American Journal of Plant Sciences, **2011, 2, 702-715**
doi:10.4236/ajps.2011.25085 Published Online November 2011 (http://www.SciRP.org/journal/ajps)

Influence of Planting Date on Seed Protein, Oil, Sugars, Minerals, and Nitrogen Metabolism in Soybean under Irrigated and Non-Irrigated Environments*

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Received August 17th, 2011; revised September 21st, 2011; accepted October 15th, 2011.

ABSTRACT

Information on the effect of planting date and irrigation on soybean [*Glycine max* (*L.*) *Merr.*] *seed composition in the Early Soybean Production System* (*ESPS*) *is deficient, and what is available is inconclusive. The objective of this research was to investigate the effects of planting date on seed protein, oil, fatty acids, sugars, and minerals in soybean grown under irrigated* (*I*) *and non-irrigated* (*NI*) *conditions. A 2-yr field experiment was conducted in Stoneville, MS in* 2007 *and* 2008. *Soybean was planted during second week of April* (*early planting*) *and second week of May* (*late planting*) *each year. Results showed that under irrigated condition, early planting increased seed oil* (*up to* 16% *increase*) *and oleic acid* (*up to* 22.8% *increase*), *but decreased protein* (*up to* 6.6% *decrease*), *linoleic* (*up to* 10.9% *decrease*) *and linolenic acids* (*up to* 27.7% *decrease*) *compared to late planting. Under I conditions, late planting resulted in higher sucrose and raffinose and lower stachyose compared with early planting. Under NI conditions, seed of early planting had higher protein* (*up to* 4% *increase*) *and oleic acid* (*up to* 25% *increase*) *and lower oil* (*up to*10.8% *decrease*) *and linolenic acids* (*up to* 13% *decrease*) *than those of late planting. Under NI, stachyose concentration was higher than sucrose or raffinose, especially in early planting. Under I, early planting resulted in lower leaf and seed B, Fe, and P concentrations compared with those of late planting. Under NI, however, early planting resulted in higher accumulation of leaf B and P, but lower seed B and P compared with those of late planting. This research demonstrated that both irrigation and planting date have a significant influence on seed protein, oil, unsaturated fatty acids, and sugars. Our results suggest that seed of late planting accumulate more B, P, and Fe than those of early planting, and this could be a beneficial gain. Limited translocation of nutrients from leaves to seed under NI is undesirable. Soybean producers may use this information to maintain yield and seed quality, and soybean breeders to select for seed quality traits and mineral translocation efficiency in stress environments.*

Keywords*: *Mineral Nutrition*, *Oligosaccharides*, *Raffinose*, *Stachyose*, *Seed Composition*, *Sucrose

1. Introduction

Soybean is a major crop in the word and a source of protein, oil, carbohydrates, and other nutrients for humans and animals [1]. Seed contains about 40% protein, 20% oil, and 33% carbohydrates [2]. Soybean seed soluble carbohydrates, including disaccharides (sucrose) and oligosaccharides (raffinose and stachyose), contribute to seed quality [3]. The average soybean seed contains 9% to 12% total soluble carbohydrates, of which 4% to 5% are sucrose ($C_{12}H_{22}O_{11}$), 1 to 2% are raffinose ($C_{18}H_{32}O_{16}$), and 3.5% to 4.5% are stachyose ($C_{24}H_{42}O_{21}$) [4]. Raffinose and stachyose are undesirable seed quality traits because they have detrimental effects on food and feed quality, causing flatulence or diarrhea in nonruminants [3], and soybean with low raffinose and stachyose is desirable because of increased feed energy efficiency, mineral uptake, and reduced flatulence for nonruminant animals. Soybean seed with high sucrose is desirable because it

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improves taste and flavor in tofu, soymilk, and nato [1].

Although the Early Soybean Production System (ESPS) showed yield benefit under irrigated and non-irrigated conditions [5], lower seed quality and substandard germination of seed [6,7] and variability of seed composition constituents [8-10] are still a challenge. Planting date was used as a management strategy to optimize yield and seed quality. This is because changing of the planting date would lead to a change in environmental factors, including temperature and rainfall. Although it is well established that a late planting date results in higher seed quality (seed germination), in ESPS for maturity group IV and V, effects of planting date on seed composition constituents (protein, oil, fatty acids, and sugars) have not been well investigated.

Previous research showed that oil concentration increased with early planting, but this increase pattern was not consistent across locations [11-13]. On the other hand, it was found that protein concentration increased and oil concentration decreased with late planting [12,13]. This variability in seed composition constituents across environment and location is still a challenge for normal and novel modified seed constituent lines. For example, it was reported that the instability of high oleate germplasm lines across environments was due to mainly to the effect of temperature on the enzymes controlling biosynthesis of soybean seed fatty acids, especially at a seed-fill (R5-R6) stage [14-16]. It was suggested that palmitic and linolenic acids may decrease with later planting date, but stearic acid may increase, and this may be due to temperature changes during seed maturation at later planting [15]. On the other hand, oleic acid levels increased and linoleic and linolenic acid levels decreased when soybeans were grown in warmer environments [17]. Higher oleic acid and lower linoleic and linolenic acids in soybean seed oil are desirable because of their contribution to the stability of the oil. The effect of temperature on oleic and linolenic acid was explained previously in that temperature may affect oleate and linoleate desaturases [18], decrease oleyl and linoleyl desaturase activities at 35˚C [19], decrease ω-6 desaturase enzyme, encoded by the FAD2-1A gene, and desaturases degraded at high growth temperatures of 30˚C [20].

Based on the above literature, effects of planting date on seed composition are still inconclusive. Therefore, the objective of this research was to further investigate effects of planting date and irrigation on seed protein, oil, fatty acids, sugars, and mineral nutrition. Since nitrogen is an essential nutrient for seed protein, and nitrogen metabolism in legumes is a result of both N_2 fixation and assimilation [21,22], the effect of planting date and irrigation on nitrogen fixation and assimilation were also investigated.

