Review

Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars

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ABSTRACT

Crop biomass production is a function of the efficiencies with which sunlight can be intercepted by the canopy and then converted into biomass. Conversion efficiency has been identified as a target for improvement to enhance crop biomass and yield. Greater conversion efficiency in modern soybean [*Glycine max* (L.) Merr.] cultivars was documented in recent field trials, and this study explored the physiological basis for this observation. In replicated field trials conducted over three successive years, diurnal leaf gas exchange and photosynthetic CO_2 response curves were measured in 24 soybean cultivars with year of release dates (YOR) from 1923 to 2007. Maximum photosynthetic capacity, mesophyll conductance and nighttime respiration have not changed consistently with cultivar release date. However, daily carbon gain was periodically greater in more recently released cultivars compared with older cultivars. Our analysis suggests that this difference in daily carbon gain primarily occurred when stomatal conductance and soil water content were high. There was also evidence for greater chlorophyll content and greater sink capacity late in the growing season in more recently released soybean varieties. Better understanding of the mechanisms that have improved conversion efficiency in the past may help identify new, promising targets for the future.

Key-words: *Glycine max*; conversion efficiency; photosynthesis; respiration; stomatal conductance.

INTRODUCTION

Soybean is an important source of protein for food and feed throughout the world and is second only to maize in planted area in the USA (FAOSTAT 2013, http://faostat3.fao.org 2013). The annual rate of genetic and on-farm soybean yield improvement is now faster than it was 40 years ago (Rincker *et al.* 2014; Specht *et al.* 2014), and soybean seed yields do not appear to be stagnating in most regions of the USA

(Ray *et al.* 2012; Rowntree *et al.* 2013). Still, the current rate of yield gain is thought to be insufficient to meet growing demand and the United Nation's 2050 target of doubling crop yields (Ray *et al.* 2013). Climate change further challenges yield progress as environmental stressors such as increased heat, drought and vapour pressure deficit negatively impact crop production (Lobell *et al.* 2014; Ort & Long 2014). Therefore, there is a need to strategically evaluate targets for improvement, which may be informed by a better understanding of the physiological basis for past improvements in crop yield.

Analysis of historical soybean germplasm has documented that breeders have increased yields by improving plant harvest index (HI), the seed fraction of all aboveground biomass in terms of weight or energy, and to a lesser extent, seasonal canopy light interception and seasonal conversion efficiency, the effective utilization of solar energy to produce plant biomass (Morrison *et al.* 1999; Koester *et al.* 2014). The historical improvements in HI and canopy light interception efficiency have put these two physiological yield components close to their proposed theoretical limits (Hay 1995; Zhu *et al.* 2010). Conversion efficiency, on the other hand, is below the estimated theoretical limit for photosynthetic CO_2 fixation and sensitive to environmental variables (Slattery *et al.* 2013); thus is a proposed target for sustaining future improvement in crop productivity (Gifford & Evans 1981; Zhu *et al.* 2010).

Recent reviews have proposed that improving conversion efficiency through greater photosynthetic carbon fixation could provide the bump in crop performance needed to sustain and improve yields for a growing population (von Caemmerer & Evans 2010; Zhu *et al.* 2010; Parry *et al.* 2011; Raines 2011). While our previous work with historical soybean germplasm showed that long-term selection for greater seed yield has improved conversion efficiency (Koester *et al.* 2014), the mechanistic basis for the enhancement in conversion efficiency is not known. There are different possibilities for how breeding may have improved conversion efficiency, which have consequences for resource-use efficiency by the plant. One possibility is that breeding has selected for increased investment in Rubisco and other limiting enzymes of photosynthesis, which would require additional nitrogen (Jin *et al.* 2010). Another possibility

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is that selection has resulted in greater stomatal conductance (g_s) to CO_2 and water vapour, allowing modern lines to have greater photosynthetic and transpiration rates when water is in plentiful supply (Fisher *et al.* 1998). In Canadian and Chinese soybean germplasm, increased leaf-level photosynthesis has accompanied genetic yield improvement (Morrison *et al.* 1999; Jin *et al.* 2010; Liu *et al.* 2012), and in wheat, historical improvements in yield have been associated with greater g_s as well as greater intrinsic sink strength (Fischer *et al.* 1998) and greater leaf chlorophyll content (Sadras *et al.* 2012).

This study provides an analysis of how photosynthesis has been affected by breeding for seed yield improvement in US soybean germplasm. In replicated field trials conducted over 3 years (2011–2013), we took over 4000 measurements of leaf gas exchange in 24 soybean cultivars of Midwestern US adaptation with year of release dates (YOR) spanning from 1923 to 2007 (Table 1). We sought to understand the mechanisms driving the improvements in conversion efficiency by investigating if daily carbon gain has increased with soybean YOR, and how photosynthetic capacity (i.e. the investment in Rubisco protein activity and electron transport capacity), dark respiration rate, leaf-level chlorophyll and starch content have been altered by 84 years of soybean genetic yield improvement.

MATERIALS AND METHODS

Experimental field design

Research was conducted at the Crop Research and Education Center in Urbana, IL (40°N, 88°14′W), in 2011, 2012 and 2013.

Twenty-four indeterminate, maturity group III soybean cultivars were chosen to represent the past 84 years of soybean breeding (Table 1). Soybeans were grown in replicated plots as previously described in Koester *et al.* (2014). In 2011, the experimental plots were not thinned to a uniform density, and so data from 2011 were not used to calculate light interception efficiency, conversion efficiency, HI or seed yield. Plots were thinned to a uniform density in 2012 and 2013. Daily meteorological data including solar radiation (S_t, Fig. S1a–c), temperature (Fig. S1d–f) and precipitation (Fig. S1g–i) were collected approximately 1.5 km from the field site by the Illinois Climate Network monitoring station (Angel 2009). Plots were irrigated using drip-line tubing four times during the 2012 season to relieve water stress (Fig. S1h).

