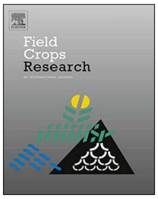
Field Crops Research 213 (2017) 204–212

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr



Is soybean yield limited by nitrogen supply?

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ARTICLE INFO

Keywords:
Soybean
Nitrogen
Yield potential
Protein
Oil

ABSTRACT

As soybean yield continues to increase, it seems critical to know if there is a yield level at which potential contribution of indigenous nitrogen (N) sources (N fixation and soil mineralization) becomes insufficient to meet crop N requirements for high yields, while still maintaining or increasing protein and oil concentration. We have hypothesized that, in absence of other limiting factors, degree of N limitation increases with increasing yield potential (Yp) of the production environment. To test this hypothesis, we developed a novel protocol to ensure an ample N supply during the entire crop season (full-N treatment). That protocol was applied to field-grown irrigated soybean in Balcarce (Argentina) and Nebraska (USA), where measured full-N seed yields were ± 15% of their simulated Yp in 92% of the cases. The combination of locations, years, sowing dates, and N treatments resulted in a wide range of seed yields, from 2.5 to 6.5 Mg ha^{-1}. Overall, full-N seed yield averaged 11% higher than seed yield without N addition (zero-N). However, magnitude of yield difference between full-N and zero-N depended upon Yp, ranging from no detectable yield difference in low-Yp (*ca.* 2.5 Mg ha^{-1}) to up to 900 kg ha^{-1} in high-Yp environments (*ca.* 6 Mg ha^{-1}). Seed yield differences were associated with higher aboveground dry matter, seed number, and seed weight in the full-N *versus* zero-N treatments. Seed protein (but not oil) concentration was higher in the full-N treatment, and both protein and oil yields were higher in the full-N *versus* zero-N treatments. Findings from this study indicate that (i) N limits soybean seed yield (as well as protein yield, and oil yield) in environments with high Yp, where indigenous N sources seem insufficient to fully satisfy crop N requirements, and (ii) yield response to N fertilizer can occur above a 2.5 Mg ha^{-1} Yp threshold and has an upper limit of 250 kg seed per Mg increase in Yp.

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the most important legume crop globally, with a respective harvested area and total production of 118 million ha and 307 million Mg (FAOSTAT 2017, http://faostat3.fao.org), accounting for 56% of total global oilseed production (Wilson, 2008). Soybean is a key component of global food security as a source of protein for human food and animal feed, and oil for cooking and biofuel. Meeting soybean demand on existing cropland area for a global population of 9.7 billion people by year 2050 will put pressure on narrowing the existing gap between average producer yield and yield potential (Cassman et al., 2003; van Ittersum et al., 2013). Yield potential (Yp) is defined as the yield of a well-adapted cultivar when grown without limitations in water and nutrient supply and kept free of biotic stresses (weeds, diseases, and insect pests) (Evans, 1993). Hence, for a given site-year, soybean Yp is determined by solar radiation, temperature, and other factors that influence the length of time during which the crop was grown, such as variety maturity group and sowing date. For example, soybean Yp has been postulated to range between 6 and 8 Mg ha^{-1} in favorable environments of the US Corn Belt (Specht et al., 1999; Sinclair and Rufty, 2012).

Relatively high amounts of nitrogen (N) must be taken up by all crops to achieve high seed yields, particularly legumes, because of their high seed protein content (Sinclair and De Wit, 1976; Giller and Cadisch, 1995). On average, a soybean crop accumulates ca. 79 kg N ha^{-1} in its aboveground biomass per each additional Mg seed yield, with the latter expressed at standard seed moisture of 0.130 kg H_2O kg^{-1} seed (Salvagiotti et al., 2008; Tamagno et al., 2017). This ratio can be used to estimate the N uptake requirement over a range of soybean seed yields. For example, seed yields ranging from 6 to 8 Mg ha^{-1} would be expected to have an associated N uptake requirement range of 480–640 kg N ha^{-1}. In contrast, only 240 kg N uptake ha^{-1} would be required for a soybean yield of 3 Mg ha^{-1}, which is equivalent to the average soybean yield during the last 5 years in the United States (US) and Argentina (USDA-NASS, 2010–2014; https://www.nass.usda.gov/Quick_Stats/; https://datos.magyp.gob.ar/).

