High-Yielding Soybean: Genetic Gain × Fertilizer Nitrogen Interaction

I. A. Ciampitti  
*Kansas State University*, ciampitti@ksu.edu

J. Kimball  
*Kansas State University*, jkimball@ksu.edu

Eric Adee  
*Kansas State University*, eadee@ksu.edu

*See next page for additional authors*

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Abstract
The U.S. accounts for 35% of the global soybean production. Potential soybean yields are determined by the interaction of genotype, environment, and management practices (G × E × M). The question "Do high yielding soybean need to be fertilized with nitrogen (N)?" is still a valid one. The overall objective of this project is to study the contribution of N via utilization of varying N strategies under historical and current soybean genotypes. Two field experiments were conducted during the 2015 growing season at Ottawa (east central KS) and at Ashland Bottoms (central KS). Three soybeans varieties were used (1990s = non-RR, 2000s = RR-1, and 2010s = RR-2) under three N systems (non-N applied; late-N, 50 lb N/a; and 550 lb N/a, split in 3 timings) with all seeds inoculated. At Ottawa, the study was planted in an area without soybean history, with yields ranging from 14 to 37 bushels per acre. Superior yields were recorded for the modern soybean variety Roundup Ready (RR-2) relative to the RR-1 and non-RR materials. As related to the N management approach, slightly higher soybean yields occurred when N nutrition was based on fertilizer N application. At the Ashland Bottoms site, yields ranged from 44 to 76 bushels per acre. High yields were with the oldest soybean genotype (non-RR) when N nutrition was based on the fertilizer N application; while low yields were when the N nutrition of the modern soybean variety (RR-2) was based on the inoculation. There was no variety by N factor interaction with yield. The variety (P < 0.05) was the main significant single effect, which presented the following order from high to low productivity: non-RR >> RR-1 = RR-2. A conclusion from the first year of this experiment was the field where soybean had not been previously planted (Ottawa) had a lower yield capacity compared to the site with a soybean history (Ashland Bottoms).

Keywords
soybean, nitrogen, inoculation, fertilization, genotypes

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Authors
I. A. Ciampitti, J. Kimball, Eric Adee, O. Ortez, and G. I. Carmona
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I.A. Ciampitti, J. Kimball, E.A. Adee, O. Ortez, and G.I. Carmona

Summary
The U.S. accounts for 35% of the global soybean production. Potential soybean yields are determined by the interaction of genotype, environment, and management practices ($G \times E \times M$). The question “Do high yielding soybean need to be fertilized with nitrogen (N)?” is still a valid one. The overall objective of this project is to study the contribution of N via utilization of varying N strategies under historical and current soybean genotypes. Two field experiments were conducted during the 2015 growing season at Ottawa (east central KS) and at Ashland Bottoms (central KS). Three soybeans varieties were used (1990s = non-RR, 2000s = RR-1, and 2010s = RR-2) under three N systems (non-N applied; late-N, 50 lb N/a; and 550 lb N/a, split in 3 timings) with all seeds inoculated. At Ottawa, the study was planted in an area without soybean history, with yields ranging from 14 to 37 bushels per acre. Superior yields were recorded for the modern soybean variety Roundup Ready (RR-2) relative to the RR-1 and non-RR materials. As related to the N management approach, slightly higher soybean yields occurred when N nutrition was based on fertilizer N application. At the Ashland Bottoms site, yields ranged from 44 to 76 bushels per acre. High yields were with the oldest soybean genotype (non-RR) when N nutrition was based on the fertilizer N application; while low yields were when the N nutrition of the modern soybean variety (RR-2) was based on the inoculation. There was no variety by N factor interaction with yield. The variety ($P < 0.05$) was the main significant single effect, which presented the following order from high to low productivity: non-RR >> RR-1 = RR-2. A conclusion from the first year of this experiment was the field where soybean had not been previously planted (Ottawa) had a lower yield capacity compared to the site with a soybean history (Ashland Bottoms).

Introduction
The U.S. accounts for 35% of the global soybean production (FAOSTAT & USDA-NASS, 2007-2012). 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.