2. Materials and Methods

2.1. Field and Growth Conditions

A 2-yr field study was conducted during 2007 and 2008 at the USDA-ARS Crop Production Systems Research farm, Stoneville, MS (33˚26'N latitude), The soil was a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoqualf) with pH 6.7, 1.1% organic matter, a cation exchange capacity of 15 cmol/kg, and soil textural fractions of 26% sand, 55% silt, and 19% clay. The experimental area was disked, subsoiled, disked, and bedded in the fall of the previous year. Prior to planting, the raised beds were smoothed as needed. Soybean was planted in 102-cm wide rows using a MaxEmerge 2 planter (Deere and Co., Moline, IL) at 285,000 seeds/ha. Soybean cultivar "AG4604RR/S" was planted April 9 and May 10 in 2007 and April 8 and May 12 in 2008. Pendimethalin at 1.12 kg·ai/ha plus paraquat at 1.12 kg·ai/ha were applied to the entire experimental area immediately after each planting. Paraquat was applied to kill existing weeds at planting, Pendimethalin was used to provide early-season weed control. Glyphosate at 0.84 kg·ae/ha was applied at 4 - 5 weeks after planting soybean to the entire experimental area for postemergence weed control. Herbicides were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray nozzles (TeeJet Spraying Systems Co., Wheaton, IL), delivering 187 L/ha water at 179 kPa. All plots were hand weeded periodically throughout the season to keep weed-free. No fertilizer nitrogen was applied and the crop was irrigated on an as-needed basis each year. Each treatment plot consisted of twelve rows spaced 102-cm apart and 15.2 m long. At harvest about 200 soybean pods were randomly sampled from the middle four rows for seed. Soybean from middle eight rows in each plot were harvested using a combine, and grain yield was adjusted to 13% moisture.

2.2. Seed Analysis for Protein, Oil, and Fatty Acids

Mature seed collected at harvest were analyzed for protein, oil, and fatty acids. Approximately 25 g of seed from each plot were ground using a Laboratory Mill 3600 (Perten, Springfield, IL). Analyses were conducted by near infrared reflectance [23] using a diode array feed analyzer AD 7200 (Perten, Springfield, IL). Calibrations were developed by the University of Minnesota, using Perten's Thermo Galactic Grams PLS IQ software. The calibration curve has been regularly updated for unique samples according to AOAC methods [24,25]. Analyses of protein and oil were performed based on a seed dry matter basis [23,26].

2.3. Seed Analysis for Sucrose, Raffinose, and Stachyose

Matured seed collected at harvest from each planting date were analyzed for sucrose, raffinose, and stachyose concentrations [10]. About 25 g of seed from each plot were ground using a Laboratory Mill 3600 (Perten, Springfield, IL). Analyses were conducted by near infrared reflectance [9,23] using an AD 7200 array feed analyzer (Perten, Springfield, IL). Calibrations were developed by the Department of Agronomy and Plant Genetics, University of Minnesota St Paul, MN, using Thermo Galactic Grams PLS IQ software, developed by Perten company (Perten, Springfield, IL). Analyses of sugars were performed based on a seed dry matter basis [23,26].

2.4. Seed N, S, and Mineral Composition

Mature seed collected at harvest were analyzed for N, S, Ca, Mg, and Zn concentrations at The University of Georgia's Soil, Plant, and Water Laboratory, Athens, GA. Seed Ca, Mg, and Zn concentrations were analyzed by digesting 0.5 g of dried ground seed in HNO_3 in a microwave digestion system. Values were then determined using inductively coupled plasma spectrometry. Nitrogen and S were measured in a 0.25-g sample using an elemental analyzer (LECO CNS-2000, LECO Corporation, MI). For seed B, Fe, and P, concentrations were determined as indicated in the following sections.

2.5. Nitrate Reductase Activity

Nitrate reductase activity (NRA) was measured in the fully expanded leaves at R1-R2 from each plot. The measurement of NRA was made according to the method of [27] and was described for soybean in detail by others [28]. To determine potential NRA (PNRA) where nitrate availability is not limited, nitrate at a concentration of 10 mM as KNO_3, was added to the incubation solution. Nitrate reductase activity was expressed as μmol NO_2^-·g fwt^{-1}·$hour^{-1}$).

2.6. Acetylene Reduction Assay

Ten soybean plants were randomly sampled from each plot at R1-R2 for nitrogenase activity (nitrogen fixation activity, NFA) measurement. Plants were excavated with roots and shoot and transported to the laboratory for NA. Nitrogenase activity was assayed within 30 min of collection using the acetylene reduction assay to measure NA [29,30]. Roots with nodules intact were excised and incubated in 1 L Mason jars (two jars per plot). Six roots were placed in the Mason jars and sealed, and a 10% volume of acetylene was added. After 1 h of incubation at room temperature, gas samples were removed and analyzed by gas chromatography using a flame ionization detector (FID) and a thermal conductivity detector (TCD) for determination of ethylene.

2.7. Boron Measurement

Boron concentration was measured in seed from each plot using the Azomethine-H method [31]. Calcium carbonate powder was added to 1.0 g seed samples before ashing at 500˚C for 8 hours to prevent losses of volatile B compounds. Ashed samples, then, were extracted with 20 ml of 2 M HCl at 90˚C for 10 min, filtered and transferred to plastic vials. A 2 ml sample of the solution was added to 4 ml of buffer solution (containing 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid) and 4 ml of freshly prepared azomethine-H solution (0.45% azomethine-H and 1% of ascorbic acid) [32]. Samples were left at room temperature for at least 45 min for color development, and B concentration was determined using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA, USA) at 420 nm.