Gas exchange and chlorophyll fluorescence measurements

Diurnal measurements of gas exchange were conducted on 14 d across the 2011–2013 growing seasons. Over each diurnal period, leaf CO_2 and water vapour exchange were measured approximately every 2–3 h using infrared gas analysers (LI-6400, Li-Cor, Lincoln, NE), which controlled temperature, the photosynthetic photon flux density (PPFD), CO_2 concentration and relative humidity in the cuvette. Temperature and PPFD were held at ambient conditions measured immediately before each time-point and were kept constant throughout each time-point. Ambient PPFD was measured with a quantum line sensor (LP-80,

Table 1. List of maturity group III soybean cultivars investigated in these studies, with their respective year of release and plant introduction (PI) number

Cultivar	Year of Release	PI number
Dunfield	1923	PI548318
Illini	1927	PI548348
AK (Harrow)	1928	PI548298
Mandell	1934	PI548381
Lincoln	1943	PI548362
Adams	1948	PI548502
Ford	1958	PI548562
Shelby	1958	PI548574
Ross	1960	PI548612
Adelphia	1964	PI548503
Wayne	1964	PI548628
Calland	1968	PI548527
Williams	1971	PI548631
Woodworth	1974	PI548632
Zane	1984	PI548634
Private 3-2	1986	n/a
Resnik	1987	PI534645
Private 3-9	1989	n/a
Private 3-19	1994	n/a
Private 3-11	1996	n/a
IA 3010	1998	n/a
IA 3023	2003	n/a
Private 3-13	2004	n/a
Private 3-14	2007	n/a

n/a, not available.

Li-Cor), and temperature was measured using the thermocouple within the cuvette of the infrared gas analyser. The concentration of CO_2 in the reference cuvette was set at 400 ppm, and relative humidity was adjusted to 60–65%. Two to three sunlit, fully expanded leaves from different plants in each cultivar plot were measured at each time-point. Leaf photosynthesis (A), stomatal conductance (g_s) and intercellular concentration of CO_2 (c_i) were calculated using the equations of von Caemmerer & Farquhar (1981). The total daily CO_2 uptake (A') was calculated from the instantaneous A measurements by summing the trapezoidal area under the curve. When dew was present on the leaves during the morning time-points, the leaves were dried before gas exchange was measured. During the time-points where dew was present, g_s was not calculated to avoid over estimates arising from residual moisture on the leaf surface.

Maximum rates of Rubisco carboxylation ($V_{c,max}$) and electron transport (J_{max}) were estimated at two stages of reproductive growth (flowering and pod fill) by measuring A at 12 CO_2 concentrations. In 2012 and 2013, measurements of chlorophyll fluorescence were made at the same time as gas exchange using a leaf chamber fluorometer (Li-6400-40 LCF, Licor). Petioles were excised under water from two to three plants per plot pre-dawn and were cut again under water and transported to the laboratory for gas exchange measurements. This protocol, used previously for soybean (e.g. Ainsworth *et al.* 2004; Bernacchi *et al.* 2005; Sun *et al.* 2014), ensures that transient decreases in water potential, decreases in chloroplast inorganic phosphate concentration, and decreases in maximum photosystem II (PSII) efficiency do not affect estimates of the photosynthetic capacity of plants. The trifoliates remained in the dark until 20 min before measurement when the leaves were illuminated to adapt to light conditions. CO_2 concentration in the reference cuvette was initially 400 ppm and was reduced stepwise to 50 ppm. Thereafter, the reference CO_2 concentration was restored to 400 ppm and increased stepwise to 2000 ppm as described in Ainsworth *et al.* (2002). The measurements were made at 25 °C, PPFD of 1500 μmol m^{-2} s^{-1} in 2011 and 1750 μmol m^{-2} s^{-1} in 2012 and 2013. Chlorophyll fluorescence was measured at the same time, and the quantum yield of PSII (Φ_{PSII}) was determined by measuring steady-state fluorescence (F_s) and maximum fluorescence (F_m) during a light-saturating pulse (~8000 μmol m^{-2} s^{-1}), where $\Phi_{PSII} = (F_m - F_s)/F_m$ (Genty *et al.* 1989). The rate of electron transport (J_F) was calculated as $J_F = \alpha\beta$ PPFD Φ_{PSII}, where PPFD is the photosynthetic photon flux density (1750 μmol m^{-2} s^{-1}), α is the leaf absorbance (0.85), and β is the partitioning of photons between PSII and PSI (0.5).

Photosynthetic parameters were calculated by fitting the equations of Farquhar *et al.* (1980) and by creating an A/c_i response plot as described in Farquhar & Sharkey (1982). $V_{c,max}$ was fit using the points that fell below the inflexion point of the A/c_i plot and J_{max} determined from the points above the inflexion point. Mesophyll conductance (g_m) was estimated using the constant J method (Harley *et al.* 1992) following the approach of Sun *et al.* (2014). Estimates of g_m were used to convert values of c_i to c_c, where c_c is the CO_2 concentration in the

chloroplast. The parameters $V_{c,max}$ and J_{max} were re-estimated from A/c_c curves. In 2011, chlorophyll fluorescence was not measured, so average values of g_m for the two reproductive growth stages based on 2012 and 2013 were used to estimate c_c. Estimates of $V_{c,max}$ and J_{max} based on A/c_c curves are reported.

Nighttime rates of leaf respiratory CO_2 efflux were measured on two dates (flowering and pod fill) in the 2012 and 2013 growing seasons. Measurements were made on attached mature leaves at the top of the canopy from 2200 to 0100 h using a custom built chamber designed for the LI-6400 gas exchange system (Li-Cor) as described in Gillespie *et al.* (2012). Trifoliates were sealed in the chamber and allowed to equilibrate for approximately 5–7 min until the relative humidity within the chamber was between 60% and 65%, and steady rates of CO_2 efflux were obtained.