Soybean yield potential is genetically determined. Yield potential ($Y_p$) can be attained under “ideal” conditions (genotype × environment × 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 ($Y_f$) is primarily defined by crop management practices (e.g., row spacing, planting date, fungicide and nutrient application, among several others) and the inter-
actions 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.

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

In the long term, improving soybean yields is a must if we are to meet global food demand. Forecasts for global soybean yield, based on observed yield information from the last century, suggest improvements in productivity but at a smaller yield gain rate as compared to the yield trend needed to double crop production. Thus, further improvement in management decisions should focus on capturing benefits from utilization of all inputs and natural resources (light, water, and nutrients) in the most effective approach in order to reduce the yield gap.

In summary, for the genotype × N interaction, the main question that researchers and producers are asking 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 critical for advancing soybean yield improvement.

The objectives of this study were to 1) quantify the exploitable yield gap related to the N management strategy as related to the genotype evaluated; and 2) quantify the impact of the soybean variety × N strategy on plant traits, nodules count, and yields (end of the season).

Regarding site characteristics, the soil type at the Ottawa location was a Woodson and at Ashland Bottoms was a Crete silt loam (Figure 1). Soil samples were taken before planting at Ottawa and Ashland Bottoms to a total depth of 6 inches. Parameters analyzed were pH, Melich P, cation exchange capacity (CEC), organic matter (OM), calcium (Ca), magnesium (Mg), and potassium (K) availability (Table 1).

**Procedures**

The study was conducted in field plots with a size of 10-ft wide by 50-ft long at both sites. Target seeding rate was 180,000 seeds per acre at Ashland and 140,000 seeds per acre at Ottawa, with similar row spacing, 30 inches at both sites. Each treatment was replicated five times in a split-plot layout with a complete block arrangement (soybean variety as the main plot). Soybean varieties used were: 1) old soybean var. P93B82 (Pioneer Hi-bred, Johnston, IA) for non-RR (released 1997 yr; maturity group, MG = 3.8); 2) 93Y92 for the RR-1 (released 2009 yr; MG = 3.9); and 3) modern soybean var. P34T43R2 for the RR-2 (released 2014 yr; MG = 3.4).

Nine treatment combinations were evaluated for the genotype by N approach interaction (Table 2). Nitrogen fertilizer application (expressed in lb per acre) per treatment
is presented in Table 2. Herbicides and hand weeding were used to maintain no weed interference for the entire season, and soil nutrient concentrations (other than N) were maintained above the recommended critical levels (through inorganic P/K applications).

In-season measurements for biomass as related to sampling date and phenological stage is presented in Table 3. All treatments received inoculation. Stand counts were performed (measuring two 17.5-ft sections per plot) immediately after emergence (VE), in three out of the five replications. The fertilizer N applications were performed using liquid urea-ammonium-nitrate (UAN, 32-0-0) as needed per each treatment combination: 1) 50 lb N per acre as a single late-N season was applied at R3 stage; and 2) total of 550 lb N per acre was applied split in three times (planting, mid-flowering, and R3 stages).

The measurements of this study were all conducted at V4, R2, and R5 stages. Measurements included plant height (from ground to the last developed leaf); stem diameter (ground base); chlorophyll meter (SPAD, last developed trifoliate); light bar interception (above and below canopy); and leaf area index (above and below canopy). Biomass determination was performed from ten consecutive plants per plot at four growth stages: V4, R2, R5, and before harvest (R8) (Table 3). Each individual plant was cut at the stem base and separated into different fractions: 1) leaves and stem (vegetative); or 2) pods, grain, leaves, and stem (reproductive). Each fraction was separately chopped and dried to constant weight at 140°F. Nutrient concentrations are currently under further testing (lab analysis).

At both sites, root samples were collected at V4 stage. Root scanning and nodule count were performed to all roots in each treatment. Yield information is expressed in bushels per acre, adjusted to 13.5% moisture content. Yield was collected from the central two rows (5 × 50 ft). Seed weight was estimated from 300 seeds. Grain harvest index was calculated as the grain yield to the whole-plant biomass ratio at maturity.