2.8. Iron Measurement

Seed iron from each plot was measured after acid wet digestion, extraction, and reaction of the reduced ferrous Fe with 1, 10-phenanthroline [10,33,34]. A 2 g sample of dried ground seed was digested in nitric acid (70% m/m HNO_3). After the acids were removed by volatilization, the soluble constituents were dissolved in 2 M HCl. Standard solutions of iron were prepared in 0.4 M HCl and ranged from 0.0 to 4 μg·ml^{-1} Fe. Phenanthroline solution of 0.25% m/v was prepared in 25% v/v ethanol. A fresh quinol solution (1% m/v) reagent was prepared on the day of use. An aliquot of approximately 4 ml was pipetted into a 25 ml volumetric flask. A concentration of 0.4 M HCl solution was used to dilute the aliquot to 5 ml. A volume of quinol solution was added and mixed, and then 3 ml of phenanthroline solution and 5 ml of tri-sodium citrate solution (8% m/v) were added. The mixture solution containing the aliquot, HCl, phenanthroline, tri-sodium citrate, was diluted to 25 ml. The mixture stood for 4 h and the absorbance of the samples was read at 510 nm using a Beckman Coulter DU 800 spectrophotometer.

2.9. Phosphorus Measurement

Seed phosphorus was measured spectrophotometrically as the yellow phospho-vanado-molybdate complex [35, 36]. A dry seed sample of 2 g was ashed, then 10 ml of 6 M HCl was added. Samples were placed in a water bath at 70˚C to evaporate the solution. After drying, the samples were kept under heat, and 2 ml of 36% m/m HCl was added, and gently boiled. Then, 10 ml of water was

added and the solution was carefully boiled for about 1 min. The samples were transferred and diluted to 50 ml in a volumetric flask. After the first 2 ml were discarded, the sample solution was then filtered and kept for P analysis. A volume of 5 ml of the sample was taken, and 5 ml of 5 M HCl and 5 ml of ammonium molybdate-ammonium metavanadate (a solution of ammonium molybdate, $(NH_4)_2MoO_4$ (25 g/500 ml water), and ammonium metavanadate, NH_4VO_3) (1.25 g/500 ml water) reagent were added, diluted to 50 ml, and allowed to stand for 30 min at ambient temperature before measurement. Phosphorus standard solution (0 - 50 µg/ml of phosphorus) was prepared using dihydrogen orthophosphate dissolved in both water and 36% m/m HCl. Phosphorus concentration was measured using a Beckman Coulter DU 800 spectrophotometer at 400 nm.

2.10. Statistical Analysis

The experiment was conducted in a split plot arrangement of treatments in a randomized complete block design with irrigation as the main plot and planting date as the sub-plot with six replications. The experiment (exp) was repeated twice. Each sub-plot consisted of twelve rows of 1 - 2-cm apart and 15.2 m long. The data were subjected to analysis of variance using Proc Mixed using SAS [37]. Means were separated by Fisher's least significant difference test at the 5% level of probability. Data were averaged across years (as main effect means) if the year by treatment interactions were not significant and data were presented separately for each year when interactions were significant.

3. Results and Discussion

Analysis of variance indicated that irrigation and planting date were the major sources of seed composition changes (**Tables 1** and **2**). Seed constituents responded differently to the interactions between year, irrigation, and planting date, indicating that effect of irrigation and planting date on some seed constituents were different in each year (**Tables 1** and **2**).

3.1. Seed Yield

Seed yield of early planting (April) was greater than yield from late planting (May) under I conditions (**Table 3**), but no consistent difference in yield between early and late planting was observed under non-irrigated conditions. It was demonstrated that early planting showed higher yield under irrigated and non-irrigated plants [5,38]. Our yield results under irrigated conditions support previous research. The observation that yield under NI was higher in one year only could be due to differences in growing season environmental factors of temperature and rainfall. Weather data (**Figure 1**) showed different patterns of rainfall and temperature, and these differences could be a source of inconsistency of yield across years under NI [39]. In 2007, the rainfall pattern in June and July was favorable to Early planting and in 2008, rainfall pattern in July and August was favorable to late planting. Overall, rainfall was higher in 2008 compared to 2007 (Check the rainfall data for accuracy of this statement). In 2008, both early and late planted soybean under NI produced relatively high yields with a narrow difference.

Table 1. Analysis of variance (F-value and level of significance) of the effect of year, planting date (Planting), irrigation (Irri), and their interactions for seed protein, oil, and fatty acids ($g \cdot kg^{-1}$) and seed sugars ($mg \cdot g^{-1}$)*.

Source of variability	Protein	Oil	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Sucrose	Raffinose	Stachyose
Year	16***	NS	NS	NS	4.37*	12**	NS	NS
Exp	NS	NS	NS	NS	NS	NS	NS	NS
Irri	89***	3.8*	121***	64***	18**	150***	68***	18**
Planting	52***	150***	148***	84***	35***	188***	11**	9.2**
Year × exp	NS	NS	NS	NS	NS	NS	NS	NS
Year × irri	13***	NS	4.3*	NS	NS	NS	NS	NS
Year × planting	4.8*	NS	11**	NS	NS	NS	NS	4.1*
Exp × irri	4.8*	NS	NS	NS	NS	NS	NS	NS
Exp × planting	NS	NS	NS	NS	NS	NS	NS	NS
Irri × planting	NS	NS	NS	30***	NS	11**	NS	3.9**
Year × irri × planting	NS	NS	NS	NS	5.4*	NS	NS	8.8**
Year × block × irri × planting	NS	NS	NS	NS	2.4*	2.9*	NS	2.7**

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$. NS = non-significant at the $P < 0.05$.

Table 2. Analysis of variance (F-value and level of significance) of the effect of year, planting date (Planting), irrigation (Irri), and their interactions for boron (B), iron (Fe) in leaves and seed (mg·kg^{-1}), phosphorus (P) in leaves and seed (%), weight (wt) (kg·ha^{-1}), nitrogen fixation activity (NFA, µmol C_2H_2·plant^{-1}·h^{-1}), nitrate reductase activity(NRA, µmol NO_2^-·g·fwt^{-1}·h^{-1})*.

