

RESEARCH

Genetic Gain × Management Interactions in Soybean: II. Nitrogen Utilization

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] grain yield has annually increased nearly 23 kg ha⁻¹, but the interaction of genetic advancement and improved agronomic practices has not been well quantified, including N utilization and fertilization. A field study with soybean cultivars released from 1923 to 2008 in maturity group (MG) II and MG III was conducted in multiple environments with a nonlimiting supply of fertilizer N to examine the main effects and interactions of N supply and release year on grain yield and seed quality. We hypothesized that grain yield and seed quality would be improved with the nonlimiting supply of N, especially for the modern cultivars. Supplemental N totaled 560 kg N ha⁻¹ with 40% applied at planting and 60% applied at V5. Grain yield increased with release year in MG II (17.2 kg ha⁻¹ yr⁻¹). Application of N to MG II cultivars increased seed protein by 10 to 19.5 g kg⁻¹ across all release years, but grain yield and seed oil was not affected. Grain yield gains of MG III cultivars fertilized with N was 27.4 kg ha⁻¹ yr⁻¹, which was 20% better than unfertilized (22.8 kg ha⁻¹ yr⁻¹). Application of N to MG III cultivars increased seed mass (11%) across release years with no changes in seed protein and oil. The nonlimiting supply of N increased seed protein across all release years in MG II cultivars, and the N supply from the soil and biological N fixation was insufficient to maximize grain yield in modern, MG III cultivars in the tested environments.

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Abbreviations: BNF, biological N fixation; ESN, Environmentally Smart Nitrogen; MG, maturity group; NASS, National Agricultural Statistics Service; nonnod, nonnodulating; OM, organic matter.

FROM 1924 to 2011, annual on-farm soybean yields in the United States have increased 23.4 kg ha⁻¹ on average (USDA-NASS, 2011). The yield increases are attributed to genetic improvement and grower implementation of improved agronomic practices (Specht et al., 1999; Specht and Williams, 1984) as well as increased atmospheric CO₂ levels (Specht et al., 1999). The impact of genetic improvement on yield gains can be measured by growing cultivars released over time together in yield tests. Cultivars from approximately the 1920s to the 1970s annually increased yield 9.3 kg ha⁻¹ within maturity groups (MGs) 000 to 0 (Voldeng et al., 1997), 12.5 kg ha⁻¹ within MGs 00 to IV (Specht and Williams, 1984), 16.1 kg ha⁻¹ within MGs I to IV (Luedders, 1977), 14 kg ha⁻¹ within MGs V to VII (Ustun et al., 2001), and 13.7 kg ha⁻¹ within MGs VI to VIII (Boerma, 1979). More recent (1971 to 1974) MG II and III cultivars yielded 25% (11.7 kg ha⁻¹ annually) more than

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older (1923 to 1932) cultivars (Wilcox et al., 1979). Rowntree et al. (2013) recently reported annual genetic yield gains of 18.5 kg ha⁻¹ in MG II and 22.7 kg ha⁻¹ in MG III from cultivars released in the 1920s to 2000s and planted in May.

Genetic improvements of soybean have increased yield through changes in seed quality and physiology across release years. Soybean yield and oil increased as protein concentration decreased with the advancement of soybean cultivars over time (Rowntree et al., 2013; Wilcox et al., 1979). Boerma (1979), Morrison et al. (2000), and Voldeng et al. (1997) reported seed mass was not well correlated with cultivar release year, but Specht and Williams (1984) noted an annual increase in seed mass (0.10 g per 100 seeds). In contrast to the results of Specht and Williams (1984), Morrison et al. (2000) speculated that soybean yield increases were from more seeds per plant rather than increases in seed size.

Improved pest resistance (Heatherly and Elmore, 2004; Johnson, 1987), decreased pod shattering (Heatherly and Elmore, 2004; Johnson, 1987; Specht et al., 1999), and decreased lodging (Luedders, 1977; Ustun et al., 2001; Voldeng et al., 1997) are a few specific genetic improvements to soybean and likely contributed to yield increases. Agronomic technique, technology, and practices have also contributed to soybean yield gain such as earlier planting dates, narrower row spacing (Heatherly and Elmore, 2004; Johnson, 1987; Specht et al., 1999), higher seeding rates (Heatherly and Elmore, 2004; Johnson, 1987), and improved weed control (Heatherly and Elmore, 2004; Johnson, 1987; Pike et al., 1991; Specht et al., 1999). Although many practices were widely adopted by growers, yield contributions from agronomic changes relative to genetic changes have not been estimated.

In corn (*Zea mays* L.), many specific practices or traits such as adoption of single cross hybrids, upright leaf angle, and increased tolerance to higher plant densities contributed to yield increases in the last century (Duvick, 2005). From 1907 to 2011 annual on-farm corn yield gains were approximately 72.6 kg ha⁻¹ (USDA-NASS, 2011). This rate is approximately three times greater than that of soybean on an absolute basis. A potential underlying cause for this difference in rates of yield gain for these two crops is their N requirements. Soybean (0.029 g N) is estimated to require nearly three times more N than corn (0.011 g N) per gram of photosynthate to produce grain biomass (Sinclair and de Wit, 1975).

