Ashland, ranged from 30 to 86% and its average 60%. Average stand count efficiency at Rossville was 66% as well. All US locations efficiency in stand count was observed between 60 and 66% on overall.

Table 3. Final stand counts per repetition block for Ottawa, Ashland, and Rossville-US sites, during 2016 growing season.

Field Sites	Repetitions ($\times 1,000$ plants/acre)					
	1	2	3	4	5	6
Ottawa	94	94	93	97	82	81
Ashland	104	112	97	111	88	119
Rossville	67		72		68	

Nodules Information

Nodules information was compiled for Ottawa and Ashland (US-locations) during the 2016 growing season, nodules information was expressed in nodules per plant and at the V4 stage. Nitrogen strategies showed statistical effects ($P<0.05$) while genotypes did not present differences in final nodules number. On overall, Ashland presented higher number of nodules per plant (Figure 3) than Ottawa (no soybean history for the last 20 years). As it was expected, S2 resulted with the lowest number of nodules per plant at both locations (5 nodules per plant at Ottawa and 6 nodules per plant at Ashland) when compared to S1 and S3 treatments.

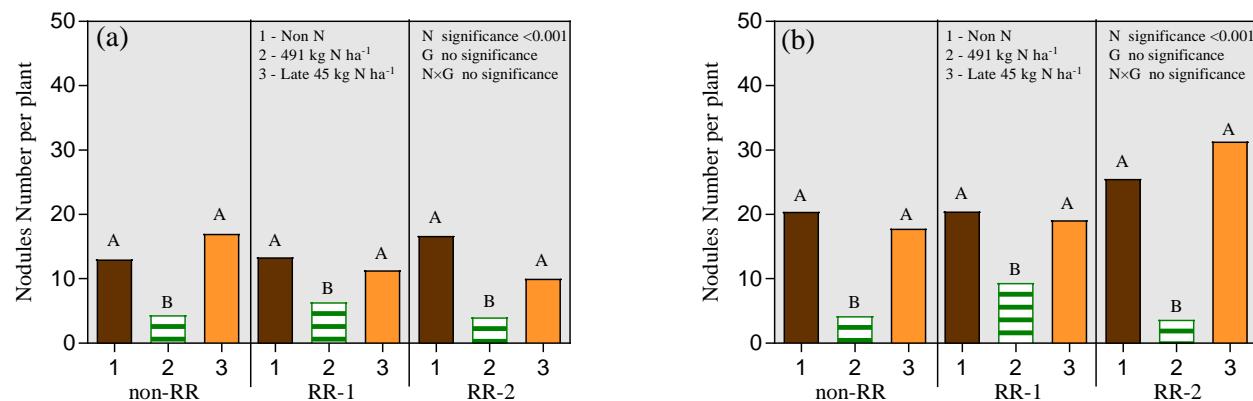


Figure 3. Per-plant nodule number affected by soybean genotype and N interaction at Ottawa (a) and Ashland (b) sites, U.S. at V4 stage during the 2016 growing season.

Genetic Gain

Twenty-one soybean genotypes from different releases were utilized in this experiment. At Rossville, 13 genotypes released in the decades of the 1980's, 1990's, 2000's and 2010's were tested. At Oliveros, 8 genotypes (two from each of the previous listed decades). At both locations, maximum yield was recorded for the modern variety (2010's), with relative yields improving with the year of release of the commercial material (Figure 4).