Source of variability	B leaves	B seed	Fe leaves	Fe seed	P leaves	P seed	Wt	NFA	NRA
Year	NS	9.9*	12**	24**	NS	NS	67***	NS	7*
Exp	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irri	244***	96***	143***	284***	180***	61***	88***	114***	134***
Planting	120***	40***	51***	24***	15**	NS	NS	21***	12**
Year × exp	NS	NS	NS	15**	NS	NS	NS	NS	NS
Year × irri	NS	NS	4.3*	5.0*	NS	NS	7.0**	NS	4.08*
Year × planting	NS	NS	NS	NS	NS	NS	7.2**	NS	NS
Exp × irri	NS	NS	NS	7.3*	4.2*	NS	NS	NS	4.1*
Exp × planting	NS	NS	NS	5.5*	NS	NS	NS	NS	NS
Irri × planting	NS	NS	NS	NS	41***	NS	16.1***	9.2**	7.6**
Year × irri × planting	NS	5.2*	NS	NS	NS	NS	28.9**	NS	NS
Year × exp × irri × planting	NS	NS	NS	NS	NS	NS	6.29**	NS	NS

*Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$. NS = non-significant at the $P < 0.05$.

Table 3. Effect of planting date on soybean yield, seed protein, oil, and fatty acids (oleic, linoleic, and linolenic under irrigated (IR) and non-irrigated (NI) conditions. The experiment was conducted in 2007 and 2008 at Stoneville, MS, USA. *

Irrigation	Planting	Yield (kg·ha^{-1})	Protein (g·kg^{-1})	Oil (g·kg^{-1})	Oleic (g·kg^{-1})	Linoleic (g·kg^{-1})	Linolenic (g·kg^{-1})
			2007				
I	April	4705 a	409 b	243 a	270 a	531 b	60 b
	May	3801 b	413 a	214 b	220 b	585 a	83 a
NI	April	4255 a	432 a	249 a	308 a	523 b	62 b
	May	3123 b	415 b	222 b	246 b	544 a	69 a
			2008				
I	April	5832 a	405 b	243 a	259 a	525 b	74 b
	May	4479 b	429 a	209 b	221 b	589 a	83 a
NI	April	4043 b	453 a	245 a	301 a	512 b	61 b
	May	4256 a	437 b	213 b	275 b	521 a	73 a

*Means within a column and within each irrigation treatment (I or NI) followed by the same letter are not significantly different at the 5% level as determined by Fishers' LSD test.

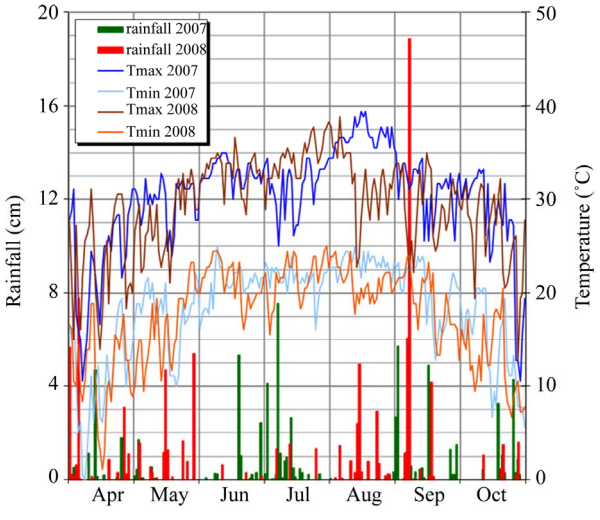

Figure 1. Rainfall (cm) and maximum temperature (Tmax) and minimum temperature (Tmin) in ºC in 2007 and 2008.

3.2. Seed Composition

Under I conditions, early planting resulted in higher oil and oleic acid, but lower protein, linoleic, and linolenic acids (**Table 3**). Late planting resulted in higher protein, linoleic and linolenic acids, but lower oil and oleic acid. Late planting resulted in higher sucrose and raffinose concentrations and lower stachyose concentration compared with early planting under irrigation (**Figure 2**). Combined sugar (sucrose + stachyose + raffinose) concentration was not consistent across years (**Figure 3**). Results from previous research on the effect of planting date on seed composition were inconsistent. For example, it was found that early planting resulted in higher oil (1.54 g·kg^{-1} increase) concentration at Arlington, WI, USA than late planting, but planting date did not affect protein concentration at Hancock, WI, USA [40]. On the other hand, planting date did not affect oil or protein content at Hancock [40]. Other researchers found the oppo-