Nitrogen is needed for normal plant functions where it is metabolized and used in constructing amino acids, proteins, and lipids (Sinclair and de Wit, 1975). Soybean is usually grown without N fertilizers, providing the soil or seed was inoculated with *Bradyrhizobium japonicum* to promote biological N fixation (BNF). This symbiotic relationship typically supplies 25 to 75% of the N for

developing soybean plants with the remaining N supplied by the soil (Varco, 1999). Improved N utilization may be a contributing factor to improved soybean yields. A greater demand is placed on BNF to supply more N as soybean yields continue to increase.

In a review of N fertilization rate, method, and timing in soybean, yields increased 50% of the time with an average yield increase of 520 kg ha⁻¹ from responsive sites (Salvagiotti et al., 2008). Nitrogen fertilization may be necessary to maximize soybean yield potential in high-yielding conditions (>4500 kg ha⁻¹) (Salvagiotti et al., 2009; Wesley et al., 1998) where ample moisture was present to facilitate nutrient uptake (Harper, 1974). This is because soil N and BNF may not fully meet the N requirement of soybean under high yielding conditions. Nitrogen application to soybean before planting (Bhangoo and Albritton, 1976; Ferguson et al., 2000; Osborne and Riedell 2006; Salvagiotti et al., 2009) and during mid to late reproductive development (Afza et al., 1987; Harper, 1974; Salvagiotti et al., 2009; Wesley et al., 1998) has been shown to increase yields from 2 to 26%.

As stated by Salvagiotti et al. (2008), N application does not always provide a yield benefit. Soil applications of N provided no yield advantage regardless of N source including NH₄NO₃ (ammonium nitrate) (Welch et al., 1973), NaNO₃ (sodium nitrate) (Deibert et al., 1979), (NH₄)₂SO₄ (ammonium sulfate) (Chesney, 1973; Pal and Saxena, 1976), CO(NH₂)₂ (urea) (Flavio et al., 2003; Schmitt et al., 2001), or polymer-coated CO(NH₂)₂ (Schmitt et al., 2001). Application methods (broadcast, sidedress, or banding), timing (planting or R1 through R5) (Fehr and Caviness, 1977), and rates (0 to 200 kg ha⁻¹) did not improve soybean yields (Chesney, 1973; Deibert et al., 1979; Flavio et al., 2003; Hoeft et al., 2000; Pal and Saxena, 1976; Varco, 1999). Additionally, N fertilization was unnecessary under adequate soil and environmental conditions (pH, soil moisture, and/or organic matter [OM]) (Hoeft et al., 2000; Varco, 1999).

Data from the USDA-National Agricultural Statistics Service (NASS) provides an estimate of combined genetic, cultural, and environmental contributions to yield. The previously mentioned studies quantified yield effects from genetic improvement and agronomic management individually. High-yielding soybean has been noted to respond to additional N, which could imply that residual soil N sources and BNF did not meet the N demands of modern cultivars. In this study, we provided a nonlimiting supply of N to MG II and MG III cultivars released from 1923 to 2008 to examine the main effects and interactions of N supply and release year on soybean yield and seed quality. We hypothesized that the N needs of modern cultivars are not being satisfied by residual soil N sources and BNF, and thus, seed yield and seed quality would be improved simultaneously when N supply was not limiting.

Table 1. Growing location, soil classification, planting dates, and harvest dates for 2010 (Wisconsin, Minnesota, Illinois, and Indiana) and 2011 (Wisconsin, Illinois, and Indiana) growing seasons.

State	Wisconsin	Minnesota	Illinois	Indiana
Research Site	Arlington Agricultural Research Station	Southern Research and Outreach Center	Crop Sciences Research and Education Center	Throckmorton Purdue Agricultural Center
Location	Arlington, WI 43°18' N, 89°20' W	Waseca, MN 44°4' N, 93°31' W	Urbana, IL 40°3' N, 88°14' W	Lafayette, IN 40°17' N, 86°54' W
Soil classification	Plano silt loam	Webster clay loam	Flanagan silt loam and Drummer silty clay loam	Throckmorton silt loam
2010	Fine-silty, mixed, superactive, mesic Typic Argiudoll	Fine-loamy, mixed, superactive, mesic Typic Endoaquoll	Fine-silty, mixed, superactive, mesic Typic Endoaquoll	Fine-silty, mixed, superactive, mesic mollic Oxyaquic Hapludalf
2011	Fine-silty, mixed, superactive, mesic Typic Argiudoll	n/a [†]	Fine, smectitic, mesic Aquic Argiudoll	Fine-silty, mixed, superactive, mesic mollic Oxyaquic Hapludalf
2010 previous crop	Corn (silage)	Corn	Corn	Corn
2011 previous crop	Corn (silage)	n/a	Corn	Corn
Planting (2010)	4 May	18 May	15 May	10 May
Planting (2011)	5 May	n/a	12 May	17 May
Harvest (2010)	12 Oct.	15 Oct.	27 Sept.	24 Sept.
Harvest (2011)	7 Oct.	n/a	11 Oct.	10 Oct.

