Impact of First True Leaf Photosynthetic Efficiency on Peanut Plant Growth under Different Early-Season Temperature Conditions

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ABSTRACT

Selecting planting dates with optimal temperatures for plant growth and development is important for successful crop production. Photosynthetic rates of peanut leaves under adverse environmental conditions have been widely studied; however, characterization of photosynthetic efficiency of first true leaves as well as its contribution to plant growth is not well elucidated. The objectives of this research were to assess the influence of first true leaves of peanut cultivars on plant growth under different temperature conditions during early growth and at the onset of flowering and to identify the photosynthetic components more closely linked with photosynthetic efficiency of the first true leaves. Experiments were conducted with April (early), May (optimum), and June (late) planting dates in 2017 and 2018. Cultivars Georgia-06G, Georgia-14N, and TifNV-High O/L were evaluated. Measurements were taken at three and five wks after planting, early season and the onset of flowering, respectively. Rapid development of first true leaves of peanut plants contributed to whole-plant growth in the early season and at the onset of flowering across the temperature conditions. Net photosynthesis of first true leaves was not impacted by temperature conditions in the early season or at the onset of flowering primarily due to greater activity of non-stomatal components associated with the thylakoid reactions. Whole-plant growth was more considerably associated with first true leaf area development than photosynthetic efficiency of those leaves in peanut plants.

Key Words: First true leaf, photosynthetic efficiency, net photosynthesis, thylakoid reactions, temperature

Peanut (*Arachis hypogaea* L.) is an important oilseed crop grown worldwide. This crop originated in South America and is grown in mostly tropical, subtropical and relatively warm temperate conditions (Hammons *et al.*, 2016). Variable environmental conditions encountered from the time of planting until harvest, from the broad regions peanuts are cultivated, can greatly influence stand establishment, crop development, and yield. Air and soil temperatures as well as soil moisture affect germination and early seedling growth. Considering adequate soil moisture at planting and in the early season along with proper management practices, air and soil temperatures play an important role in stand establishment and seedling development. Thus, selection of planting date is a critical factor for production. Peanut seed emergence occurs between 6 to 11 d after planting, depending upon soil and air temperatures (Canavar and Kaynak, 2010). As a general rule, peanut germination is considered optimum in the soil temperature range of 20 to 35 C at a 10 cm soil depth for three consecutive d (Kvien *et al.*, 2019) and air temperatures between 27 and 32 C are considered optimum for peanut growth and yield (Boote *et al.*, 1989; Williams and Boote, 1995).

Gardner and Auma (1989) reported a negative effect of delaying peanut planting on plant growth such as plant height and leaf area. Similarly, Prasad *et al.* (2006) evaluated the effect of different planting dates with soil temperatures ranging from 15 to 32 C on emergence and early season growth of six peanut cultivars. The authors reported the lowest emergence rate and smallest seedlings for the planting date with the coolest temperature conditions.

Temperature is the driving force for several chemical reactions occurring in plant cells at different stages throughout the life cycle. Decreases in temperature below the optimum range generally decrease the rate of chemical reactions. Suboptimal temperature increases the probability of early-season seedling injury from pests and pathogens in peanuts with potential seedling death, ultimately resulting in reduced plant populations and lower yield potential (Prasad *et al.*, 2006; Bell *et al.*, 1993; Bell, 1986). Leong and Ong (1983) reported a linear increase in rate of peanut development with increasing temperature from 19 to 31 C. Low temperature in the early season can also have pronounced effects on the physiology and biochemistry of seedlings, resulting in reduced plant growth and development due to lower photosynthetic rates (Allen and Ort, 2001). Re-

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duced net assimilation rate under low temperature can be due to stomatal factors, such as stomata closure or non-stomatal factors, such as deactivation of Rubisco (Holaday *et al.* 1992; 2016). Bagnall *et al.* (1988) reported 50 to 70% decrease in net photosynthesis within the first two d with a change in temperature from 30 to 19 C. The authors further indicated that the decrease in net photosynthesis under sub-optimal temperature conditions was due to non-stomatal limitations.

Supra-optimal temperature at planting and in the early season can also negatively affect germination and seedling growth (Challinor *et al.*, 2005; Hamidou *et al.*, 2013). For peanuts, air temperatures near 30 C are considered optimal for growth and physiological processes such as photosynthesis (Wood, 1968; Cox 1979; Ong, 1984). Current evidence suggests that high temperature limits photosynthesis by inhibiting rubisco activity through deactivation of rubisco activase, by limiting photosynthetic electron transport via disruptions in the thylakoid membrane, or by decreased affinity of rubisco for carbon dioxide and low carbon dioxide to oxygen ratio inside the leaf, thereby increasing photorespiration rate (Feller *et al.*, 1998; Crafts-Brandner and Salvucci, 2000; Salvucci and Crafts- Brander, 2004; Schrader *et al.*, 2004; Wise *et al.*, 2004).

Following emergence, first true leaves are the first green tissues in peanuts that are photosynthetically active, contributing substantially to production of photoassimilates used for further whole plant growth and development. The amount of energy produced by the leaves through photosynthesis depends upon leaf size and photosynthetic efficiency. The net carbon available to drive growth is influenced by photosynthetic pigments available for light energy capture, rate of thylakoid reactions, efficiency of carbon fixation reactions and rate of dark respiration (Taiz and Zeiger, 2010).

The first true leaves develop at the node next to node zero, designated as node one (Boote, 1982). During the early stage of crop growth, leaf area index of peanut seedlings is low (Taiz and Zeiger, 2010). However, as growth progresses, plants develop more leaves and expand existing leaves, increasing the leaf area index. At a later stage, such as the onset of flowering, peanut plants have a substantial number of leaves above the first true leaves. Variation in photosynthetic rate in different leaves within a peanut plant was reported, with higher photosynthetic rates in young, fully expanded leaves than older lower-canopy leaves (Henning *et al.*, 1979). However, studies characterizing the contributions of first true leaves to peanut plant growth at different developmental stages or growth temperature conditions are limited. Therefore, the objectives of this study were to evaluate the contribution of first true leaves of modern peanut cultivars to whole-plant growth under different temperature conditions during the early season and at the onset of flowering as well as to identify the photosynthetic components more closely associated with photosynthetic efficiency of the first true leaves. It was hypothesized that first true leaf development will have a pronounced effect on peanut plant growth under supra-optimal temperature conditions, irrespective of plant developmental stage. In addition, non-stomatal components associated with the thylakoid reactions will more closely drive photosynthetic efficiency of first true leaves in modern peanut cultivars than stomatal factors.