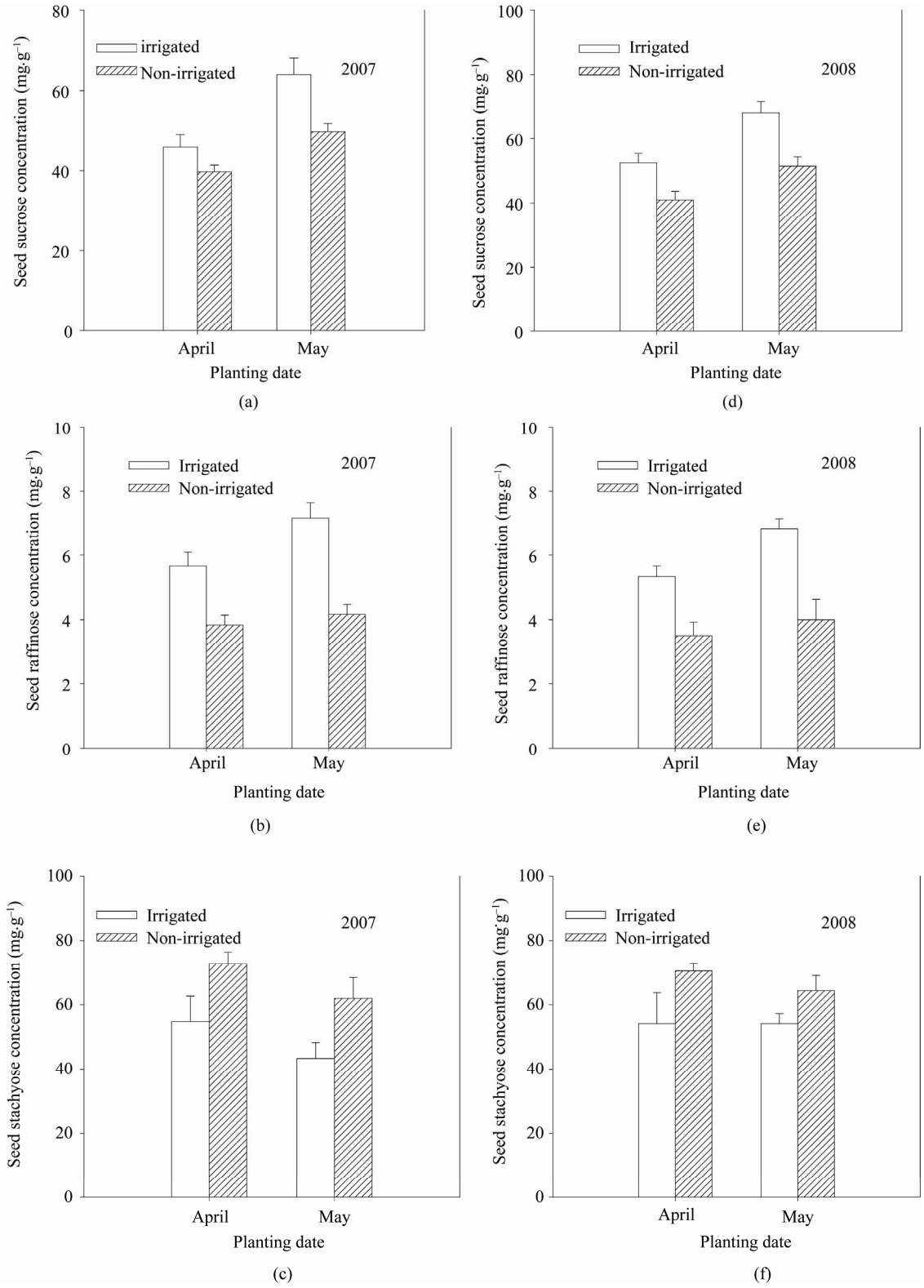

Figure 2. Effect of early planting (April planting) and late planting (May planting) and irrigated and non-irrigated environments in 2007 (a, b, c) and in 2008 (d, e, f) on seed concentrations of sucrose (a, d), raffinose (b, e), and stachyose (c, f). Bar values are means ± SE.

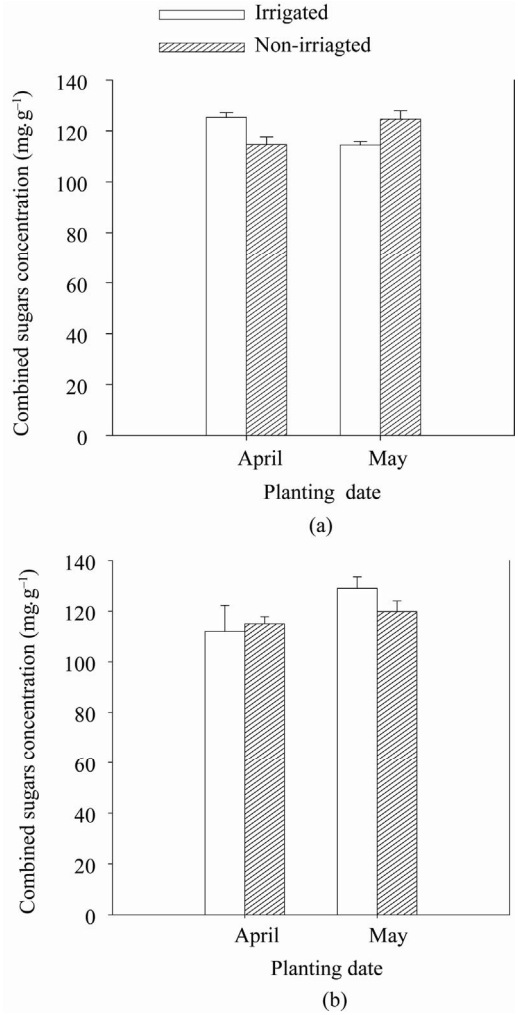

Figure 3. Effect of early planting (April planting) and late planting (May planting) and irrigated and non-irrigated environments on the seed concentrations of combined sugars (sucrose + raffinose + stachyose) in 2007 (a) and in 2008 (b). Bar values are means ± SE.

site in that delayed planting increased protein concentration, but decreased oil concentration [12].

In our experiment, the increase of oil and oleic acid in the early planting may be due to higher temperature during the seed-fill stage, especially from July to August. The higher increase in protein, linoleic, and linolenic acids at late planting may be due to lower temperature coinciding with the seed-fill stage from August to September. The effect of temperature on seed composition was previously reported [9,41-43]. It was reported that the inconsistency of seed composition constituents in previous research could be due to the range of temperatures under which soybean grow. This was explained by [43], who suggested that differences in protein levels in a given genotype could depend on the range of temperature during the seed-fill. They proposed that later maturing genotypes may accumulate more protein than early maturing, and this is because the late maturing soybean may have developed its seed under mean daily temperature of less than 20˚C. Since maximum temperature in Mississippi Delta can exceed 36˚C during flowering and seed fill, it is possible that the inconsistency of the results in the literature could be due to environment, genotype, and their interactions.