[†]n/a, not applicable.

MATERIALS AND METHODS

The experiment was conducted at the same fields with the same cultivars as the companion study by Rowntree et al. (2013) with the addition of Waseca, MN, during 2010. Fifty-nine MG II cultivars (released from 1928 to 2008) were planted at the Minnesota and Wisconsin locations and 57 MG III cultivars (released from 1923 to 2007) were planted at the Illinois and Indiana locations. Cultivars were planted at a rate of 370,650 seeds ha⁻¹ in each plot, which were four 76-cm rows wide by 4.6 m long. Information for growing site, soil texture, harvest and planting dates, and previous crop information is presented in Table 1. Cultivars were randomly placed in two adjacent blocks and fertilized with 560 kg N ha⁻¹ as CO(NH₂)₂ and polymer-coated CO(NH₂)₂ (Environmentally Smart Nitrogen [ESN]; Agrium Ltd. of Calgary) or without fertilizer N supply as a control treatment. Nitrogen was split into two broadcast applications with 224 kg N ha⁻¹ [112 kg N as CO(NH₂)₂ and 112 kg N as ESN] at planting and 336 kg N ha⁻¹ at V5 [168 kg N as CO(NH₂)₂ and 168 kg N as ESN]. Urea was treated with the urease inhibitor, N-(n-butyl)thiophosphoric triamide (Agrotain) (Agrotain International), for the V5 application at a rate of 4.2 mL kg⁻¹. Nonnodulating (nonnod) isolines developed from the nodulating cultivars were planted in 2011 to assess soil N supply and BNF in comparison to its original cultivars. Harosoy 63 (MG II released in 1963) and the nonnod isoline of it were planted in Wisconsin. Williams (MG III released in 1971) and the nonnod isoline of it were planted in Illinois and Indiana. The nonnod Harosoy 63 and nonnod Williams were used for soil N evaluation only and were not included in the statistical analysis. The following equation by Weber (1966) was used to determine the N contribution of BNF to grain yield at each location:

yield contribution from BNF =

$$1 - \frac{\text{grain yield of nonnod isoline (unfertilized)}}{\text{grain yield of nodulating line (unfertilized)}}$$

Early and harvest plant stands, lodging before harvest, grain yield (adjusted to moisture of 130 g kg⁻¹), seed mass (100-seed weight), and seed quality were collected as described by Rowntree et al. (2013). Grain subsamples (approximately 500 g) were collected from each plot to determine seed protein and oil with a Perten DA 7200 Feed Analyzer (Perten Instruments). Soil samples were collected at planting to assess soil fertility (0 to 20 cm) and residual N (0 to 30 and 30 to 60 cm) (Table 2).

Statistical analysis was similar to Rowntree et al. (2013), which included the mixed-effect regression analysis using PROC MIXED procedure in SAS version 9.2 (SAS Institute,

Table 2. Soil N, K, P, organic matter, and pH information for Wisconsin, Minnesota, Illinois, and Indiana locations in 2010 and Wisconsin, Illinois, and Indiana locations in 2011.

Soil fertility	Wisconsin		Minnesota		Illinois		Indiana	
	2010	2011	2010	2011	2010	2011	2010	2011
P, mg kg ^{-1†}	44	56	32	23	34	66	39	
K, mg kg ^{-1†}	173	166	184	122	122	146	138	
pH [‡]	7.1	6.9	7.1	6.1	5.8	6.1	6.0	
Organic matter, g kg ^{-1§}	3.2	3.2	6.3	4.1	3.6	3.0	2.9	
Preplant soil N								
0–30 cm depth								
NH ₄ -N, mg kg ^{-1¶}	4	5	n/a [#]	n/a	4	3	4	
NO ₃ -N, mg kg ^{-1¶}	22	10	n/a	n/a	10	40	10	
30–60 cm depth								
NH ₄ -N, mg kg ^{-1¶}	4	5	n/a	n/a	4	n/a	4	
NO ₃ -N, mg kg ^{-1¶}	12	10	n/a	n/a	5	n/a	7	

[†]Mehlich-3 extraction.

[‡]Sikora buffer method.

[§]Loss of ignition test.

[¶]KCl extraction.

[#]n/a, not applicable.

Table 3. Mean monthly air temperature, total monthly precipitation, and 30 yr averages (1980–2010) at Wisconsin, Minnesota, Illinois, and Indiana during the 2010 and Wisconsin, Illinois, and Indiana 2011 growing seasons.