Leaf chlorophyll and starch measurements

In 2012 and 2013, leaf samples were taken from three plants per genotype during the R2 and R5 developmental stages, corresponding to measurements of photosynthetic potential. In 2012, leaf discs were taken pre-dawn, at midday, and dusk, and in 2013 at dawn and dusk. Leaf discs (1.4 cm^2 dia.) were excised from fully expanded leaves at the top of the canopy and immediately plunged into liquid N, then stored at −80 °C. Chlorophyll was extracted in 80% (v/v) buffered ethanol (2 mM HEPES, pH 7.8). Extracts were measured at 470, 649 and 665 nm using a spectrophotometer (Synergy 2 Microplate Reader, BioTek Instruments), and chlorophyll content was quantified using the equations of Porra *et al.* (1989). Leaf discs were then ground in liquid N, solubilized by heating to 95 °C in 0.1 M NaOH, and acidified to pH 4.9. Starch was converted to glucose by overnight incubation with exoamylases and endoamylases, and starch content was determined from glucose equivalents using a continuous enzymatic substrate assay previously described (Hendriks *et al.* 2003).

Modelling soil moisture and statistical analysis

Soil volumetric water content was modelled using the Soil Temperature and Moisture Model (STM^2; USDA-ARS; http://www.ars.usda.gov/services/software/download.htm?softwareid=209). The model predicts soil temperature and moisture conditions based on soil type, incident solar radiation, maximum and minimum temperatures, and precipitation. The soil type at our site was a silt loam at the surface with a silty clay loam beneath. Weather parameters used in the model were collected from the Illinois Climate Network monitoring station, and soil moisture data were averaged for the top 30 cm of the soil profile.

Correlations between variables and cultivar YOR were tested for significance using least square regression (PROC MIXED, SAS version 9.2, SAS Institute Inc. Cary, NC, USA) or first order linear regression (SigmaPlot, Systat Software, Inc., Richmond, CA, USA). A student's t-test was used to determine if average A and g_s values were significantly different among the five oldest and five most recently

released cultivars. Correlation matrices for photosynthetic, biomass and yield traits were constructed using R (R Foundation for Statistical Computing, Vienna, Austria). Outliers were detected using PROC UNIVARIATE (SAS version 9.2, SAS Institute Inc., Cary, NC, USA) by testing if the residuals determined from linear regression fell outside the 95% confidence interval.

RESULTS

Diurnal gas exchange and cultivar year of release

Instantaneous rates of carbon uptake (A) were measured from dawn to dusk on 14 d throughout the 2011, 2012 and 2013 growing seasons allowing us to calculate the daily integral carbon gain of a leaf (A'). On eight of the 14 d, there was a significant linear increase in A' with cultivar YOR (Fig. 1). On days where a significant trend of increased A' was observed, more recently released cultivars had on average 12% greater daily carbon uptake than older cultivars (Fig. 1). The gain was similar across all 3 years with the exception of the last measurement day in 2013

(Fig. 1n). On this date, older cultivars were in the early stages of senescence and had significantly lower rates of photosynthesis and daily C gain, and therefore the increase in A' with YOR (i.e. the slope of the linear correlation) was greater (Fig. 1n).

In order to examine diurnal trends in A, we compared the five oldest and five most recently released soybean cultivars (Figs 2, S2 & S3). Modern cultivars had greater rates of A primarily in the early afternoon when A often peaked (Figs 2f–j, S2f–j & S5e–h). During these periods, newer cultivars had up to 23% greater rates of A than older cultivars. Although the increases in A at an individual time-point were often insignificant, the cumulative difference across the day led to significant increases in A' with cultivar YOR. Across the 3 years of study, diurnal A and A' did not vary consistently with plant developmental stage. In 2011, A' was significantly lower in R5 and R6 (Fig. 1d,e), likely because of dry conditions. However, in 2012 and 2013, A and A' were relatively consistent across the growing season (Figs 1, 2 & S3).