The variability in the relationship between seed composition constituents and temperature may depend on the range of temperature. For example, it was found that the range of maximum temperature during filling period for Harosoy early isolines was from 31.6˚C to 33.6˚C in 2004 and from 33.5˚C to 35.5˚C in 2005 [9]. However, for Clark late isolines the maximum temperature was from 31.8˚C to 33.5˚C in 2004 and from 33.2˚C to 36˚C in 2005. The differences in temperature ranges between the two years resulted in protein decrease as temperature increased in 2004, but protein increased as temperature increased further in 2005 [9]. This observation was also found by other researchers. Piper and Boote found that protein was high between 20˚C and 25˚C, but higher when temperature was lower than 20˚C or greater than 25˚C [42]. Oil concentration increased as temperature increased up to a point, then oil concentration decreased as temperature increased [44-46]. The increase of oil with maximum temperature was also observed in by others [8,14].

The increase in sucrose and raffinose in late planting under irrigated and non-irrigated conditions (**Figure 1**) could be due to lower temperature at the seed-fill stage that coincided with late planting. Since planting date leads to temperature changes during the critical stages of growth, seed sugar concentrations can be discussed in the context of planting date and temperature. Generally, late planting moves the seed fill period to a cooler temperatures compared with early planting for maturity group IV and V, and this can be observed from the weather data for maximum and minimum temperatures (**Figure 1**). Literature indicated that the effect of temperature on sugars was not consistent. For example, it was found that low and high temperatures (from 18˚C/13˚C - 33˚C/28˚C) had no effect on raffinose levels, slightly decreased stachyose at the highest temperature 33˚C/28˚C, and significantly decreased sucrose content as the temperature increased [47]. In a growth chamber experiment on mid-high oleic acid breeding line N98-4445A (MG III), combined sugars (sucrose, raffinose, and starchyose) in mature seed grown under high temperature (37˚C/30˚C) did not change compared with those grown under 27˚C/18˚C [48]. Recently, Bellaloui *et al*. conducted a field experiment on Clark and Harosoy isolines and found that

sucrose, stachyose, and combined sugars had a signifycant positive linear relationship with maximum temperature in 2004, but negative relationship in 2005 [10]. The inconsistency was suggested to be due to differences in temperatures between years since the range of maximum temperature during the last 20 d before maturity (filling period) for the Clark isoline set ranged from 31.7˚C to 33.4˚C in 2004 and from 33.3˚C to 36.12˚C in 2005 [10]. It was concluded that the increase in galactinol synthase activity and galactinol content, and the decrease in myo-inositol during sugar partitioning during late seed development may be associated with sugar metabolism in soybean seeds [49]. In addition, the regulation of raffinose oligosaccharide accumulation may also depend on galactosyl transferase activity [49]. Further research is needed to reconcile the controversial literature results on the effects of temperature on seed sugars.

3.3. Mineral Seed Composition

Early planting under irrigation resulted in a decrease in leaf and seed B, Fe, and P concentration compared with late planting (**Figures 4** and **5**). However, early planting under NI resulted in significant accumulation of leaf B and P, but less seed B and seed P compared with late planting (**Figures 4** and **5**). Non-irrigation conditions resulted in lower leaf and seed B and P, but there was no consistency for leaf and seed Fe. The lower concentration of B, P, and Fe in leaves and seed in early planting may indicate that the uptake and translocation of these nutrients during seed-fill stages may be affected by higher temperature. The effect of temperature on uptake and translocation of mineral nutrients were previously reported [50-52].

3.4. Nitrogen Metabolism

Both nitrogen fixation (nitrogenase activity, μmol C_2H_2 $plant^{-1}{\cdot}h^{-1}$) and nitrogen assimilation (nitrate reductase activity, μmol $NO_2^-{\cdot}gfwt{\cdot}h^{-1}$) were higher in early planting than in late planting in 2007 and 2008 (**Figure 6**), reflecting that nitrogen metabolism activity is associated with yield [53]. Non-irrigation resulted in lower nitrogen fixation and assimilation, indicating the sensitivity of nitrogen metabolism to drought or water stress [54-56]. Adding nitrate to the assay medium of leaves of plants grown under NI resulted in a striking increase in NRA, indicating that there was limitation of nitrate availability in leaf cells (data not shown). The increase of NRA by adding nitrate to the assay medium indicates that water stress inhibited nitrate uptake and translocation to leaves. Our results show that nitrogen metabolism activity may explain the yield benefits of early planting in the ESPS.

4. Conclusions

Planting date altered seed composition and mineral nutrition in soybean. Late planting tends to increase oil and oleic acid, but decrease protein, linoleic and linolenic acid under irrigated conditions. Under non-irrigation, protein and oleic acid tend to increase, but linoleic and linolenic acids tend to decrease to decrease. Total seed constituents were higher at early planting than late planting because of the higher yield at early planting (**Table 4**). Late planting under irrigated conditions also tends to increase sucrose and raffinose, but decrease stachyose. Under non-irrigated conditions sucrose tends to decrease. Planting date effects on seed composition may be associated with the shift in temperatures. Early planting is as-

Table 4. Effect of planting date on total seed constituents (protein, oil, and fatty acids) under irrigated (IR) and non-irrigated (NI) conditions. The experiment was conducted in 2007 and 2008 at Stoneville, MS, USA.*

Irrigation	Planting	Protein ($kg{\cdot}ha^{-1}$)	Oil ($kg{\cdot}ha^{-1}$)	Oleic ($kg{\cdot}ha^{-1}$)	Linoleic ($kg{\cdot}ha^{-1}$)	Linolenic ($kg{\cdot}ha^{-1}$)
			2007			
I	April	1924 a	1142 a	1269 a	2496 a	282 b
	May	1571 b	813 b	835 b	2222 b	314 a
NI	April	1841 a	1059 a	1310 a	2225 a	265 a
	May	1297 b	694 b	767 b	1698 b	214 b
			2008			
I	April	2361 a	1415 a	1512 a	3060 a	434 a
	May	1920 b	938 b	991 b	2638 b	373 b
NI	April	1833 a	990 a	1209 a	2078 b	245 b
	May	1858 a	904 b	1169 a	2217 a	311 a

*Means within a column and within each irrigation treatment (I or NI) followed by the same letter are not significantly different at the 5% level as determined by Fishers' LSD test.