	Wisconsin			Minnesota		Illinois			Indiana		
	2010	2011	30 yr.	2010	30 yr.	2010	2011	30 yr.	2010	2011	30 yr.
Air temperature	°C										
Apr.	10.4	6.2	7.1	11.7	7.7	15.1	11.9	11.1	14.9	11.6	10.7
May	15.3	13.4	13.2	15.1	14.7	18.3	16.9	16.9	18.1	17.1	16.6
June	19.7	19.6	18.7	19.3	20.1	23.8	22.8	22.3	23.3	22.6	21.8
July	22.9	24.0	20.8	22.5	22.1	25.2	26.8	23.8	24.4	26.0	23.4
Aug.	22.2	21.0	19.6	22.9	20.7	25.1	24.1	23.0	24.3	22.7	22.4
Sept.	15.6	14.5	15.2	15.2	16.1	19.7	17.5	19.0	19.4	17.1	18.8
Oct.	11.1	11.1	8.6	10.6	8.9	13.9	12.7	12.2	13.2	12.7	12.1
Precipitation	mm										
Apr.	107.5	106.4	88.9	84.3	82.9	48.5	214.6	93.5	72.9	192.6	86.6
May	88.9	55.4	93.7	83.1	100.1	78.5	121.9	124.2	72.6	113.4	117.9
June	169.4	98.8	118.9	327.9	119.4	198.6	106.7	110.2	95.0	92.8	115.6
July	222.8	64.3	105.7	167.9	115.5	90.7	39.9	119.4	66.3	45.5	103.6
Aug.	114.0	39.9	99.1	61.7	99.8	40.1	44.7	99.8	42.2	26.3	100.1
Sept.	50.5	96.5	89.9	383.3	90.8	76.7	70.9	79.5	24.1	82.8	71.2
Oct.	49.8	39.1	64.8	25.9	66.1	27.4	67.1	82.8	12.4	46.0	68.9

2008). Individual models were developed for each MG. In this study, the main effects of release year, N supply, and release year \times N supply were considered fixed effects. The performance of specific cultivars was not the goal of this study, and therefore we used the corresponding release years. Environment, defined as location \times testing year, and the interactions of environment with treatments (release year, N supply, and release year \times N supply) were considered random effects. Fixed effects were tested for significance ($P < 0.05$) using the appropriate F test. Final models for the effects of N and release year on grain yield, seed mass, seed protein, seed oil, and protein yield were a function of the model fit statistics (Akaike information criterion, Bayesian information criterion, and -2 residual log likelihood).

RESULTS AND DISCUSSION

Environment

Environmental conditions for each growing environments were the same as in research by Rowntree et al. (2013). The additional growing environment of Waseca, MN, in 2010 was near the 30-yr average in temperature except higher in August. Precipitation was above the 30-yr average for the growing season at this environment (Table 3).

Grain Yield

Yields increased with cultivar release year within and across both MGs. All cultivars were grown together at a fixed time and space; therefore, the exhibited increase over release year represented the amount of genetic improvement obtained through breeding, adopted agronomic practices, and increased atmospheric CO₂ (Luedders, 1977; Specht and Williams, 1984; Ustun et al., 2001; Voldeng et al., 1997; Wilcox et al., 1979).

Yield of cultivars within MG II were not affected by fertilizer N and increased linearly over release year (17.2 ± 1.5 kg ha⁻¹ yr⁻¹) (Fig. 1A). Our regression analysis showed

that yield increased 1376 kg ha⁻¹ from MG II cultivars released in 1928 compared to 2008 (2286 vs. 3662 kg ha⁻¹). Yield gain for MG II cultivars (17.2 kg ha⁻¹ yr⁻¹) in this study closely aligned with previous reports of 11.2 (Wilcox et al., 1979), 18.5 (Rowntree et al., 2013), and 29.1 (Specht and Williams, 1984) kg ha⁻¹ yr⁻¹ for MG II cultivars. High soil N and OM levels at Minnesota and Wisconsin locations (Table 2) likely supplied sufficient N in addition to BNF to meet the requirements of the unfertilized MG II cultivars in comparison to fertilized cultivars.

In a comparison of nodulating and nonnod isolines, BNF contributed only 23% of needed N for yield development in MG II cultivars. Thus, soil supply of N was abundant (77% contribution to total yield) in Wisconsin during 2011 and additional N was not needed. Previous research has found the process of BNF to be more energetically expensive in comparison to N assimilation (Finke et al., 1982; Minchin et al., 1981; Ryle et al., 1979; Sinclair, 2004). Therefore, soybean grown in conditions with a large supply of N from soil, residual sources, and/or fertilizer will allow more photosynthate to be available for dry matter production and/or protein accumulation rather than expenditure on BNF. These MG II cultivars may also depend less on BNF to supply N than our MG III cultivars and therefore did not differ in yield response to N. Other researchers have also reported soybean yield to be unresponsive to N fertilization (Flavio et al., 2003; Freeborn et al., 2001; Schmitt et al., 2001; Welch et al., 1973).