Daily patterns of stomatal conductance (g_s) and intercellular [CO_2] (c_i) were also compared across the three
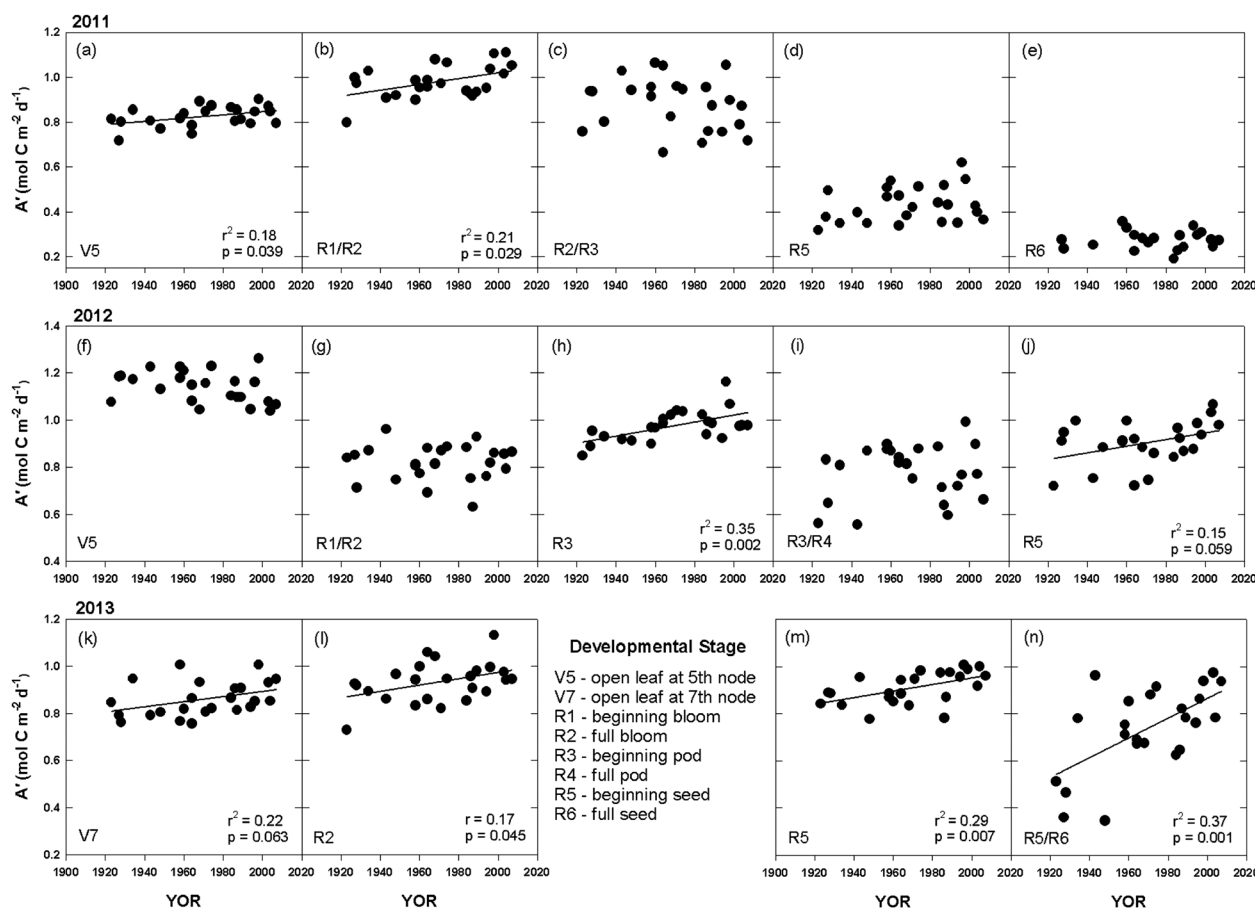

Figure 1. The daily carbon uptake (A') plotted against cultivar YOR for 2011 (a–e), 2012 (f–j) and 2013 (k–n), with the successive soybean developmental stage (Fehr *et al.* 1971) at each of the measurement dates given at the bottom left of the panel. The modelled soil volumetric water content on each day is shown, except for the last day of 2013 when historic soybean cultivars were in very different stages of senescence. For those panels in which least squares linear regression between A' and YOR was significant ($P < 0.1$), the coefficient of determination (r^2) and P are provided.

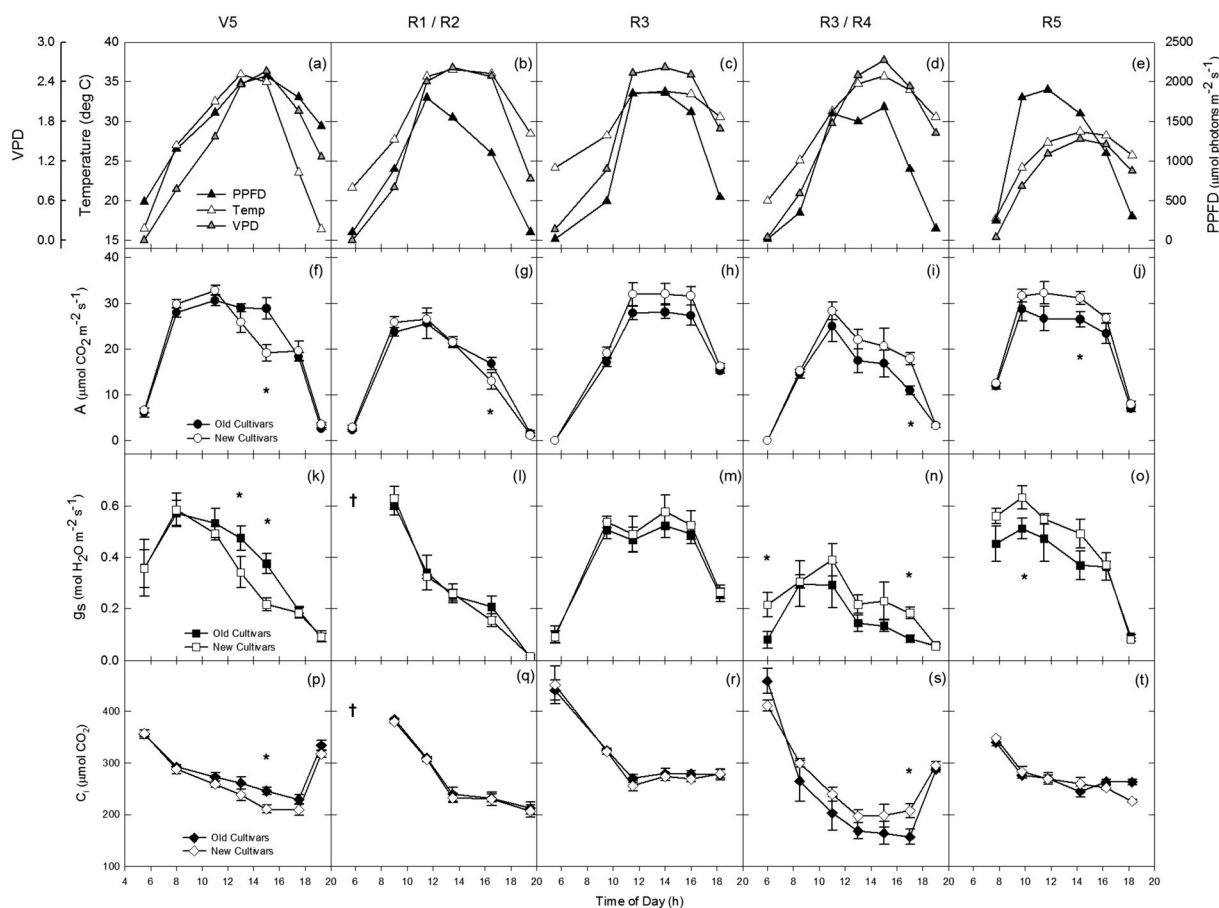© 2016 John Wiley & Sons Ltd, *Plant, Cell and Environment*, **39**, 1058–1067