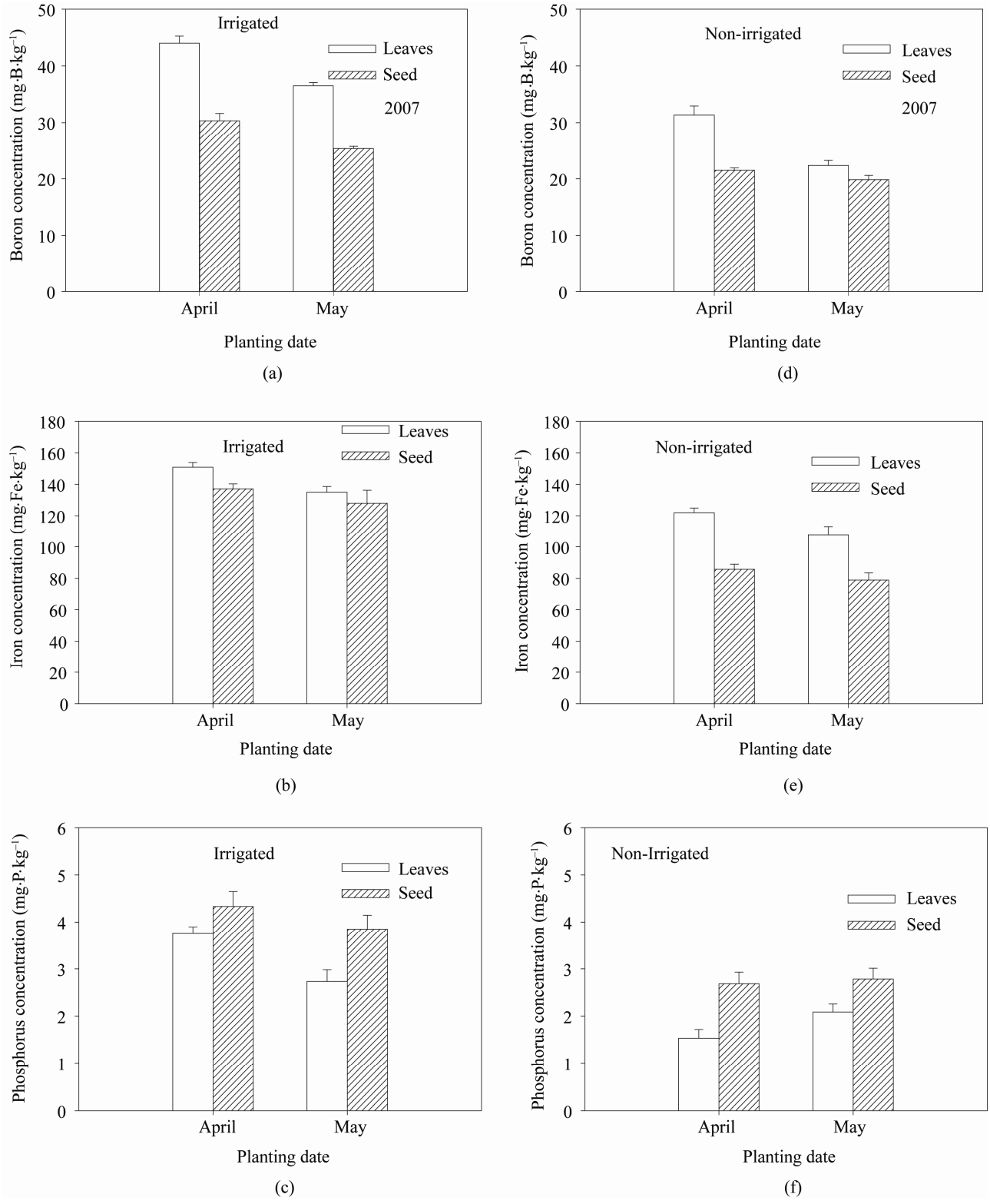

Figure 4. Effect of early planting (April planting) and late planting (May planting) and irrigated (a, b, c) and non-irrigated (d, e, f) environments on seed concentrations of boron (B), iron (Fe), and phosphorus (P) in 2007. Bar values are means ± SE.