Materials and Methods

Plant material and experimental layout

A two-year field experiment was conducted at the Lang-Rigdon Research Farm of the University of Georgia, Tifton, GA (N 31°52′, W 83°55′, 108 masl) during the growing seasons in 2017 and 2018. The soil series at the site is Tifton loamy sand (fine-loamy, kaolinitic, thermic, Plinthic Kandiudults). Three runner-type peanut cultivars, Georgia-06G (Branch, 2007), Georgia-14N (Branch and Brenneman, 2015) and TifNV-High O/L (Holbrook *et al.*, 2017), were planted on three different dates corresponding to April (early), May (optimal) and June (late) in order to generate differences in temperature conditions at planting and in the early season. For the 2017 growing season, the selected planting dates were April 17, May 10, and June 5 and for the 2018 season, the planting dates were April 25, May 14, and June 11. A split-plot design with planting date as the whole-plot and cultivar as the sub-plot was used, and treatments were replicated four times. For both growing seasons, plots were four single-rows spaced 91 cm apart and 11 m long. Seeds were sown at a depth of approximately 5 cm with a seeding rate of 19 seeds per linear meter. Soil and air temperatures as well as precipitation data were collected from the University of Georgia weather station closest to the experimental location. Fungicide applications followed the schedule for a high-risk category to help protect the trial from damaging level from disease incidence (Kemerait *et al.*, 2018). Overhead sprinkler irrigation was used to irrigate the field according to the University of Georgia Extension recommendations (Porter, 2019). Plant growth and

physiological parameters were collected at two sampling dates, three and five weeks after planting (WAP), for each planting date.

Measurements and sampling protocol

Seedling emergence index. Emergence was recorded daily during the first 14 d after planting (DAP) from a 3 m section of the two middle rows in each plot. The total number of emerged seedlings were counted, with seedlings being considered emerged only when visible first true leaves were completely above the soil surface. The seedling emergence index (SEI) was calculated according to equation described in Maguire (1962).

$$\text{SEI} = \sum \left(\frac{\text{Et}}{\text{Dt}} \right) \qquad [1]$$

where E_t is the number of seedlings emerged and D_t is the number of d after planting at which the seedlings were counted.

Whole plant growth. Whole plants (not including tissues below the cotyledons) from a total of 1.8 m were destructively sampled from the two middle rows of each plot between 0700 and 0800 h at 3 and 5 WAP. Plastic bags with moist paper tower were used to store the harvested plants in order to avoid wilting. The samples were kept in a refrigerator at 4 C until further processing. Measurements included number of plants per 1.8-m section, first true leaf area (FTLA), and tissue dry weight. The first true leaves were separated from the rest of the leaves and leaf area was recorded using a table-top leaf area meter (LI-3100; LI-COR, Lincoln, NE). Then, all the leaves (including the first true leaves) and stems were dried at 80 C for 48 h using a forced air oven and dry weight (DW) of leaves and stems per plant (in g/plant) was recorded.

Physiological Measurements

Pigment content. Pigment contents (chlorophyll A, chlorophyll B, and total carotenoids) were quantified in first true leaf samples at 3 and 5 WAP. Four 5-mm diameter leaf discs were placed in amber vials filled with 5 ml reagent grade ethanol solution (96% ethanol solution) and incubated at 4 C for fourteen d. Afterwards, sample absorbance was read at 665, 649, and 470 nm wavelengths using a multi-well plate reader (Synergy HTX, BioTek, Winooski, VT). Content of chlorophyll A, chlorophyll B, and total carotenoids per unit leaf area were calculated using the equations described in Lichtenthaler and Wellburn (1983) and expressed in $\mu g/cm^2$.

Gas exchange and fluorescence measurements. Along with first true leaf pigment content, gas exchange and fluorescence were recorded at 3 and 5 WAP on single leaflets per leaf (top leaflets further

from peduncle insertion). Light-adapted measurements of gas exchange, chlorophyll fluorescence, and leaf temperatures were determined in the first true leaves between 1100 and 1400 h using the LI-6400XT portable photosynthesis system (LI-COR, Nebraska, USA) and the fluorometer chamber (Model LI-6400-40, LI-COR, Lincoln, NE). The gas exchange measurements included net photosynthesis (A_n), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and leaf temperature (C). The reference CO_2 concentration in the leaf chamber was set as 400 μmol/mol along with photosynthetically active radiation (PAR) of 1500 μmol m/s. Other chamber settings included a flow rate of 500 μmol/s, block temperature matching the ambient air temperature at the time of measurements and relative humidity of 60 ± 10%. The 6400-40 Leaf Chamber Fluorometer was used to record chlorophyll fluorescence measurements. After the steady state net photosynthesis (A_n) and fluorescence (F_s) were reached, the multi-phase protocol was used to determine the maximum fluorescence intensity (F_m'), at which all reaction centers are closed (Earl and Ennahli, 2004). Further, as per the equations given in Maxwell and Johnson (2000) and Genty *et al.* (1989), actual quantum yield of photosystem II (Φ_{PSII}) and electron transport rate through photosystem II (ETR) were calculated as:

$$\Phi PSII = \frac{Fm' - Fs}{Fm'} \qquad [2]$$

$$\text{ETR} = \text{PSII} \times \text{PAR} \times 0.5 \times 0.84 \qquad [3]$$

where 0.5 and 0.84 are coefficients representing the energy distribution between photosystems II and I. for C3 plants and leaf absorbance ratio, respectively.

Dark-adapted measurements, including midday dark respiration (R_d), were obtained in the first true leaves by covering some plants from each plot with a structure consisting of a frame and opaque black felt. The plants were dark-adapted for approximately 2 h from 0930 to 1130 h prior to the measurements. For the dark-adapted measurements, all chamber settings were the same as those used for the light-adapted measurements, except that no illumination was provided in the dark measurements. Gross photosynthesis (A_g) was then calculated by summing R_d (obtained from the dark-adapted assessment) and A_n (obtained from the light-adapted assessment). Gross photosynthesis was expressed in μmol m/s.