In contrast, yield within MG III cultivars was influenced by the interaction of N \times release year. The rate of yield gain was greater with fertilized cultivars (27.4 ± 1.6 kg ha⁻¹ yr⁻¹) than unfertilized cultivars (22.8 ± 1.1 kg ha⁻¹ yr⁻¹) (Fig. 1B) showing that current cultivars were

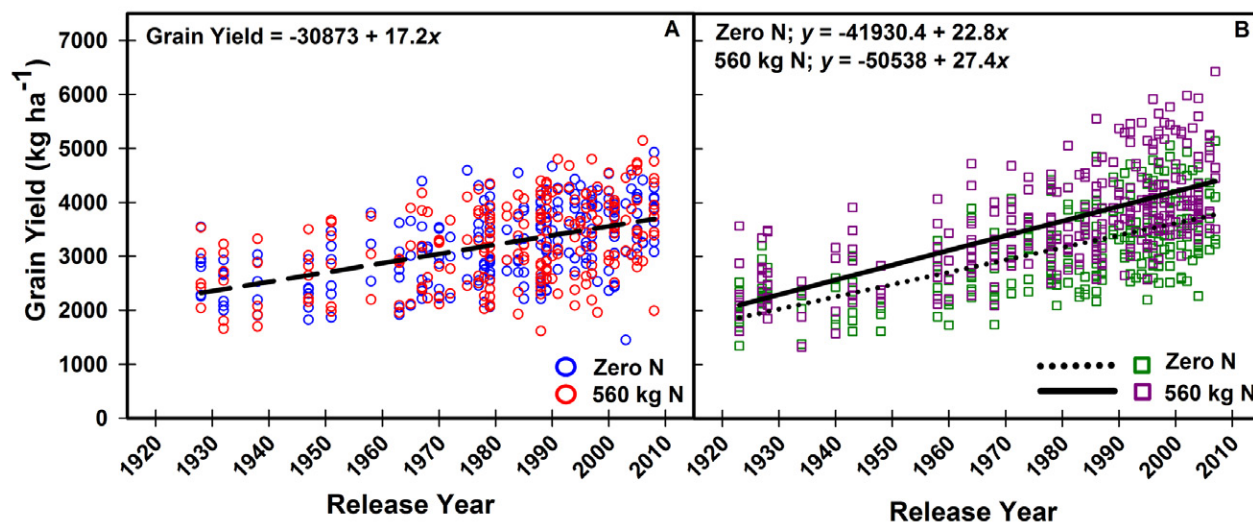


Figure 1. Grain yield (kg ha^{-1}) at 130 g kg^{-1} moisture of (A) maturity group II and (B) maturity group III cultivars released from 1923 to 2008 that were treated with zero N or 560 kg N ha^{-1} in 2010 (Minnesota, Wisconsin, Illinois, and Indiana) and 2011 (Wisconsin, Illinois, and Indiana). (A) Grain yield did not differ between N treatments but increased ($P < 0.01$) with cultivar release year. (B) Grain yield increased with N fertilization ($P < 0.01$), release year ($P < 0.01$), and the release year \times N interaction was significant ($P < 0.01$).

more responsive to additional N than older cultivars. Total yield increased 1915 kg ha^{-1} in unfertilized cultivars and 2302 kg ha^{-1} with fertilized cultivars over the 84 yr represented in the MG III cultivars (Fig. 1B). Yield advantage to fertilized cultivars was 12.4% in the release year of 1923 and 16.3% in 2007 (238 and 624 kg ha^{-1} , respectively). The N supplied from the soils of Illinois and Indiana and BNF provided almost the entire amount of N needed to maximize the yield potential of the older, MG III cultivars. Whereas the supply of N from the soil and BNF did not satisfy the entire N requirement for the modern, MG III cultivars to attain the full yield potential. We observed greater leaf retention in the modern cultivars than the older cultivars, which likely translated into more leaf biomass for photosynthetic activity and remobilization to developing seeds. Kumudini et al. (2001) also reported that 79% of genetic improvement in short season soybean was due to increased biomass accumulation.

Our yield gain over cultivar release years of $22.8 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in unfertilized MG III cultivars was slightly less than the reported USDA-NASS yield increases for Illinois and Indiana of 26.2 and $28.9 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively (USDA-NASS, 2011). Similar to MG II, the implementation of current technologies (e.g., row width, rhizobial inoculation) and growing environment (e.g., atmospheric CO_2 levels) may explain differences between the rates of on-farm yield gain and our reported yield gain. For instance, historical yield data included years with soybean fields that were not inoculated, were planted in wide rows, and were planted later than current practices. This combination created a lower starting point for yield that would increase more dramatically as these techniques were adopted. Our annual increase in MG III cultivars (22.8 kg ha^{-1}) was greater than previous reports of 12.7

kg ha^{-1} (Wilcox et al., 1979) and of 17.2 kg ha^{-1} (Specht and Williams, 1984) but less than the report of 30 kg ha^{-1} from Wilcox (2001). Rowntree et al. (2013) also reported a similar yield gain ($22.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$) for MG III cultivars. Our MG III yield increases (12 to 16%) from N were consistent with previous reports of 5 to 37% from N applications (Afza et al., 1987; Bhangoo and Albritton, 1976; Ferguson et al., 2000; Harper, 1974; Osborne and Riedell, 2006; Salvagiotti et al., 2009; Wesley et al., 1998).