Figure 2. Diurnal air temperature (open triangles), photosynthetic photon flux density (PPFD, closed triangles), vapour pressure deficit (VPD, grey triangles; a–e), average assimilation rate (A) (f–j), stomatal conductance (g_s) (k–o) and intercellular [CO_2] (c_i) (p–t) of the five oldest cultivars (closed symbols) and five most recently released cultivars (open symbols) measured in 2012. The developmental stage of each sampling date is shown at the top of the plot. Significant differences between old and new cultivars at $P < 0.05$ and $P < 0.01$ are denoted by * and **. † denotes when g_s data were not reported because leaves were damp.

growing seasons in the five oldest and five most recently released cultivars (Figs 2k–t, S2k–t & S3i–p). Patterns of g_s were similar to patterns of A, and varied over the growing season and among years. On dates when there was a significant relationship between A' and cultivar YOR, midday g_s was high (typically $>0.4\,mol\,m^{-2}\,s^{-1}$; Fig. 2m,o; Fig. S2k,l; Fig. S3i–k). Using the STM^2 model, we estimated soil volumetric water content (SWC) on the days that diurnal gas exchange measurements were taken. Modelled SWC varied from 0.26 to $0.32\,cm^3\,H_2O\,cm^{-3}$ soil, and there was a quadratic relationship between average g_s across all cultivars and SWC (Fig. S4). In general, dates with the low modelled SWC did not show a positive correlation between A' and YOR, whereas dates with higher SWC tended to show a positive correlation (Fig. 1). Furthermore, on dates that A' was not significantly correlated with YOR, average g_s in old and new cultivars was very similar ($\sim0.30\,mol\,m^{-2}\,s^{-1}$). However, when A' was significantly correlated with YOR, average g_s in older cultivars was $0.59\,mol\,m^{-2}\,s^{-1}$, while average g_s in newer cultivars was significantly greater ($0.67\,mol\,m^{-2}\,s^{-1}$; *t*-test, $P < 0.05$).

No consistent change in photosynthetic capacity or respiration rate with cultivar year of release

Photosynthetic capacity of historical soybean cultivars was measured during flowering (R2) and seed fill (R5) across the 3 years of this study. On four of the six measurement dates, there was no evidence for greater $V_{c,max}$ in more recently released cultivars (Fig. 3a,e,f,i), and J_{max} was correlated with cultivar YOR only at the end of 2013 (Fig. 3l). In 2011 and 2013, there was a positive correlation between $V_{c,max}$ and cultivar YOR late in the growing season (Fig. 2b,j). There was no evidence for change in g_m with cultivar YOR (Fig. 4), but it was generally higher in R5 compared with R2 based on paired *t*-tests ($P < 0.1$ in 2012, $P < 0.05$ in 2013). While there was no difference in $V_{c,max}$ between early and late reproductive periods (R2 versus R5 in Fig. 3), J_{max} was significantly higher in R5 compared with R2 (Figs 3 & 4; paired *t*-test $P < 0.01$ in both 2012 and 2013).

Although care was taken to measure mature green leaves at the top of the canopy and there were no visible signs of senescence in any of the cultivars, there may have been differences in leaf age that account for some of the trend or there may have been differences in source-sink balance, which impacted
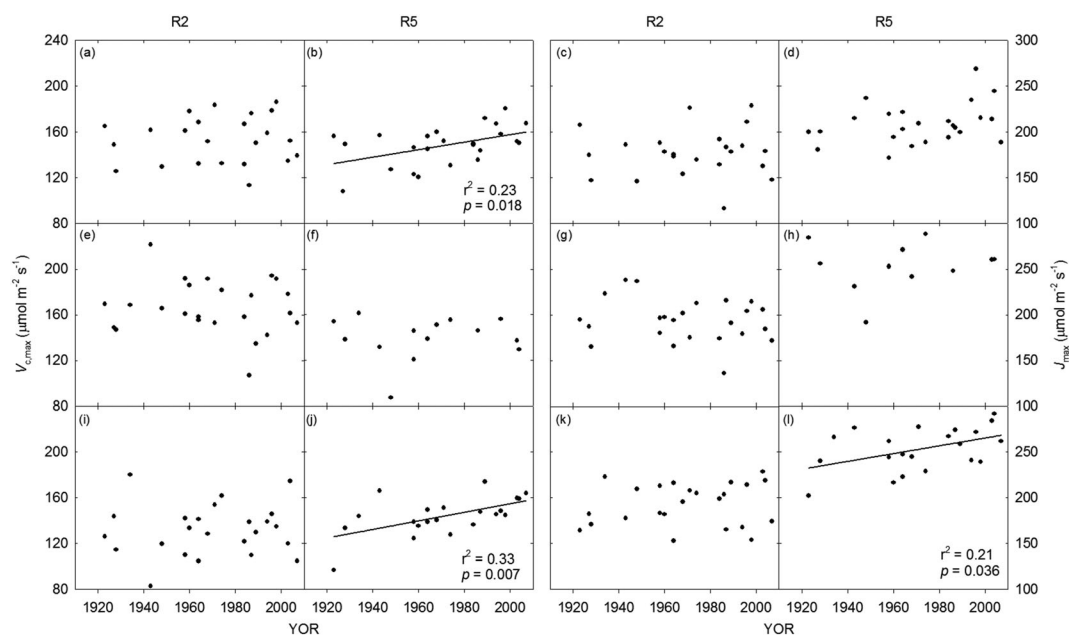
Figure 3. The maximum rate of carboxylation by Rubisco ($V_{c,max}$, a, b, e, f, i, j) and the maximum rate of the regeneration of RuBP (J_{max}, c, d, g, h, k, l) plotted against cultivar YOR for 2011, 2012 and 2013 growing seasons during full bloom (R2) and beginning seed (R5; Fehr *et al.* 1971). For those panels in which least squares linear regression was significant ($P < 0.05$), the coefficient of determination (r^2) and P are provided.