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http://dx.doi.org/10.1016/j.fcr.2017.08.009
Received 8 May 2017; Received in revised form 8 August 2017; Accepted 10 August 2017

Soybean rarely receives N fertilizer in producer fields (though a small application as 'starter' fertilizer is sometimes applied at sowing time). Still, there is currently much interest in the degree to which well-managed soybean crops, grown in favorable production environments, can meet the large N requirement for 6–8 Mg ha^{-1} seed yields by relying exclusively on soil N mineralization and N fixation.

Soybean yield continues to increase over time due to genetic and agronomic improvement (Grassini et al., 2014a,b; Specht et al., 2014). Hence, it would be useful to discern the seed yield level at which the potential contributions of N from fixation and N from soil organic matter mineralization might jointly become insufficient to satisfy crop N requirement. Acquisition of that information requires data collection from soybean crops grown in stress-free conditions, where measured yields approach the site-year-specific Yp, as determined by weather, sowing date, and variety. In such scenarios, experimental comparison of a 'zero-N' treatment receiving no N fertilizer (*i.e.*, crop must rely exclusively on the indigenous N sources including N-fixation) *versus* a full-N treatment receiving N fertilizer applied as needed to sequentially ensure ample N supply throughout the crop-growing season. This comparison would need to be repetitively conducted across a wide range of Yp production systems to generate sufficient data for determining whether there is a yield level at which those indigenous N sources are insufficient to meet crop N requirements.

Meeting crop N requirement is challenging because it requires temporal synchronization between the seasonal supply of N from indigenous N sources and seasonal crop N demand, and addition of N fertilizer when the latter exceeds the former at any time during the crop season (Cassman et al., 2002). No previous study has explicitly attempted to grow soybean in production settings of very high yields while ensuring non N-limiting conditions (Salvagiotti et al., 2008 and references cited therein). While a few studies have reported using large N fertilizer amounts in soybean (> 300 kg ha^{-1}), N fertilizer in these studies was applied as a single large dose near the sowing date, or in split applications during early vegetative stages (*e.g.*, Brevedan et al., 1978; Herridge and Brockwell, 1988; Ray et al., 2006; Wilson et al., 2014). Because soybean absorbs *ca.* 60% of total N uptake during the pod setting and seed filling phases (Thies et al., 1995; Bender et al., 2015; Gaspar et al., 2017), it is difficult to determine the degree to which these previous studies have ensured non-N limiting conditions during those phases. Moreover, the yield response (or lack of response) to N fertilizer, reported by these previous studies, was likely confounded by other non-N growth-limiting factors. For example, in water-limited conditions, the yield response to N fertilizer can be amplified by the negative effect of temporary water shortages on N fixation (Purcell et al., 2004; Ray et al., 2006). Moreover, even in absence of water limitation, other growth-reducing factors may have limited crop growth in these experiments, given that measured yields consistently fell short of the high range of 6–8 Mg ha^{-1} soybean Yp (Specht et al., 1999; Sinclair and Ruffy, 2012), and also were less than measured yields (5–6 Mg ha^{-1}) that are routinely attained by progressive soybean producers (Grassini et al., 2014a,b, 2015).

In this study, we hypothesized that, in absence of other limiting factors, the degree of N limitation increases with higher Yp. To test this hypothesis, we developed a protocol to ensure ample N supply during each phase of the soybean crop season. The protocol was applied to field-grown irrigated crops in Balcarce (Argentina) and Nebraska (USA) that were within ± 15% of their simulated Yp based on site-year specific weather, sowing date, and variety. Results were interpreted using simple eco-physiological frameworks.