Fertilized cultivars appeared to retain leaves longer than unfertilized cultivars, but measurements were not taken. Increased vegetative biomass and/or longer leaf retention may have led to more net photosynthesis over the duration of the growing season. From the models developed by Sinclair and de Wit (1976), the most efficient way to increase seed-fill period was to allocate a greater proportion of photosynthate for assimilation of additional N. An increase in N supply from the roots or vegetative tissue would potentially lengthen seed-fill duration and increase yields (Sinclair and de Wit, 1976). In contrast, increased photosynthetic rates without an increased N supply caused soybean yields to decrease due to greater demand and translocation of N to the seed that ultimately shortened seed fill duration (Sinclair and de Wit, 1976).

Major differences between MGs were likely due to growing environments and soil N availability. Biological N fixation contributed 61 and 59% (Illinois and Indiana, respectively) of N needed for yield development when nodulating and nonnod isolines were compared. These contributions are more than two times higher than those of MG II (23%) even though the soil N concentrations were similar among Wisconsin, Illinois, and Indiana before planting in 2011 (Table 2). Bhangoo and Albritton

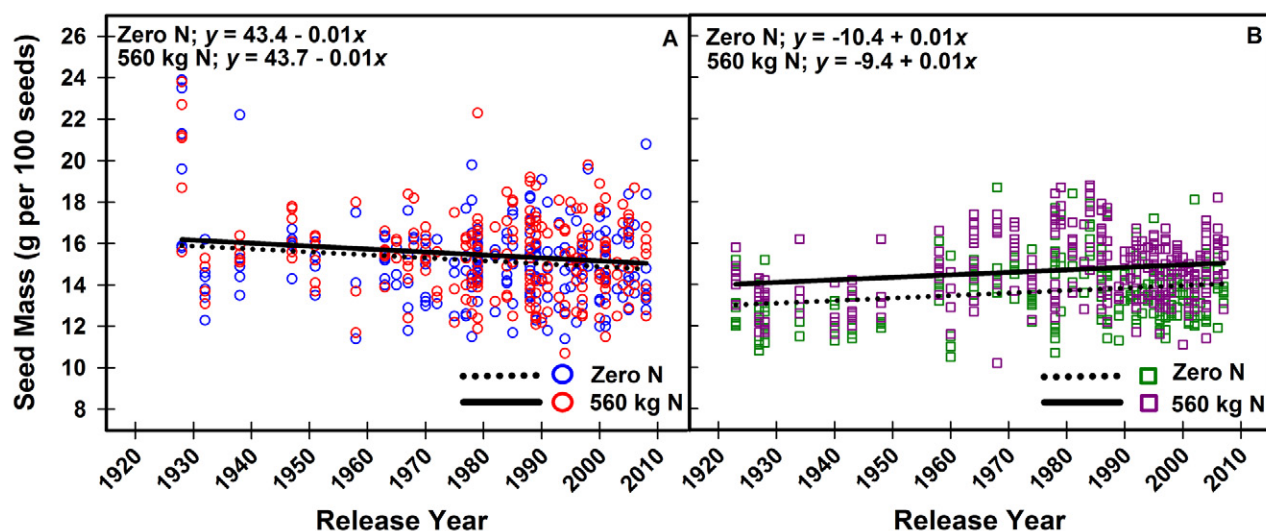


Figure 2. Seed mass (g per 100 seeds) of (A) maturity group II and (B) maturity group III cultivars released from 1923 to 2008 treated with zero N or 560 kg N ha⁻¹ in 2010 (Minnesota, Wisconsin, Illinois, and Indiana) and 2011 (Wisconsin, Illinois, and Indiana). (A) Seed mass decreased with cultivar release year ($P < 0.10$) but increased with N fertilization ($P < 0.01$). (B) Seed mass increased with cultivar release year ($P < 0.10$) and N fertilization ($P < 0.01$).

(1976) reported a 37% increase in yield due to applied N on a soil with about 1 g of OM kg⁻¹. In contrast, Schmitt et al. (2001) reported in-season N fertilization had no effect on soils ranging from medium (1 to 3 g kg⁻¹) to high (>3 g kg⁻¹) in OM. Our data suggest that OM of 2.9 to 4.1 g kg⁻¹ (MG III) (Table 2) was not a limiting factor in expression of yield responses to fertilizer N.

Seed Mass

Numerically, seed mass was affected by release year in both MG II (decreased) and MG III (increased); however, this effect was only marginally significant ($P < 0.10$). The annual rate of seed mass loss in MG II ($\beta = -0.01 \pm 0.007$ g per 100 seeds) and gains in MG III ($\beta = 0.01 \pm 0.007$ g per 100 seeds) did not differ between N treatments (Fig. 2A and 2B). Over the nine decades, total seed mass decreased 7.7% (1.14 g per 100 seeds) in MG II and increased 7.8% (1.02 g per 100 seeds) in MG III. Seed mass was poorly correlated to cultivar release year in previous research (Morrison et al., 2000; Voldeng et al., 1997; Boerma, 1979) but did increase 0.10 g per 100 seeds annually in one study across MGs 00 to IV (Specht and Williams, 1984). Rowntree et al. (2013) also reported MG III cultivars increased seed mass (0.017 g per 100 seeds yr⁻¹) with breeding advancements. Previous researchers have attributed increased grain yields to more seeds per plant via breeding and selection (Morrison et al., 2000) instead of increased individual seed mass.