photosynthesis. Leaf chlorophyll content was measured during R2 and R5 in 2012 and 2013, and there was a positive correlation between chlorophyll content and YOR on three of the four dates of measurement (Fig. 5). However, there was no significant reduction in chlorophyll content between R2 and R5 in any of the soybean varieties, suggesting that leaf senescence was not occurring when photosynthetic capacity ($V_{c,max}$ and J_{max}) was assessed in R5. Starch measurements also do not support the hypothesis that changes in source-sink balance altered photosynthetic capacity. There was no correlation between YOR and starch content in R2 in either 2012 or 2013 (Fig. 6a–d,i,j). In 2012, there was a significant negative
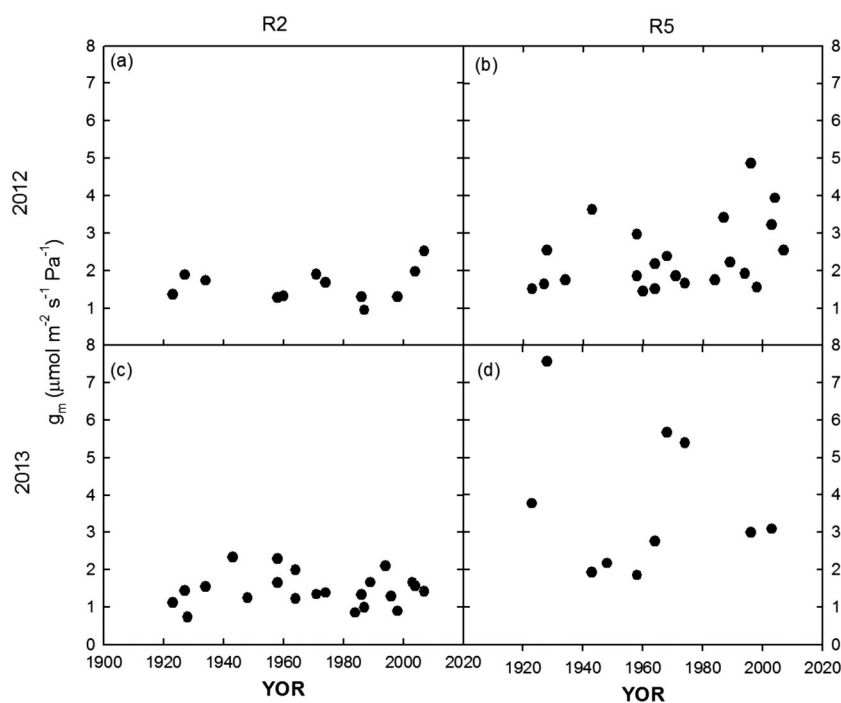

Figure 4. Mesophyll conductance (g_m) plotted against cultivar YOR for the 2012 and 2013 growing seasons during full bloom (R2) and beginning seed (R5) growth stages.
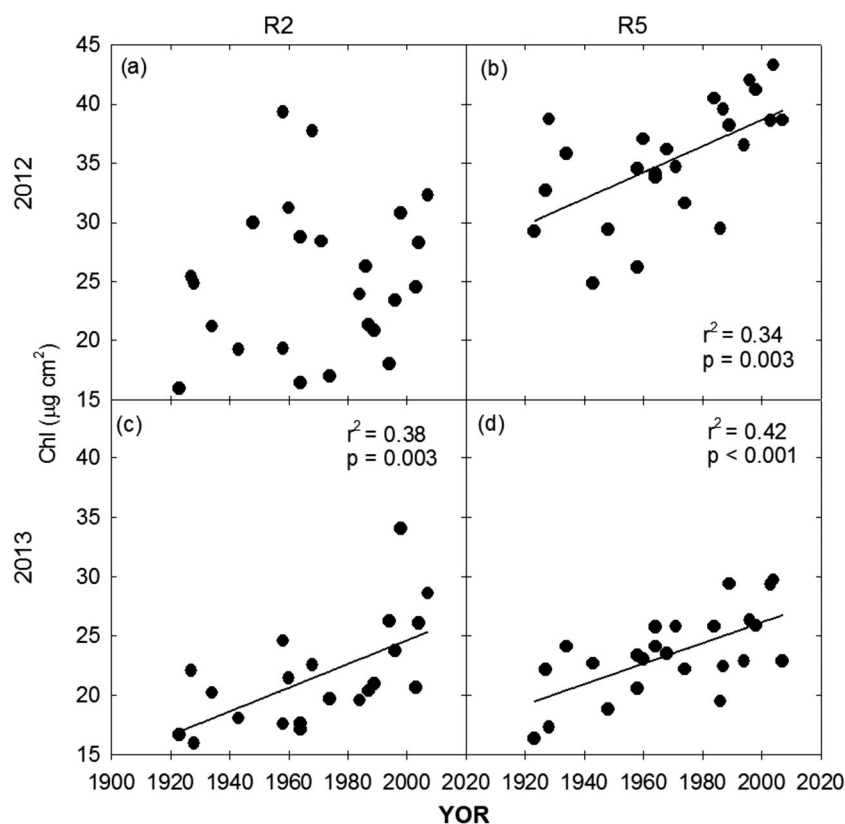
Figure 5. Leaf chlorophyll content (Chl) plotted against cultivar YOR for the 2012 and 2013 growing seasons during full bloom (R2) and beginning seed (R5) growth stages.

correlation between YOR and starch content (Fig. 5e–h), but not in 2013 (Fig. 6k,l), when greater $V_{c,max}$ in more recently released cultivars was apparent (Fig. 3j,l).