2. Materials and methods

2.1. Field experiments

Field experiments were conducted in Balcarce (BA), Argentina, during two crop seasons (BA-Y1: 2014/2015 and BA-Y2: 2015/2016), and at four sites in Nebraska (NE), USA, during one crop season (2016). The experiments in BA consisted of a combination of sowing date, variety maturity group, and N treatments (zero-N and full-N, see Section 2.2), whereas experiments in NE were replicated at four producer irrigated high-yield fields that included the same two N treatments (Table 1). For simplicity, the combinations of crop season x sowing date x variety in BA, or the producer fields in NE, are hereafter called 'environments'. In all experiments, crops were irrigated and managed to ensure optimal water and nutrient supply (except, of course, for N in the zero-N treatment, see Section 2.2) and to avoid stress from weeds, insects and pathogens. Irrigation was applied throughout the crop season with application event amounts adjusted periodically to match seasonal changes in crop water demand. In NE producer fields, soil water content in the upper 90 cm of soil was monitored using Watermark® sensors, which indicated that soil water status was consistently above 65% of total soil plant available water between emergence and physiological maturity. Several prophylactic foliar applications of herbicide, fungicide, and insecticide kept the crops free from biotic stresses in all experiments. A meteorological station located at each site provided daily weather data.

Table 1
Description of field experiments conducted in Balcarce (Argentina) and Nebraska, NE (USA).

Experiment	Crop season	Location	Variety name and maturity group (MG)	Sowing date
Balcarce (BA-Y1)	2014/2015	37.7647 S 58.3125 W 118 m a.s.l.	DM2200 (MG:2.1), DM3810 (MG:3.8), DM4612 (MG:4.6)	Nov 1, Nov 27, Dec 18, Jan 6
Balcarce (BA-Y2)	2015/2016	37.7652 S 58.3117 W 118 m a.s.l.	DM2200 (MG:2.1), DM3312 (MG:3.3), DM3810 (MG:3.8)	Nov 3, Dec 12, Jan 11
Atkinson, NE	2016	42.6372 N 98.9561 W 635 m a.s.l.	AG2723 (MG:2.7)	April 25
Mead, NE	2016	41.2441 N 96.5016 W 368 m a.s.l.	AG2723 (MG:2.7)	May 8
Saronville, NE	2016	40.6005 N 97.9658 W 538 m a.s.l.	AG2431 (MG:2.4)	April 26
Smithfield, NE	2016	40.5380 N 99.6833 W 769 m a.s.l.	P24T19 (MG:2.4)	May 13

2.1.1. Balcarce, Argentina

Field experiments were conducted on a deep fine-loamy Typic Argiudol. Topsoil (0–20 cm) organic matter, extractable phosphorous (P Bray-1 method), and soil pH were 27 g C kg^{-1}, 22 mg kg^{-1}, and 6.4 in BA-Y1, and 28 g C kg^{-1}, 25 mg kg^{-1}, and 6.6 in BA-Y2. Soil N-NO$_3^-$ in the upper 60 cm at sowing ranged from 59 to 114 kg ha^{-1} in BA-Y1 and from 61 to 105 kg ha^{-1} in BA-Y2. Previous crop was wheat and hairy vetch [*Vicia villosa* Roth.] in BA-Y1 and BA-Y2, respectively. The field was disked before sowing only in BA-Y1. Seeds were treated with fungicide and inoculated in both years using the best available product in the market and following recommended inoculation practices (Nitragin Optimize II® Pack Apron® & Jumpstart). Row spacing was 0.35 m and plants were thinned at V1 stage to *ca.* 35 plants m^{-2}. Phosphorous, sulfur, and calcium fertilizer amounts applied before sowing were 19, 21, 26 kg ha^{-1} in BA-Y1 and 20, 22, 27 kg ha^{-1} in BA-Y2, respectively.

The factorial experiments in Argentina were arranged in a split-split plot design with four replicates in both years. Sowing dates were main plots, three varieties of contrasting maturity group were subplots, and the two N treatments were sub-subplots (Table 1). Sub-subplot size was 3.8 × 10 m and 6.3 × 6 m for BA-Y1 and BA-Y2, respectively. Sowing