Nitrogen application increased seed mass less than 1% (0.28 g per 100 seeds) in MG II (Fig. 2A) but nearly 11% (0.99 g per 100 seeds) in MG III (Fig. 2B). Other research reported applications of N increased seed mass by 2% (Salvagiotti et al., 2009) and nearly 4% (Flavio et al., 2003; Ham et al., 1975; Munier-Jolain et al., 1996). Seed mass

was influenced by environment (De Bruin and Pedersen, 2008), planting date (Elmore, 1990), plant density (Ball et al., 2000), and cultivar variability (Robinson et al., 2009; Voldeng et al., 1997; Specht and Williams, 1984). Nitrogen application did not influence seed number ($P = 0.17$) (data not shown), and therefore greater seed mass (approximately 11%) in MG III cultivars was a major driving factor for the positive response in grain yield. The seed mass increase in MG II (<1%) was minimal and did not influence grain yield.

Protein and Oil

The release year \times N interaction was significant for protein in MG II (Fig. 3A). Protein concentration decreased linearly over release years, yet fertilizer N improved protein concentration (Fig. 3A). The rates of decrease differed between unfertilized ($\beta = -0.12 \pm 0.04$ g kg⁻¹ yr⁻¹) and fertilized cultivars ($\beta = -0.24 \pm 0.07$ g kg⁻¹ yr⁻¹) for MG II (Fig. 3A). Protein content decreased a total of 9.6 g kg⁻¹ (unfertilized) and decreased 19.2 g kg⁻¹ (fertilized) across the 80 yr for MG II cultivars. Nitrogen fertilization did not affect seed protein in MG III cultivars (Fig. 3B), but protein decreased across release years ($\beta = -0.25 \pm 0.05$ g kg⁻¹ yr⁻¹). Higher grain yields from modern cultivars produced more protein in given land area (Fig. 4). Nitrogen fertilization did not increase total protein harvest in MG II since N fertilization did not increase yields (Fig. 4A and 1A, respectively). However, N fertilization did increase total protein harvest across release years of MG III cultivars (9.61 ± 0.59 kg ha⁻¹ yr⁻¹) compared to unfertilized cultivars (8.06 ± 0.41 kg ha⁻¹ yr⁻¹) (Fig. 4B). This was due to the increase in grain yield from N fertilization (Fig. 1B).

Wilcox et al. (1979) reported MG II cultivars decreased in protein content (0.071 g kg⁻¹ annually from 1923 to 1974) and protein of MG III cultivars did not change across release years. Those were smaller than the rates calculated

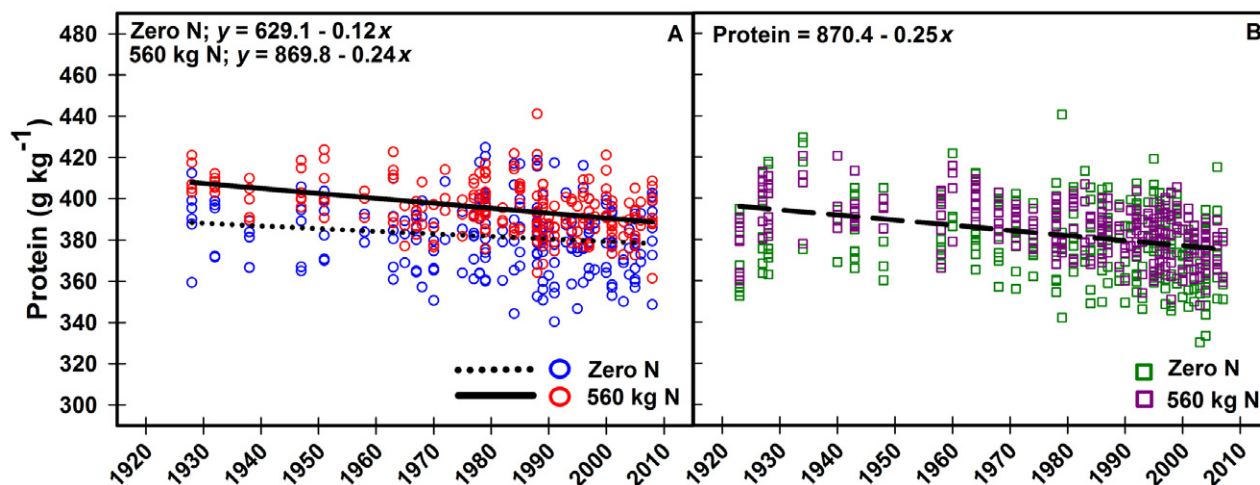


Figure 3. Grain protein concentration (g kg^{-1}) of (A) maturity group II and (B) maturity group III cultivars released from 1923 to 2008 treated with zero N or 560 kg N ha^{-1} in 2010 (Minnesota, Wisconsin, Illinois, and Indiana) and 2011 (Wisconsin, Illinois, and Indiana). (A) Protein increased with N fertilization ($P < 0.01$) but decreased with cultivar release year ($P < 0.01$), and the release year \times N interaction was significant ($P < 0.01$). (B) Protein decreased with cultivar release year ($P < 0.01$) and N fertilization had no effect.