**Weather Information**

Seasonal precipitation distribution, expressed in inches, was documented throughout the entire growing season (Figure 2). For temperature, the maximum and minimum for 2015 was very similar to the 30-year average (historical). At the Ottawa site, the largest deviation was documented for the month of May, minimum temperature was 13°F higher as compared with the historical minimum (Figure 2), which was not observed at Ashland Bottoms. Otherwise, the temperatures at Ashland Bottoms site were similar as at Ottawa, varying between 40 to 90°F.

Seasonal precipitation for 2015 was lower relative to the historical record, with exception of the month of May, for which 2015 precipitation was almost double the historical record (Figure 2). In agreement with the temperature trend, the month of May was a clear outlier for the 2015 growing season as compared with the historical weather information. At Ashland Bottoms site, similar precipitation distribution was registered as compared with Ottawa. Total precipitation for 2015 growing season was comparable at both locations, approximately 28 inches (Figure 2).
Results

Soybean grain yields at the Ottawa site, without soybean history, ranged from 14 to 37 bushels per acre (Table 5). High yields were recorded for the modern variety (2000s) when a total of 550 lb N per acre were applied, while lowest yield was related to the 1990s (RR-1) variety when plants were inoculated only. There was not interaction between variety and N source (P>0.05). Only single factors, variety and N rate were significant. For the N approach, a trend to higher yields was observed when N was applied as the main source; while low yields were documented when N nutrition depended only on inoculation (Figure 3).

Plant height, SPAD, and stem diameter improved throughout the phenological stages. Plant height ranged from 10 inches at V4 to 23 inches at R5 growth stage. The SPAD at V4 was lower than at R2 and R5, while in those last stages, SPAD measurements were basically stable, with few variations. Related to stem diameter, this plant trait has increased from the average of 0.10 inches at V4 to 0.18 inches at R5 (Figure 4). Finally, the number of nodules per soybean plant at V4 did not differ between treatments, averaging 17 nodules per plant (Figure 5).

Grain yield at the Ashland Bottoms site ranged from 44 to 76 bushels per acre (Table 5). High yields were with the oldest soybean genotype (non-RR) when N nutrition was based on the fertilizer N application; while low yields were when the N nutrition of the modern soybean variety (RR-2) was based on inoculation. Statistically, variety × N factor did not significantly affect yields. Variety did (P < 0.05) impact yields, with the following order from high to low yield: non-RR >> RR-1 = RR-2 (Figure 6).

At Ashland, plant height and SPAD increased throughout the stages, except for the stem diameter, which kept stable (Figure 7). Plant trait values were superior at this site as compared to Ottawa, which might be connected with greater plant growth and higher yield potential.

In addition, the number of nodules per plant at Ashland Bottoms were four-fold greater (averaged 50 nodules per plant) relative to the Ottawa site (Figures 5 and 8). Nodules number at Ashland Bottoms did not differ between genotypes and N sources, reflecting the variation recorded in the characterization of this trait (Figure 8).

Soybean yield at Ashland Bottoms site was greater ( ranged from 44 to 76 bushels per acre) than at Ottawa ( ranged from 14 to 37 bushels per acre), which primarily reflects weather × soil × cropping system history interaction. At Ottawa, maximum agronomical yield was documented for the modern soybean variety (RR-2) when dependent on N fertilization (plus residual soil N). On the other side, lowest yield was recorded for the RR-1 variety when it was dependent on the BNF as the main source of N nutrition (inoculation plus residual soil N). Still, good inoculation is critical for establishing new soybean ground and for increasing soybean production in a sustainable and economically efficient manner. At Ashland Bottoms, fertilizer N × variety interaction was negligible. Statistically, the non-RR variety showed greater yield when compared to RR-1 and RR-2. At Ashland Bottoms, the plant height, SPAD, stem diameter, and the number of nodules per plant were greater than at Ottawa, reflecting better growing conditions and high yield potential. In summary, further evaluation and research is needed in order
to properly dissect the “true” genetic contribution on soybean yield and its interaction with N nutrition.