From the measured gas exchange and fluorescence parameters, further parameters were derived according to the methods described in Ennahli and
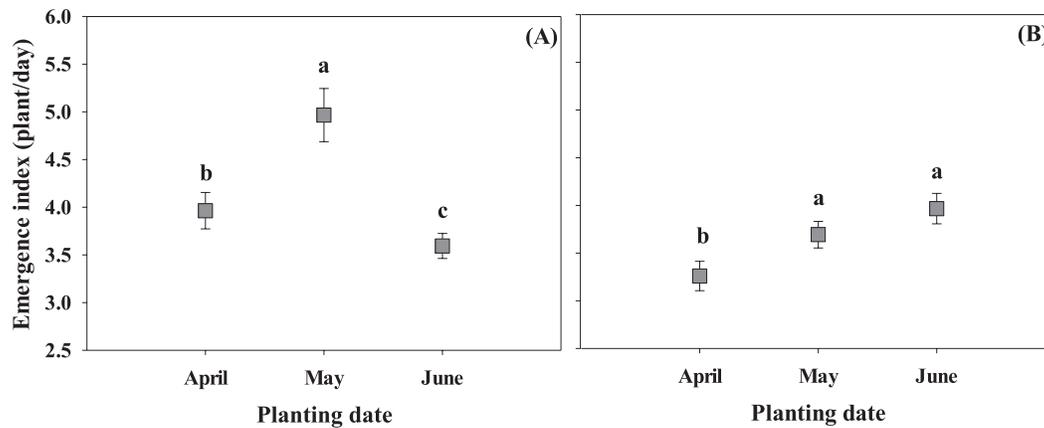
Fig. 1. Seedling emergence index (SEI) for three planting dates, April, May, and June, at 3 and 5 weeks after planting in two seasons, 2017 (A) and 2018 (B). Values represent means ± standard error with data combined across three cultivars within planting dates (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.

Earl (2005), Pilon *et al.* (2018), and Galmés *et al.* (2007). The derived parameters are the electron transport rate to carbon assimilation (ETR$_a$; in μmol m/s), electron transport rate to photorespiration or rate of oxygenation reaction of rubisco (ETR$_p$; μmol m/s), carbon dioxide concentration in chloroplast (C$_c$; μmol/mol), and mesophyll conductance (g$_m$; mol m/s).

Statistical Analysis

A two-way analysis of variance was conducted using JMP Pro 13.0 (SAS Institute, 2016). Planting date and cultivar were considered fixed effects and data from the two years were analysed separately as differences were observed between the years. Replication and replication × planting date were considered as random effects. Means were separated using Fisher's Least Significant Difference *post hoc* test at the 0.05 probability level whenever significant differences were observed. Pairwise correlations were also performed to identify associations between plant growth and the physiological component processes.

Results and Discussion

Uniform and vigorous seedling emergence ensures a successful start to the peanut crop. Along with seed characteristics, environmental conditions influence the rate and time of seedling emergence. Under optimum soil moisture, soil temperature plays a significant role in seedling emergence. For both the growing seasons, April planting dates corresponded to the lowest temperature conditions, whereas June planting dates had the highest temperatures conditions. In 2017, average 10-cm soil temperature within the first 14 d after planting ranged from 21 to 27 C for April, 24 to 30 C for

May, and 24 to 30 C for June. In 2018, temperatures were similar to 2017 in April, but they were higher for May and June, with average 10-cm soil temperatures between 22 and 28 C for April, 25 and 27 C for May, and 28 an 33 C for June.

Seedling emergence index was unaffected by planting date by cultivar interaction, but was significantly impacted by planting date (Fig. 1; *P* = 0.01) and cultivar (Fig. 2; *P* = 0.01) separately in both growing seasons. In 2017, May conditions promoted higher initial seedling emergence rate, followed by April and June (Fig. 1A). In 2018, higher emergence index was observed in May and June compared to April (Fig. 1B). In addition, GA-06G resulted in the highest seedling emergence index across all cultivars in both growing seasons (Fig. 2), which is the cultivar with higher seed weight (Tubbs, 2019). Studies on other species indicated that emergence and development of seedlings are influenced by seed size (Snider *et al.*, 2014; Ries and Everson, 1973). The lower emergence index observed for GA-14N in 2017 can be attributed to the lower seed vigor of this specific cultivar (which has smaller seeds; Tubbs, 2019) due to adverse conditions encountered during 2016 growing season. Seedlings that emerge early have a greater chance of survival and higher relative yield as compared to seedlings that emerge later (Wanjura *et al.*, 1969). Soil temperatures between 27 and 33 C, encountered by the seeds planted in May and June, promoted higher emergence rate of the peanut cultivars.

First true leaf area and whole-plant dry weight were not affected by planting date by cultivar interaction at either 3 or 5 WAP for both growing seasons. However, a significant effect of planting date was observed for the measured growth
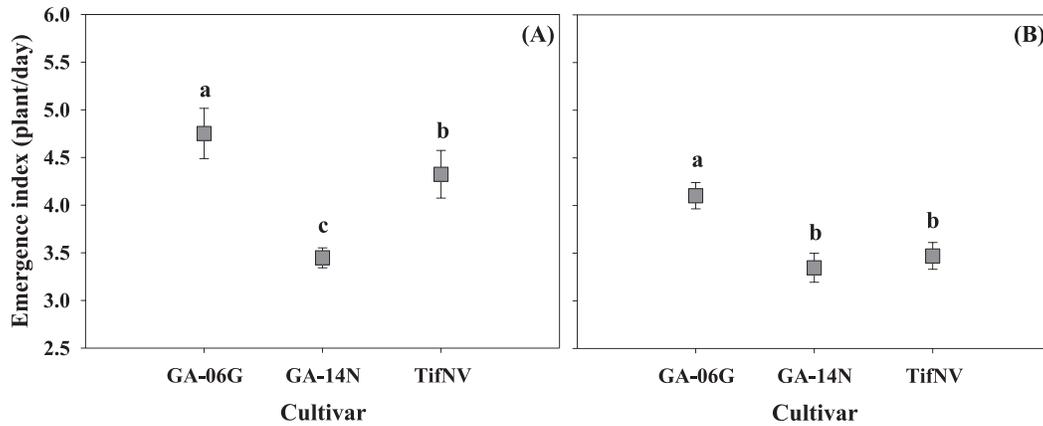
Fig. 2. Seedling emergence index (SEI) for peanut cultivars, GA-06G, GA-14N, and TifNV, at 3 and 5 weeks after planting in two seasons, 2017 (A) and 2018 (B). Values represent means ± standard error with data combined across three planting dates within cultivar (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.

parameters at both sampling times in 2017 and 2018, except for the first true leaf area at 3 WAP in 2017 (Fig. 3 and Table 1). First true leaf area was greater in June than May and April at all times (Fig. 3). For instance, in 2018, FTLA in June was, on average, 15% higher than May and April at 3 WAP, whereas at 5 WAP, June planting resulted in 36% and 30% greater FTLA than April and May, respectively (Fig. 3B). Total plant dry weight was affected by planting dates at 3 and 5 WAP in both growing seasons (Table 1). Temperature conditions for the June planting resulted in the highest dry matter accumulation, followed by May and lastly April, with the lowest dry matter accumulation. At 5 WAP in 2017, total dry weight for the June planting date was approximately 2.9-fold higher than April and 1.4-fold higher than May (Table 1). Similarly, in 2018, June resulted in 2.7-fold higher total dry weight per plant compared to April and May. Other research reported similar effect of low

temperature conditions on peanut growth and development (Leong and Ong, 1983; Allen and Ort, 2001). Leong and Ong (1983) suggested a linear increase in rate of growth and development of peanuts with increasing temperature from 19 to 31 C. Jordan *et al.* (2019) reported an increase in population and growth of peanut plants with planting shift from mid-April to end of May.