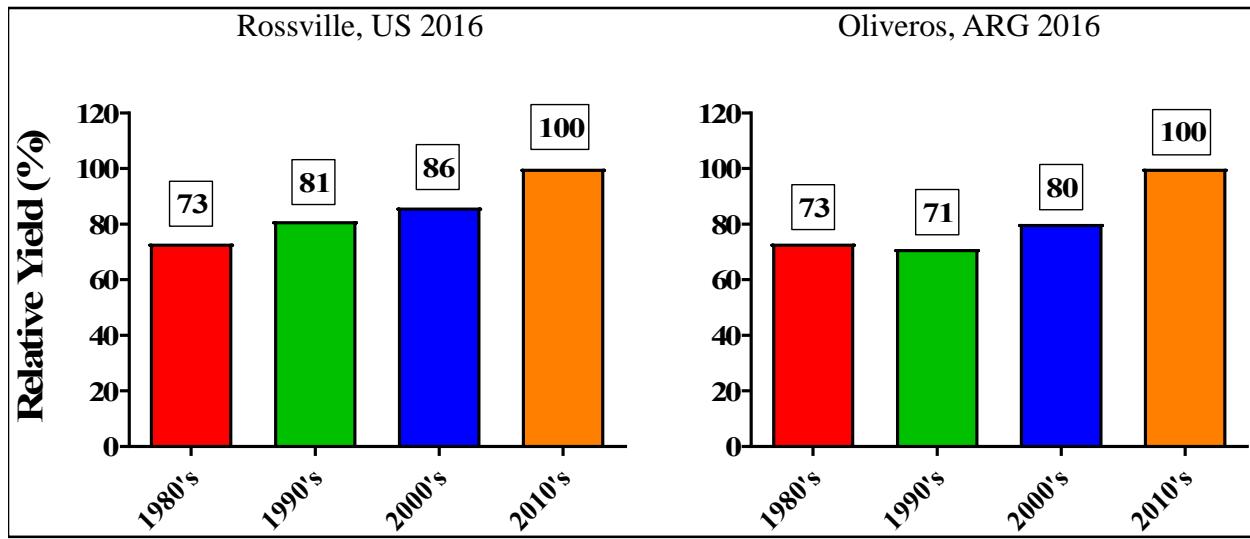


Figure 4. Genetic improvement for soybean genotypes presented in relative yields (%) for four release decades at Rossville, US and Oliveros, ARG during the 2016 growing season.

Yields

Yield information, expressed in bu ac^{-1} , was adjusted to 13.5% of moisture content. Yields were recorded with a plot combine and from the two central rows in all plots.

Soybean Genotypes by N fertilization strategies

Yields for 13 genotypes are presented for Rossville (US) and 8 genotypes for Oliveros (ARG) all considering the three N strategies. Yields were similar for both locations, ranging between 37 and 87 bu ac^{-1} . Nitrogen strategy and genotypes presented statistical significance ($P < 0.05$), but there was not interaction. Greater yields, 18% increase at Rossville and 21% increase at Oliveros, were obtained with modern soybean genotypes (release year >2000 's) (Figure 5). On the N applications, S2 (600 lbs N ac^{-1}) increased 18% yields at Rossville and 5% at Oliveros compared to S1 (non-N applied but inoculated) (Figure 5).

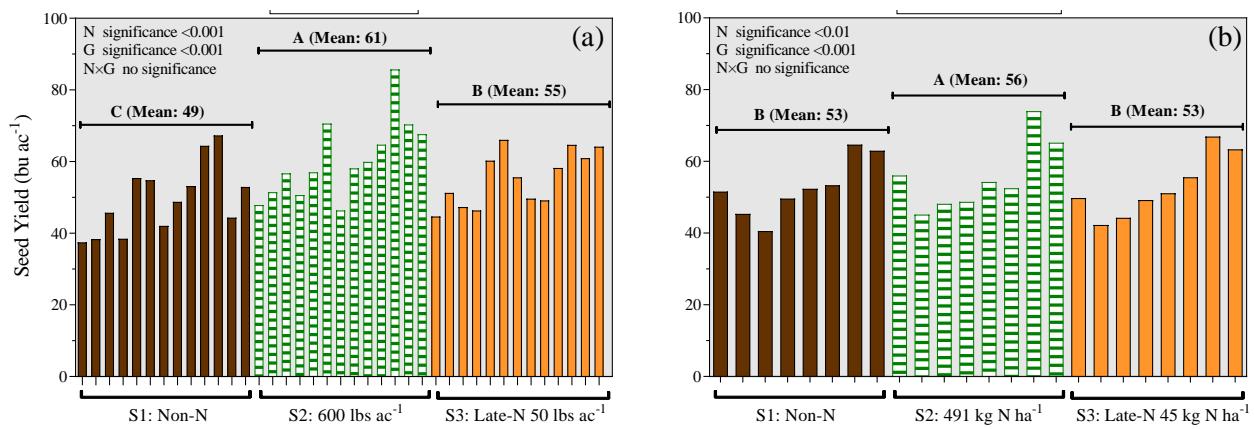


Figure 5. Seed yield for soybean genotypes with different N fertilization strategies at Rossville, US (a) and Oliveros, ARG (b) during the 2016 growing season.