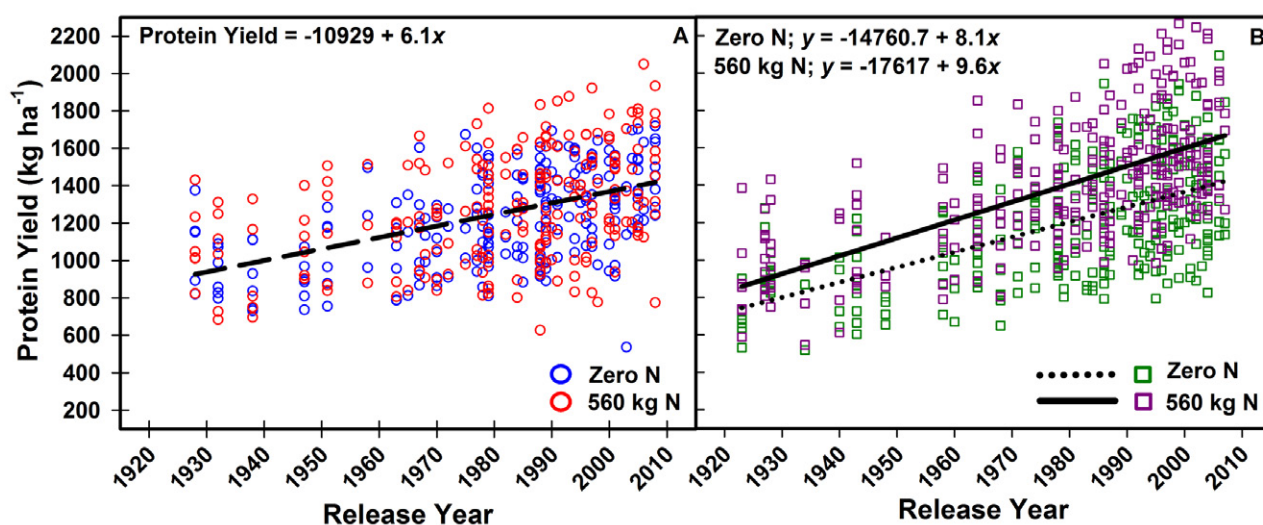


Figure 4. Protein yield (kg ha^{-1}) of (A) maturity group II and (B) maturity group III cultivars released from 1923 to 2008 treated with zero N or 560 kg N ha^{-1} in 2010 (Minnesota, Wisconsin, Illinois, and Indiana) and 2011 (Wisconsin, Illinois, and Indiana). (A) Protein yield increased with cultivar release year ($P < 0.01$). (B) Protein yield increased with cultivar release year ($P < 0.01$) and N fertilization ($P < 0.01$) and the release year \times N interaction was significant ($P < 0.01$).

in our study. However, Wilcox et al. (1979) was limited to five cultivars per MG across 51 release years (1923 to 1974), and our study included nearly 60 cultivars per MG and spanned 85 release years (1923 to 2008). Our grain protein results align with those reported by Rowntree et al. (2013) for MG II ($-0.19 \text{ g kg}^{-1} \text{ yr}^{-1}$) and MG III ($-0.24 \text{ g kg}^{-1} \text{ yr}^{-1}$) cultivars.

Fertilizer N did not influence oil concentration in either MG. Oil concentration increased linearly in MG II ($P < 0.01$) and MG III ($P < 0.01$) over release years (data not shown). Annual rates of oil gain were $0.13 \pm 0.03 \text{ g kg}^{-1}$ in MG II and $0.12 \pm 0.03 \text{ g kg}^{-1}$ in MG III. Rowntree et al. (2013) reported an annual increase in grain oil of 0.142 g kg^{-1} and 0.127 g kg^{-1} for MG II and III cultivars, respectively.

CONCLUSIONS

Across nearly nine decades, MG II cultivars appeared to use the fertilizer N for seed protein synthesis rather than gains in grain yield. Conversely, MG III cultivars utilized the nonlimited supply of N to increase total protein yield (kg ha^{-1}) and grain yield (i.e., dry matter) while BNF plus soil N sources were not sufficient to attain the full potential in grain yield. More supply of N may have fostered greater dry matter accumulation and lengthened the seed-fill period, which may explain the observed increases in seed mass of MG III cultivars. Increasing N availability to soybean from exogenous N applications, rhizobial relations, and/or breeding efforts could unlock advances in grain yield and seed quality. These efforts should simultaneously

evaluate the effects on photosynthetic activity, seed-fill period, grain yield components, and seed composition.

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