First true leaf area was also significantly affected by cultivar at 3 and 5 WAP in 2017 (Fig. 4). TifNV had the highest FTLA at both sampling times, followed by GA-06G and lastly GA-14N. More specifically, FTLA of TifNV was, on average, 25% and 34% greater than GA-06G and GA-14N, respectively (Fig. 4). Prasad *et al.* (2006) studied the effect of different soil temperatures on seedling emergence and early season growth of six peanut cultivars. The authors reported lower germination rates and early season growth for planting dates corresponding to coolest sowing

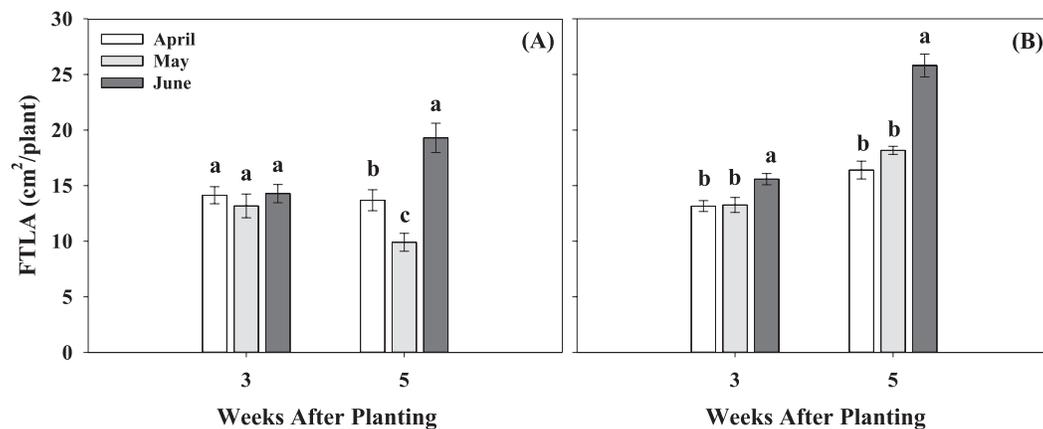**Fig. 3. First true leaf area per plant (FTLA; cm²/plant) for three planting dates, April, May, and June, at 3 and 5 weeks after planting in two seasons, 2017 (A) and 2018 (B). Values represent means ± standard error with data combined across three cultivars within planting dates (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.**

Table 1. Dry weight measurements at 3 and 5 weeks after planting (WAP) for the three selected planting dates, April, May, and June, and for three peanut cultivars, Georgia-06G, Georgia-14N, and TifNV-High O/L, during two growing seasons, 2017 and 2018.

| | Total DW[a] | | | |
| | 2017 | | 2018 | |
Source of variation	3 WAP	5 WAP	3 WAP	5 WAP
Planting Date	———(g/plant)———			
April	0.63 c[b]	2.11 c	0.88 b	3.11 b
May	0.94 b	4.48 b	0.99 b	2.99 b
June	1.53 a	6.10 a	1.88 a	8.09 a
Cultivar				
GA-06G	1.14 a	4.52 a	1.36 a	5.03 a
GA-14N	0.86 b	3.64 b	1.22 a	4.37 a
TifNV	1.10 a	4.53 a	1.53 a	4.79 a

[a]DW, dry weight.

[b]Values represent means (n= 12) and those not sharing a common letter within each year and sampling date are significantly different at P < 0.05.
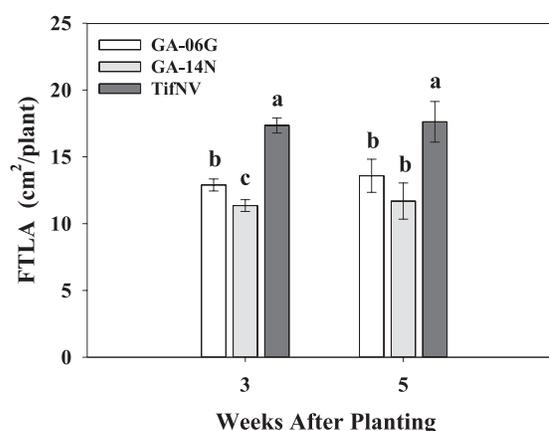

Fig. 4. First true leaf area per plant (FTLA/plant) for peanut cultivars, GA-06G, GA-14N, and TifNV, at 3 and 5 weeks after planting in 2017. Values represent means ± standard error with data combined across three planting dates within cultivar (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.

temperatures. Also, differences in early vigor among the cultivars were due to differences in rate of first true leaf differentiation, which resulted in significant differences in early seedling development among different peanut cultivars.

The current research indicates that temperature conditions substantially impacted overall peanut plant growth, including first true leaf development (Table 1 and Fig. 3). Average daily temperature calculated from each planting date to 3 WAP (2017 and 2018 averaged) varied from 13.7 to 26.7 C, 19.5 to 29.9 C, and 21.4 to 31 C for April, May, and June, respectively. Moreover, the average daily temperatures from planting to 5 WAP were 15.5 to 28.2 C for April, 19.7 to 29.9 C for May, and 21.7 to 31.6 C for June. Rapid development and differentiation of first true leaves is an indication of greater seedling vigor in other crops, such as cotton (*Gossypium hirsutum* L.; Pilon *et al*., 2016). Dry weight is also a commonly used parameter to indicate plant vigor (Snider *et al*., 2016). Pairwise correlations showed positive relationship between first true leaf area and whole-plant dry matter at early season (r = 0.42) and at the onset of flowering (r = 0.52), suggesting contribution of the first true leaf development to peanut plant vigor (Tables 2 and 3).