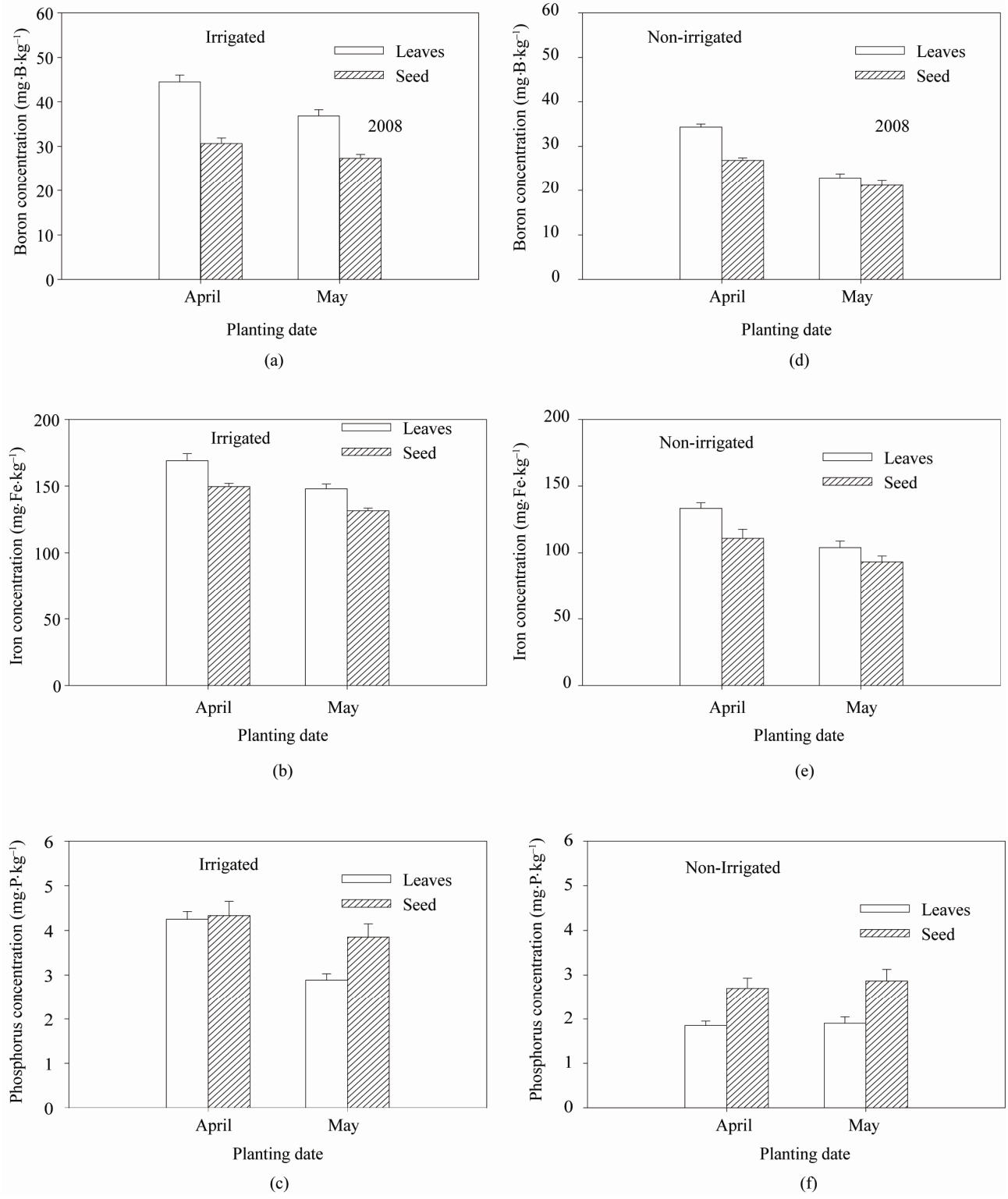

Figure 5. Effect of early planting (April planting) and late planting (May planting) and irrigated (a, b, c) and non-irrigated (d, e, f) environments on seed concentrations of boron (B), iron (Fe), and phosphorus (P) in 2008. Bar values are means ± SE.

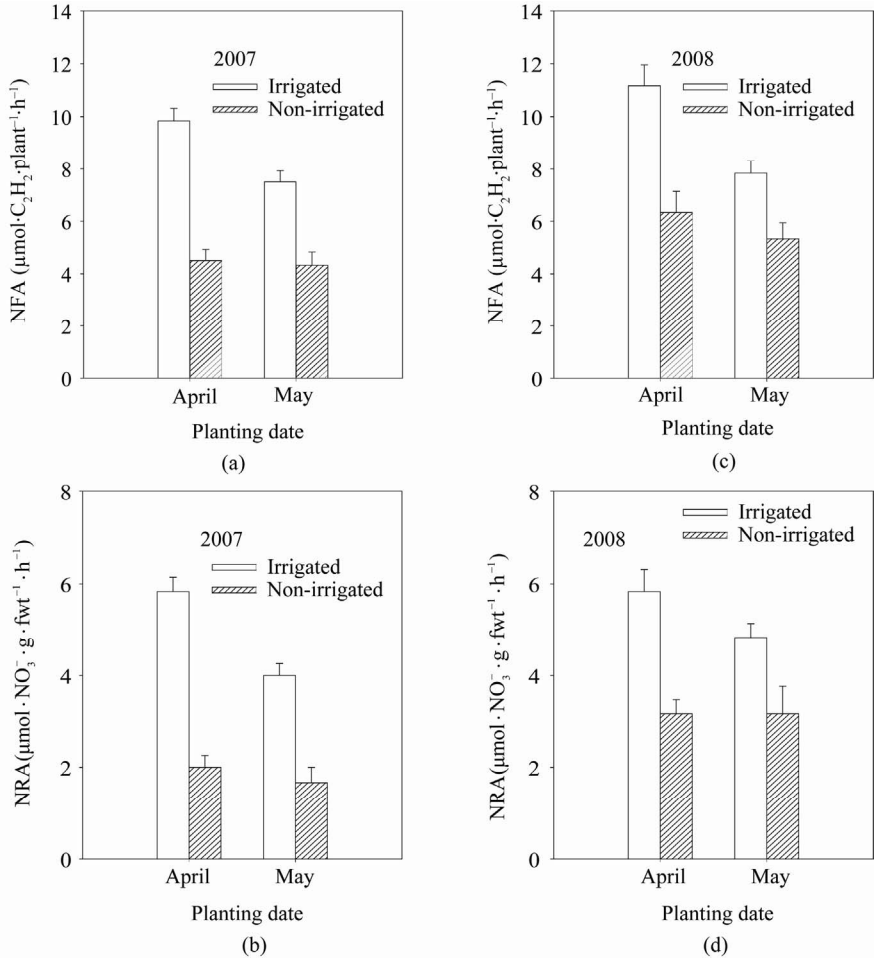

Figure 6. Effect of early planting (April planting) and late planting (May planting) and irrigated and non-irrigated environments on nitrogen fixation activity/nitrogenase activity (NFA), NA (a, c) and nitrate assimilation (nitrate reductase activity, NRA) (b, d) in 2007 and 2008. Bar values are means ± SE.

sociated with cooler temperature and Late planting is associated with hotter temperature during flowering and seed fill periods. Lack of translocation of B, Fe, and P from leaves to seed under non-irrigated conditions may suggest foliar application of these nutrients may be needed, especially under deficiencies of these nutrients in soil.

5. Acknowledgements

We thank Albert Tidwell field technical assistance, Sandra Mosley for lab technical assistance, and Debbie Boykin for statistical assistance. This research was funded by United States Department of Agriculture, Agricultural Research Service, project number 6402-21000-034-000.

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