At Ottawa yields were lower (ranging from 21 to 30 bu ac⁻¹) when compared to Ashland (47 to 65 bu ac⁻¹) (Figure 6). At Ottawa and Ashland, genotypes had statistical effect ($P<0.05$) on soybean yields and N application was also significant but just for Ottawa. At Ottawa, higher yields were observed for modern soybean genotype (2010's decade) and the S2 and S3 N management approaches relative to past varieties and the S1 treatment.

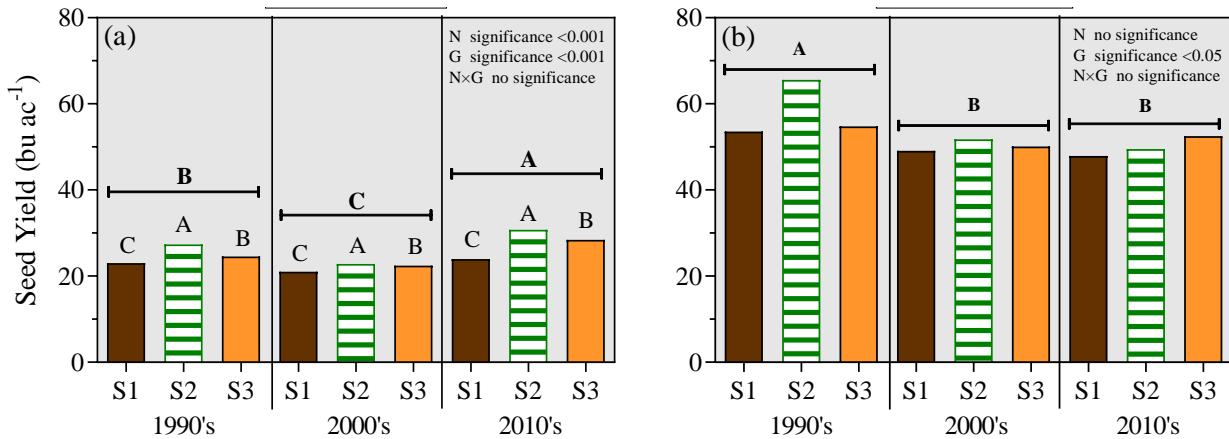


Figure 6. Seed yield for soybean genotypes with different N fertilization strategies at Ottawa (a) and Ashland (b), US during the 2016 growing season.

Historical Genotypes by N Strategies Interaction

At Oliveros, genotype by N strategy presented a significant ($P<0.05$) interaction (Figure 7). Highest yield (74 bu ac⁻¹) was observed with the modern soybean genotype (2010's release decade) and the S2 N management approach. In the other hand, lower yields were documented for the 1990's variety regardless of the N management approach.

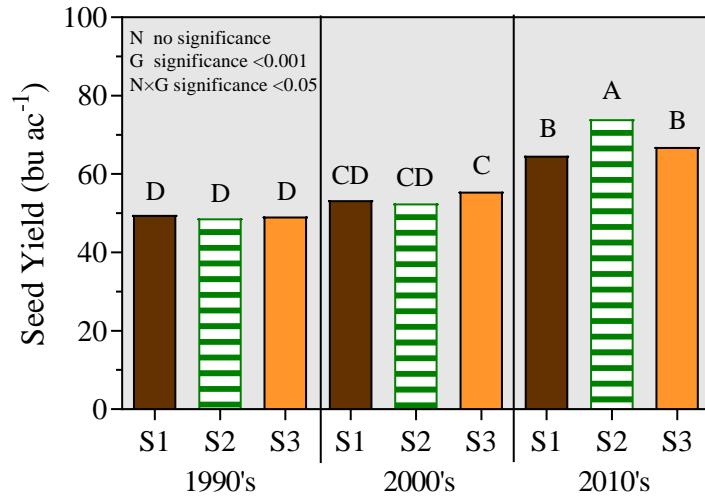


Figure 7. Seed yield for soybean genotypes with different N fertilization strategies at Oliveros, ARG during the 2016 growing season.

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