In addition to FTLA, pigment content (chlorophyll A and total carotenoids), T_{leaf}, g_s, and C_i measured on the first true leaf were also positively correlated with whole-plant dry weight during early growth stage (Table 2). However, whole-plant dry weight was negatively correlated with multiple photosynthetic variables measured on the first true leaf such as net photosynthesis, ETR, ETR_a, and ETR_p. Net photosynthesis along with ETR decreased with an increase in plant dry weight. In addition, there was a positive correlation between plant dry matter and R_d of first true leaves. At the onset of flowering, T_{leaf} (r = 0.354) was positively correlated with whole-plant dry weight, whereas negative relationship was observed between whole-plant dry weight and measured pigment concentrations and C_c (Table 3). No significant correlation was observed between whole-plant dry weight and net photosynthesis at 5 WAP. First true leaf area development is a potential indicator of whole-plant vigor at early season and the onset of flowering; however, the photosynthetic efficiency of the first true leaf does not have direct impact on whole-plant growth, because A_n was negatively associated with whole-plant dry matter at early stage and not associated at the onset of flowering, when first true leaves were fully developed and the plant had a high number of upper canopy leaves photosynthetically active.

No significant planting date by cultivar interation was observed for any of the pigment contents collected at 3 and 5 WAP in the 2017 and 2018 seasons. However, all pigment contents were significantly affected by planting date at 3 and 5 WAP in both seasons, except for chlorohyll B at 5 WAP in 2018 (Fig. 5). At 3 WAP in the 2017 season, the April planting resulted in the highest chlorophyll A and total carotenoids, followed by May and June plantings (Fig. 5A and E). Chlorophyll B at 3 WAP in 2017 was higher for the May planting date compared to April and June (Fig. 5C). A negative impact of delayed planting

Table 2. Pairwise correlation coefficients for growth parameters, pigment contents and physiological parameters at 3 WAP for three cultivars, three planting dates, and two growing seasons combined.

	Chl. B[a]	Cart.	FTLA	A_n	g_s	ETR	T_{leaf}	R_d	A_g	ETR_a	ETR_p	C_i	C_c	g_m	DW
Chl. A	0.79**[b]	0.95**	0.31*	-0.11	0.05	0.15	0.82**	0.24	-0.07	0.05	0.20	0.13	-0.10	0.14	0.33**
Chl. B		0.55**	0.29*	-0.03	0.06	0.09	0.62**	0.19	0.00	0.05	0.09	0.10	-0.03	0.16	0.22
Cart.			0.26*	-0.12	0.01	0.22	0.80**	0.22	-0.08	0.08	0.28	0.09	-0.13	0.11	0.28*
FTLA				-0.14	0.02	-0.17	0.35*	0.11	-0.13	-0.18	-0.11	0.13	0.04	0.07	0.42**
A_n					0.56**	0.42**	-0.46**	-0.22	0.98**	0.84**	-0.09	0.04	0.07	0.12	-0.32*
g_s						-0.10	-0.20	0.13	0.60**	0.30*	-0.44**	0.52**	0.08	0.02	0.32*
ETR							-0.15	-0.09	0.41**	0.84**	0.86**	-0.32*	-0.51**	0.11	-0.48**
T_{leaf}								0.30*	-0.42**	-0.34*	0.07	0.12	0.04	0.13	0.41**
R_d									-0.04	-0.07	-0.07	0.22	0.029	0.3	0.30*
A_g										0.84*	-0.11	0.08	0.08	0.05	-0.28
ETR_a											0.44**	-0.14	-0.25	0.02	-0.45**
ETR_p												-0.39**	-0.60**	-0.11	-0.37**
C_i													0.07	0.14	0.50**
C_c														0.05	-0.01
g_m															-0.02

[a]Abbreviations: first true leaf area per plant, FTLA; dry weight per plant, DW; chlorophyll A, Chl. A; chlorophyll B, Chl. B; total carotenoids, Cart.; net photosynthesis, A_n; stomatal conductance, g_s; electron transport rate, ETR; leaf temperatures, T_{leaf}; dark respiration, R_d; gross photosynthesis, A_g; electron transport to carbon assimilation, ETR_a; electron transport to photorespiration, ETR_p; intercellular CO_2 concentration inside leaf, C_i; carbon dioxide concentration in chloroplast, C_c; mesophyll conductance; g_m.

[b]* = $P < 0.05$, ** = $p < 0.01$

was observed at 3 and 5 WAP, where all the measured pigment contents were the lowest for June. For example, at 5 WAP, chlorophyll A was 38% lower in June compared with April (Fig. 5A). Similarly, total carotenoids were 52% lower in June than April planting (Fig. 5E). In 2018, samples for pigment analysis were not collected at 3 WAP for the first planting date (April) due to rainy conditions. June resulted in substantially higher content of all pigments measured when

Table 3. Pairwise correlation coefficients for growth parameters, pigment contents and physiological parameters at 5 WAP for three cultivars, three planting dates, and two growing seasons combined.

	Chl. B[a]	Cart.	FTLA	A_n	g_s	ETR	Tl_{eaf}	R_d	A_g	ETR_a	ETR_p	C_i	C_c	g_m	DW
Chl. A	0.22	0.95**[b]	0.02	0.08	0.06	0.03	-0.12	0.33	0.15	0.09	-0.05	-0.21	0.07	-0.02	-0.42**
Chl. B		-0.06	-0.52**	0.20	-0.13	0.09	-0.56**	0.10	0.23	0.16	-0.01	-0.31*	0.11	-0.10	-0.34**
Cart.			0.18	0.02	0.09	0.01	0.02	0.33*	0.09	0.05	-0.05	-0.14	0.02	0.02	-0.34**
FTLA				-0.21	0.01	-0.22	0.58**	0.02	-0.21	-0.23	-0.18	-0.08	-0.13	0.26*	0.52**
A_n					0.53**	0.74**	-0.42**	-0.20	0.98**	0.92**	0.43**	0.02	-0.02	0.01	-0.11
g_s						0.36**	-0.10	0.09	0.56**	0.49**	0.16	0.60**	0.09	-0.16	0.16
ETR							-0.32*	-0.19	0.71**	0.94**	0.92**	0.17	0.14	0.06	-0.25
T_{leaf}								0.06	-0.42**	-0.40**	-0.19	-0.03	-0.11	0.14	0.54**
R_d									0.01	-0.11	-0.25	0.15	0.02	-0.16	-0.11
A_g										0.91**	0.38**	0.06	-0.02	-0.03	-0.14
ETR_a											0.73**	0.13	0.07	0.02	-0.21
ETR_p												0.19	0.20	0.09	-0.25
C_i													0.07	-0.15	0.03
C_c														-0.00	-0.16*
g_m															0.26