Table 1. Pre-plant soil characterization at 0-6 inch depth at Ashland and Ottawa locations

| Soil parameters | Ashland | Ottawa |
|-----------------|---------|--------|
| pH              | 7.9     | 6.5    |
| Mehlich P (ppm) | 60      | 7.4    |
| CEC (meq/100g)  | 13.2    | 25.9   |
| OM (%)          | 1.58    | 3.32   |
| K (ppm)         | 264     | 191    |
| Ca (ppm)        | 2145    | 3273   |
| Mg (ppm)        | 71.1    | 532    |

Table 2. Treatment description for both field sites during the 2015 growing season

| Treatment | Varieties | N application       |
|-----------|-----------|---------------------|
| 1         | non-RR    | non-N               |
| 2         | non-RR    | 550 lb N            |
| 3         | non-RR    | late-N (50 lb N)    |
| 4         | RR-1      | non-N               |
| 5         | RR-1      | 550 lb N            |
| 6         | RR-1      | late-N (50 lb N)    |
| 7         | RR-2      | non-N               |
| 8         | RR-2      | 550 lb N            |
| 9         | RR-2      | late-N (50 lb N)    |

Table 3. Planting and sampling dates for Ashland and Ottawa sites, 2015 growing season

| Field sites | Planting date | Sampling date | Phenological stage |
|-------------|---------------|---------------|--------------------|
| Ashland     | 05/15/2015    | 06/22/2015    | V4                 |
|             |               | 07/16/2015    | R2                 |
|             |               | 08/17/2015    | R5                 |
|             |               | 09/18/2015    | R8                 |
| Ottawa      | 06/15/2015    | 07/20/2015    | V4                 |
|             |               | 08/12/2015    | R2                 |
|             |               | 09/09/2015    | R5                 |
|             |               | 10/12/2015    | R8                 |
Table 4. Final stand counts per treatment for Ashland and Ottawa sites, 2015 growing season

| Field sites | Treatments (× 1,000 plants per acre) |
|-------------|-------------------------------------|
|             | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Ashland     | 157 | 159 | 149 | 162 | 157 | 167 | 170 | 161 | 161 |
| Ottawa      | 62 | 65 | 62 | 74 | 72 | 77 | 83 | 78 | 86 |

Table 5. Descriptive statistics (mean, minimum, maximum, and coefficient of variation (CV)) on yield parameter, expressed in bushels per acre, for Ashland and Ottawa sites during the 2015 growing season

| Field sites | Mean yield | Min. yield | Max. yield | CV |
|-------------|------------|------------|------------|----|
|             | bushels per acre | bushels per acre | bushels per acre | %   |
| Ashland     | 52         | 44         | 76         | 14.0 |
| Ottawa      | 25         | 14         | 37         | 21.9 |

Figure 1. Map of the state of Kansas identifying field studies conducted during the 2015 season.
Figure 2. Monthly precipitation (from January to December) (upper panels) and seasonal minimum and maximum temperatures (bottom panels) for the historical averages (1985-2015 period) and 2015 growing season at Ottawa and Ashland Bottoms (Manhattan), KS.

Figure 3. Grain yield (13.5% moisture) for soybean variety by N interaction at Ottawa site (KS, U.S.) during the 2015 season. N application (N), variety (V), Interaction (N×V).
Figure 4. Plant height, SPAD and stem diameter for soybean variety by N interaction at Ottawa site (KS, U.S.) during the 2015 season.

* Phenological stage.
Figure 5. Per-plant nodule number affected by soybean variety and N interaction at the Ottawa site (KS, U.S.) during the 2015 season.
* Phenological stage.

Figure 6. Grain yield (13.5% moisture) for soybean variety by N interaction at the Ashland Bottoms site (KS, U.S.) during the 2015 season. N application (N), variety (V), Interaction (N×V).
Figure 7. Plant height, SPAD and stem diameter for soybean variety by N interaction at the Ashland Bottoms site (KS, U.S.) during the 2015 season.
* Phenological stage.
Figure 8. Per-plant nodule number affected by soybean variety and N interaction at the Ashland Bottoms site (KS, U.S.) during the 2015 season.

* Phenological stage.
Figure 9. Ashland Bottoms Location, 2015 season.