Nighttime measurements of CO_2 efflux (dark respiration, R) were measured during the same developmental stages as the A/c_i analysis in 2012 and 2013. R did not change with cultivar YOR at any time (Fig. S5) and also did not correlate with peak biomass, conversion efficiency or yield (Figs S6 & S7).

DISCUSSION

This study investigated the physiological mechanisms driving improvement in conversion efficiency in historical soybean cultivars (Koester *et al.* 2014). Diurnal C assimilation was greater in modern soybean cultivars on approximately half of the dates of measurement across three field seasons (Fig. 1), and we investigated the physiological and environmental basis for greater A. Across the 3 years of study, A increased with cultivar YOR during both vegetative and reproductive stages, although there was variation from year to year. Maximum photosynthetic capacity, measured as $V_{c,max}$ or J_{max}, was greater in modern soybean cultivars only in R5 (Fig. 3), and there was also no evidence that g_m or R changed with soybean YOR (Figs 4 & 7). Therefore, biochemical improvements in photosynthesis or respiration did not entirely explain the observed trends in A or conversion efficiency with soybean cultivar YOR. Instead, the most consistent response was that modern

cultivars had greater A when g_s was high, and greater daily C assimilation (A) was observed under times of ample soil water content (Fig. 1). This is consistent with the idea that conventional breeding in many crops has inadvertently increased g_s (Roche 2015).

In C_3 plants under light-saturating conditions and well-watered conditions, photosynthesis is limited by the kinetics of carbon fixation and substrate regeneration. Rubisco is the primary carboxylating enzyme in soybean and other C_3 plants, and the rate at which Rubisco attaches CO_2 to its substrate, RuBP, can limit carbon gain (Farquhar *et al.* 1980; Farquhar & Sharkey 1982). When $V_{c,max}$ is no longer limiting, the electron transport rate supporting RuBP regeneration (J_{max}) can determine A (Farquhar & Sharkey 1982). Within the historical soybean germplasm measured in this study, neither $V_{c,max}$ nor J_{max} increased consistently with cultivar YOR. However, there was a significant increase in $V_{c,max}$ with YOR late in the growing season in 2011 and 2013 (Fig. 3b,j). We investigated changes in chlorophyll content as an indicator of leaf senescence and found that chlorophyll content linearly increased with soybean cultivar YOR in both R2 and R5, but chlorophyll content was not significantly lower in leaves of older varieties measured later in the season (Fig. 5b,d versus 5a,c). Therefore, it is unlikely that changes in leaf senescence contributed to the observed trend in $V_{c,max}$.

We also investigated that possibility that greater sink strength in modern lines improved photosynthetic capacity late
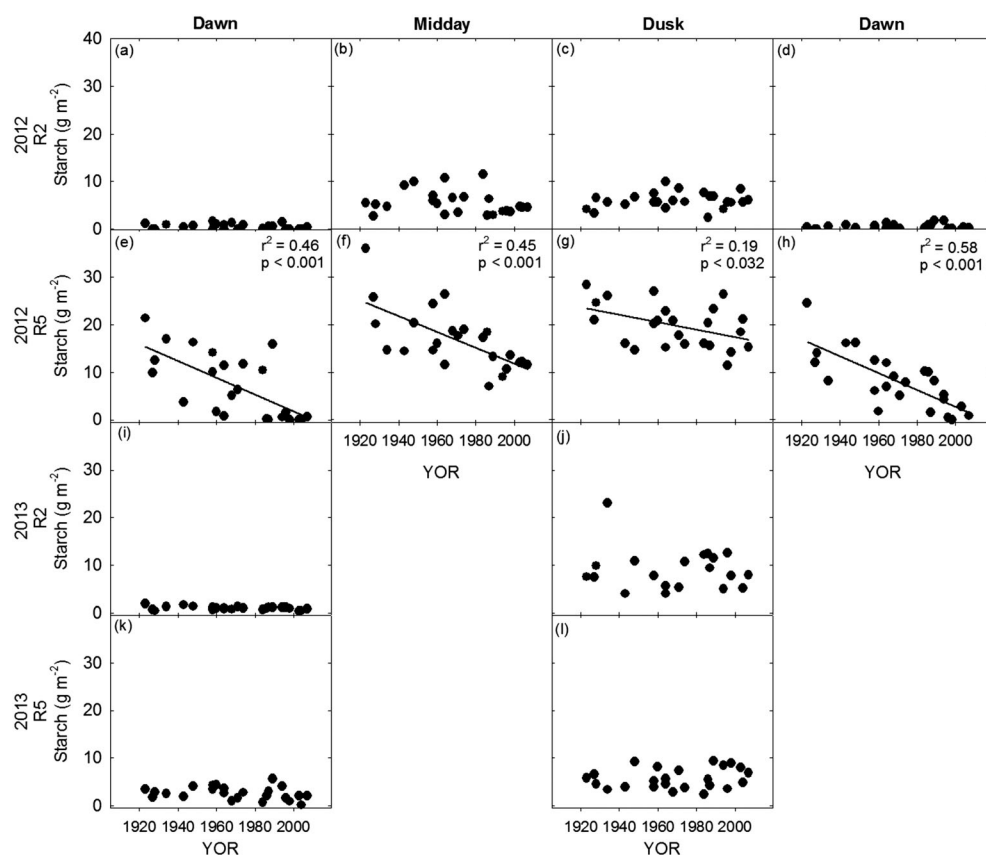
Figure 6. Leaf starch content measured at dawn, midday and dusk plotted against cultivar YOR for the 2012 and 2013 growing seasons during full bloom (R2) and beginning seed (R5) growth stages.