[a]Abbreviations: first true leaf area per plant, FTLA; dry weight per plant, DW; chlorophyll A, Chl. A; chlorophyll B, Chl. B; total carotenoids, Cart.; net photosynthesis, A_n; stomatal conductance, g_s; electron transport rate, ETR; leaf temperatures, T_{leaf}; dark respiration, R_d; gross photosynthesis, A_g; electron transport to carbon assimilation, ETR_a; electron transport to photorespiration, ETR_p; intercellular CO_2 concentration inside leaf, C_i; carbon dioxide concentration in chloroplast, C_c; mesophyll conductance; g_m.
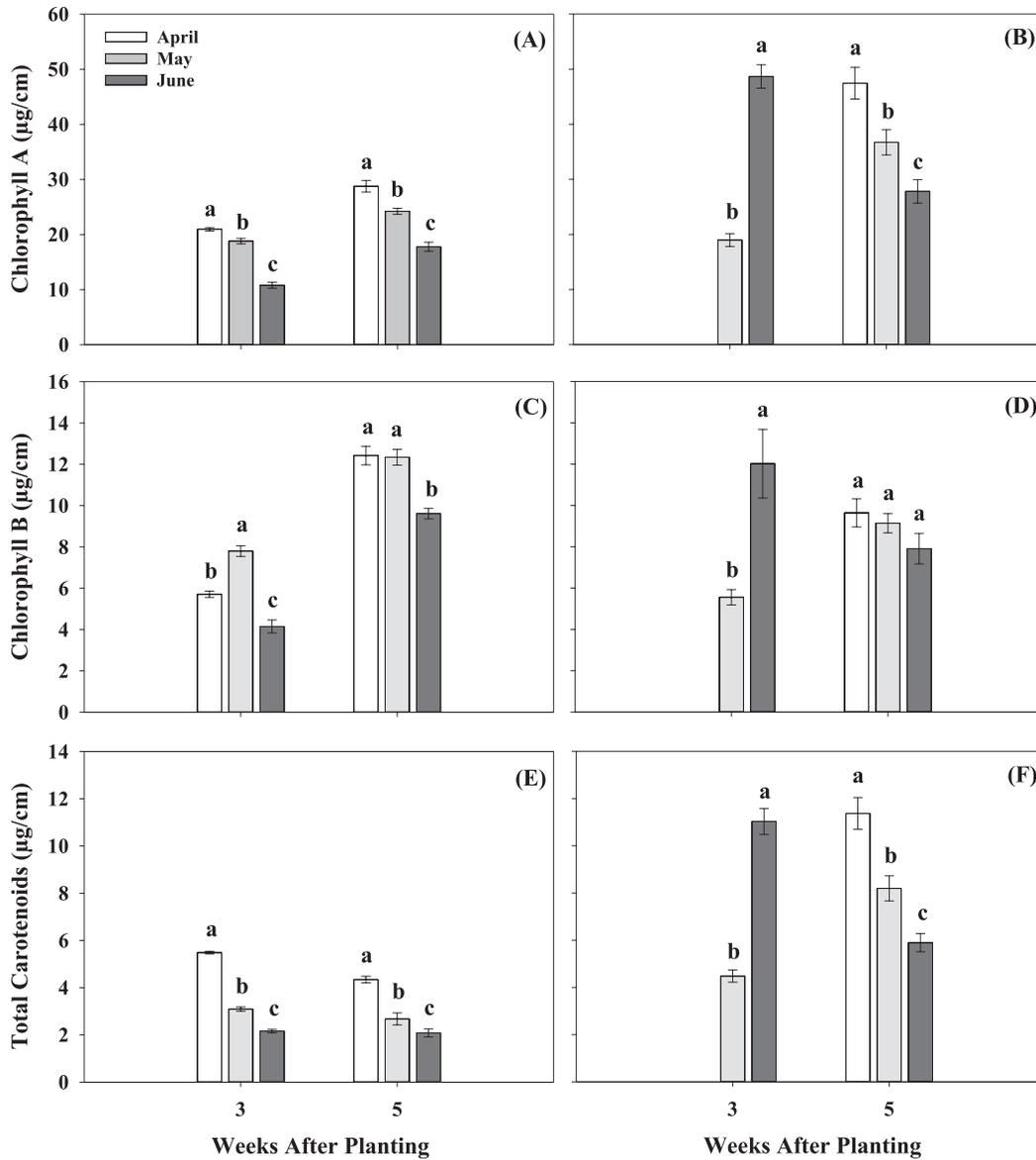
[b]* = $P < 0.05$, ** = $p < 0.01$

Fig. 5. Pigment contents, chlorophyll A, chlorophyll B, and total carotenoids, for the three planting dates, April, May, and June, at 3 and 5 weeks after planting for two growing seasons, 2017 (A, C, E) and 2018 (B, D, F). Values represent means ± standard error with data combined across three cultivar within planting date (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.

compared to May at 3 WAP (Fig. 5B, D and F). Similar to trends observed in the 2017 season, a negative effect of delayed planting on pigment contents was observed at 5 WAP for the 2018 season. Specifically, chlorophyll A and chlorophyll B were 1.7- and 1.2-fold higher, respectively, for the April planting compared to June (Fig. 5B and D). Synthesis of leaf chlorophyll is known to decrease with increase in temperature, especially above 32 C, which likely accounted for the lower pigment content in plants planted in June compared with those planted in April. In addition, pigment content was affected by cultivar in 2018, except for chlorophyll A and total carotenoids at 3 WAP

(Fig. 6). At 3 WAP, TifNV had, on average, 62% higher chlorophyll B than GA-06G and GA-14N (Fig. 6B). At 5 WAP, all measured pigment contents were greater for GA-06G and TifNV compared to GA-14N (Fig. 6).

Leaf temperature was also significantly impacted by different planting dates (Fig. 7). Increases in leaf temperature were observed with delayed planting. For example, at 5 WAP in 2017, leaf temperature for the June planting was, on average, 10 C higher than April (Fig. 7A). In addition, at 5 WAP in 2018, albeit at a lesser extent, the June planting resulted in the highest leaf temperature of 33.1 C compared to average leaf temperature of 30
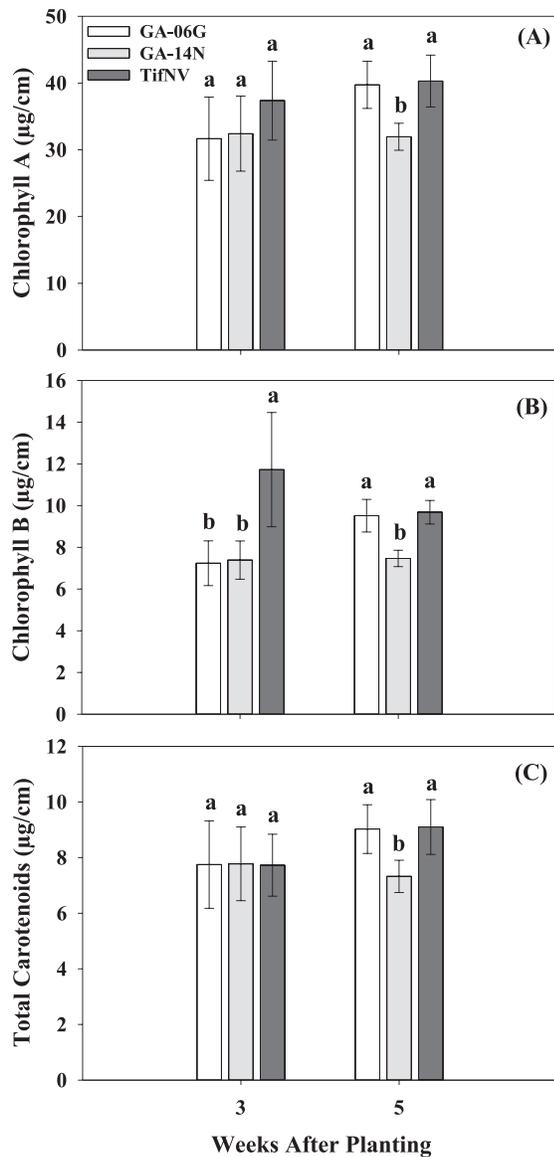
Fig. 6. Pigment contents, chlorophyll A, chlorophyll B, and total carotenoids, at 3 and 5 weeks after planting for the three peanut cultivars, Georgia-06G, Georgia-14N, and TifNV-High O/L, for the 2018 growing season. Values represent means ± standard error with data combined across three planting dates within cultivar (n = 12), except for data across 3 WAP, in which n = 8 Values not sharing a common letter within each week are significantly different at P < 0.05.

C in April (Fig. 7B). Extreme temperatures (soil, air, and leaf) can affect photosynthesis, thus affecting growth and development. Ketring (1986) reported a reduction in photosynthesis in peanut leaves with further reduction in growth when leaf temperature was above 35 C. June plantings resulted in the highest temperatures and, although none of the planting conditions promoted leaf temperatures above 35 C, significant negative correlations were observed between leaf temperature and net photosynthesis (Tables 2 and 3). Net

photosynthesis decreased with the increase in leaf temperature at 3 WAP (r = -0.46) and 5 WAP (r = -0.42) when the data was combined across both growing seasons. Cox (1979) suggested 28 C as optimum temperature for growth during early development and onset of flowering in peanut plants. Additionally, the authors indicated absence of growth at night temperatures of 15.5 C. The temperatures corresponding to the April planting were closer to optimal temperatures (28 C) for early growth and development of peanuts compared to larger deviation from optimum temperatures for the May and June plantings. Electron transport rate to carbon assimilation of the first true leaf was also negatively correlated with leaf temperature, irrespective of sample date (Tables 2 and 3). Higher temperatures encountered by plants in the June planting resulted in negative impact on photosynthetic efficiency of first true leaves, mainly affecting the thylakoid reactions.

Net photosynthesis of the first true leaf was unaffected by planting date, cultivar, and planting date x cultivar interaction at all sampling dates on both growing seasons (Table 4). However, a significant planting date effect was observed for various photosynthetic components, including ETR, ETR_a, ETR_p, C_i, C_c, and g_s for the 2017 season, and ETR_p, C_i, and g_s for the 2018 season (Tables 4 and 5). At early season, ETR, ETR_a, and ETR_p were greater for the April planting compared to June planting, whereas C_i, C_c, and g_s were higher for June compared to April in 2017 (Tables 4 and 5). In 2018, ETR_p, C_i and g_s were greater for June than those for May planting date. However, at the onset of flowering, planting date affected only C_i in 2017, with higher values for June planting compared to April, and ETR_p in 2018, with higher values for April and May compared to June (Tables 4 and 5). Although photosynthetic components of first true leaves were affected by different temperature conditions promoted by the different planting dates, net photosynthesis remained unaffected. In order to identify which photosynthetic components were more closely associated with photosynthesis maintenance under different environmental conditions, pairwise correlations were obtained among photosynthetic components of the thylakoid reactions as well as the carboxylation reactions on the first true leaves. Positive correlations were observed between net photosynthesis of the first true leaves and multiple photosynthetic components, such as ETR, ETR_a, ETR_p, and g_s (Tables 2 and 3). At early growth, net photosynthesis was strongly associated with A_g (r = 0.98) and ETR_a (r = 0.84), while a weaker correlation was observed between A_n and g_s (r =
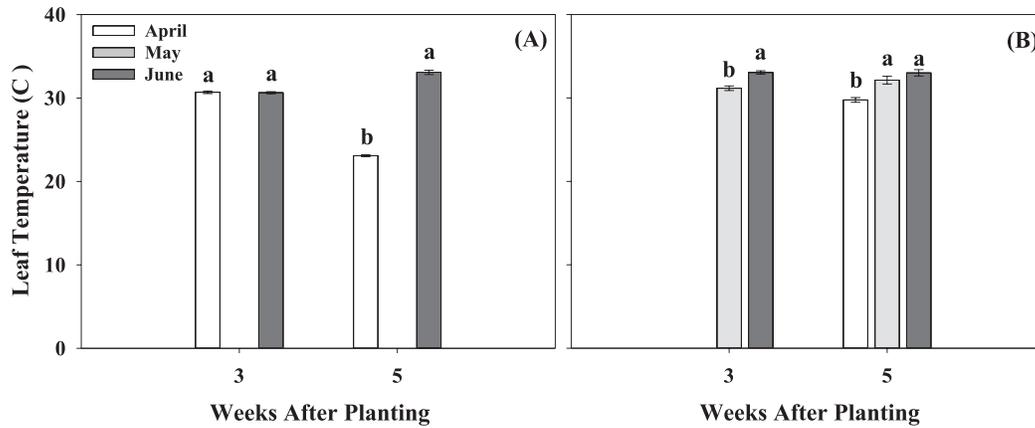
Fig. 7. Leaf temperature at 3 and 5 weeks after planting for the three planting dates, April, May, and June, for two growing seasons, 2017 (A) and 2018 (B). Values represent means ± standard error with data combined across three cultivars within planting date (n = 12). Values not sharing a common letter within each week are significantly different at P < 0.05.