in the growing season, which resulted in the observed trend in $V_{c,max}$. Soybeans are often co-limited by sink strength and source strength depending on environmental conditions and growth stage (Borras *et al.* 2004). Determining whether a plant is currently sink-limited or source-limited is difficult, but measuring starch content at the end of the night can be used as a proxy. If starch pools are not completely exhausted by the end of the night, then plants have excess C and are considered sink-limited (Pilkington *et al.* 2015). This excess starch can then accumulate and negatively feed back on photosynthesis (Grimmer *et al.* 1999; Paul & Foyer 2001; Paul & Pellny 2003). Within our study, starch pools were almost completely utilized during flowering in 2012 (Fig. 6a–d), but older cultivars had excess starch at dawn and other times of day during pod fill (Fig. 6e–h). The negative linear relationship between YOR and starch content during pod fill indicates that older cultivars were more sink limited than more recent cultivars. However, this was not the case in 2013 when starch pools were exhausted during flowering and pod fill (Fig. 6i,k). These starch measurements were coordinated with the diurnal photosynthesis measurements and measurements of photosynthetic capacity, and there was no congruency in times when starch content was accumulated in older lines (2012) and when photosynthetic capacity was lower in older lines (2013). Thus, changes in the apparent sink strength based on leaf carbohydrate content do not appear to explain the variation in photosynthetic capacity and daily C gain observed in this study.

When averaged across 2012 and 2013, neither $V_{c,max}$ nor J_{max} was correlated with conversion efficiency or total biomass (Figs S2 & S3). There was also no correlation with cultivar YOR and dark respiration rate. Studies of diverse wheat varieties have reported significant variation in photosynthetic capacity, but similar to this study, neither $V_{c,max}$ nor J_{max} was correlated with wheat biomass (Driever *et al.* 2014), nor was there evidence for increased dark respiration in historical wheat germplasm (Sadras *et al.* 2012). Driever *et al.* (2014) suggested that the instantaneous measurements of gas exchange on a single date in the growing season would rarely match the average values achieved by the crop over a dynamic growing season where naturally fluctuating conditions alter light, temperature and water availability, and thus, it is perhaps not surprising that there was no correlation between average leaf-level photosynthetic capacity and soybean biomass or seed yield (Figs S5 & S6).

Although we did not measure consistently greater biochemical capacity for photosynthesis ($V_{c,max}$ or J_{max}), the integrated total daily carbon uptake (A) increased linearly with cultivar YOR on approximately half of the soybean growth stage-specific measurement dates. To achieve this increase in daily carbon gain, modern cultivars had up to 23% greater A than older cultivars at times during the day. In similar studies of Canadian and Chinese soybean germplasm, slightly higher gains in leaf-level A with cultivar YOR were detected; although the absolute values of photosynthesis

and seed yield were much lower (Morrison *et al.* 1999; Jin *et al.* 2010). On dates when *A* was correlated with YOR, g_s was 13.5% greater in the five most recently released cultivars compared with the five oldest cultivars, suggesting that improvements in *A* with cultivar YOR may have been supported by greater CO_2 uptake and transpirational water use through greater g_s. Similar results of increased g_s in modern cultivars have been found previously within historical soybean (Morrison *et al.* 1999; Lui *et al.* 2012) and wheat (Fischer *et al.* 1998; Zheng *et al.* 2011; Sadras & Lawson 2011) germplasm. The mechanisms behind greater g_s in modern cultivars remain unknown, and future studies are needed to investigate those mechanisms (Roche 2015). It could be that modern soybean cultivars have greater stomatal sensitivity to environmental conditions or that they have altered maximum stomatal pore size or density. However, in wheat, there is no evidence that stomatal pore size and density have been altered by selection for greater seed yield (Sadras *et al.* 2012). Mesophyll conductance (g_m), the diffusion of CO_2 from substomatal cavities to the carboxylation sites in the chloroplasts, is variable within wheat germplasm (Jahan *et al.* 2014), and apparent mesophyll conductance (defined as A/c_i) increased with YOR in historical Chinese soybean germplasm (Liu *et al.* 2012). However, in the historical soybean cultivars studied here, there was no evidence that g_m varied with YOR (Fig. 4).

The environmental conditions that the soybean canopy experienced largely influenced average g_s. During the 3 years of the study, the experimental plots experienced a wide variety of environmental conditions including periods of drought and above average temperatures (Fig. S1). The times when a significant correlation between *A* and cultivar YOR were detected were times when there was high soil moisture availability. Recent research has shown that modern soybean cultivars can take advantage of high-yielding environments more than older cultivars, so yield improvement and genetic gain are greater in better environments (Rincker *et al.* 2014). We believe that these data may provide a physiological explanation for that observation. While modern cultivars have greater yields as a result of improved HI and greater light interception under both favourable and less favourable environments (Rincker *et al.* 2014; Koester *et al.* 2014), only under adequate soil moisture conditions do modern cultivars also have greater g_s and *A*. Thus, in selecting for maximum seed yield, we hypothesize soybeans have been bred to take advantage of replete soil moisture conditions. This has implications for future improvements in soybean conversion efficiency if climate change leads to warmer summers with less available soil moisture. Different strategies for improving conversion efficiency that do not demand additional water may be needed.

CONCLUSION

Although increases in $V_{c,max}$ and J_{max} have been suggested as a target for improving crop yields (Zhu *et al.* 2010; Parry *et al.* 2011; Evans 2013), these parameters have not changed with selection for greater seed yield in soybean germplasm. More

complete understanding of why these parameters have not changed is key to determining if photosynthetic capacity is truly an untapped target for increasing soybean yields in the future. This study suggests that improvements in conversion efficiency in historical soybean lines were apparent when soil water availability was ample. Globally, the majority of crops are grown under water-limiting conditions (Boyer 1982), and in the USA, irrigation is limited to less than 10% of soybean production area (2007 USDA Census of Agriculture). Increasing irrigation to improve crop conversion efficiency is unlikely to be a sustainable solution to improving soybean yields, so alternative targets may be needed.

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