0.56; Table 2). Similarly, at the onset of flowering, net photosynthesis showed a strong correlation with A_g (r = 0.98) and ETR_a (r = 0.92) and was weakly associated with g_s (r = 0.52; Table 3). Stomatal and non-stomatal factors affected photosynthetic responses of first true leaves to temperature; however, stronger influence of thylakoid components was observed on net photosynthesis at early season and the onset of flowering, as evidenced by strong, positive correlations with ETR_a and weak association with g_s as well as no significant relationship with C_i and C_c (Tables 2 and 3).

Summary and Conclusions

It is concluded that a rapid development of first true leaves of peanut plants contribute to whole-plant growth at early season and at the onset of flowering under adverse temperature conditions due to increased surface area available for light interception. However, the photosynthetic efficiency of the first true leaf has no direct impact on first true leaf surface area, irrespective of the growth stage. Furthermore, net photosynthesis of first true leaves was unaffected by temperature conditions at early season and even at the onset of flowering, when the plants have multiple upper leaves

Table 4. Net photosynthesis (A_n), gross photosynthesis (A_g), electron transport rate (ETR), electron transport to carbon assimilation (ETR_a), and electron transport to photorespiration (ETR_p) at 3 and 5 weeks after planting (WAP) for the three planting dates, April, May, and June, and three peanut cultivars, Georgia-06G, Georgia-14N, and TifNV-High O/L, for 2017 and 2018 seasons.

Year	Effect	A_n		A_g		ETR		ETR_a		ETR_p	
		3 WAP	5 WAP	3 WAP	5 WAP	3 WAP	3 WAP	5 WAP	3 WAP	5 WAP	3 WAP
2017	Planting Date						μmol/m²/s				
	April	21.6 a[a]	21.8 a	23.9 a	23.4 a	266.3 a	158.0 a	152.6 a	115.2 a	113.7 a	42.8 a
	May	–	–	–	–	–	–	–	–	–	–
	June	17.7 a	17.1 a	20.9 a	18.7 a	162.8 b	139.2 a	110.0 b	96.3 a	52.8 b	42.9 a
	Cultivar										
	GA-06G	17.9 a	22.0 a	20.6 a	23.3 a	217.2 a	148.1 a	127.4 a	111.5 a	89.9 a	36.6 a
	GA-14N	20.5 a	15.9 a	23.2 a	17.7 a	223.1 a	132.6 a	136.1 a	91.5 a	87.0 a	41.1 a
	TifNV	20.5 a	20.5 a	23.5 a	22.2 a	203.2 a	165.1 a	130.4 a	114.2 a	72.9 a	50.9 a
2018	Planting Date										
	April	–	18.2 a	–	21.0 a	–	127.9 a	–	98.7 a	–	29.3 ab
	May	21.2 a	17.9 a	23.6 a	19.7 a	173.5 a	156.2 a	120.8 a	104.5 a	52.6 b	51.8 a
	June	18.4 a	17.3 a	22.1 a	19.4 a	214.5 a	97.1 a	130.4 a	84.0 a	84.0 a	13.1 b
	Cultivar										
	GA-06G	22.0 a	17.7 a	24.7 a	19.6 a	202.4 a	137.5 a	133.5 a	98.2 a	69.0 a	39.3 a
	GA-14N	17.3 a	20.2 a	20.9 a	22.0 a	185.0 a	142.8 a	117.5 a	106.3 a	67.5 a	36.5 a
	TifNV	20.1 a	15.4 a	22.9 a	18.4 a	194.4 a	100.9 a	125.9 a	82.6 a	68.5 a	18.3 a

[a]Values represent means (n=12) and those not sharing a common letter within each treatment and year are significantly different at P < 0.05.

Table 5. Midday dark respiration (R_d), intercellular CO_2 concentration inside leaf (C_i), CO_2 concentration in the chloroplast (C_c), stomatal conductance (g_s), and mesophyll conductance (g_m) at 3 and 5 weeks after planting (WAP) for the three planting dates, April, May, and June, and three cultivars, Georgia-06G, Georgia-14N, and TifNV-High O/L, for both 2017 and 2018 seasons.

Year	Effect	R_d		C_i		C_c		g_s	
		3 WAP	5 WAP	3 WAP	5 WAP	3 WAP	5 WAP	3 WAP	5 WAP
2017	Planting Date	——$\mu mol/m^2/s$——		——$\mu mol/mol$——		——$\mu mol/mol$——		——$\mu mol/m^2/s$——	
	April	2.35 a[a]	1.61 a	220.4 b	266.6 b	153.4 b	274.8 a	0.29 b	0.34 a
	May	–	–	–	–	–	–	–	–
	June	3.16 a	1.62 a	323.7 a	308.7 a	283.6 a	319.8 a	0.52 a	0.41 a
	Cultivar								
	GA-06G	2.68 a	1.30 a	291.3 a	282.6 a	165.5 a	347.6 a	0.35 a	0.40 a
	GA-14N	2.65 a	1.85 a	237.8 a	309.3 a	217.0 a	273.7 a	0.45 a	0.38 a
	TifNV	2.93 a	1.70 a	287.0 a	271.1 a	273.0 a	270.5 a	0.41 a	0.36 a
2018	Planting Date								
	April	–	2.80 a	–	286.3 a	–	490.0 a	–	0.46 a
	May	2.44 b	1.79 a	268.4 b	276.5 a	462.6 a	403.0 a	0.35 b	0.33 a
	June	3.71 a	2.08 a	317.8 a	271.8 a	199.9 a	-4621.2 a	0.48 a	0.45 a
	Cultivar								
	GA-06G	2.72 a	1.95 ab	303.3 a	265.0 a	244.0 a	256.9 a	0.52 a	0.39 a
	GA-14N	3.65 a	1.76 b	301.2 a	304.8 a	456.1 a	307.5 a	0.37 b	0.50 a
	TifNV	2.86 a	2.95 a	274.7 b	264.8 a	293.7 a	-4292.5 a	0.35 b	0.36 a

[a]Values represent means (n=12) and those not sharing a common letter within each treatment and year are significantly different at $P < 0.05$.

photosynthetically active, mainly due to improved activity of non-stomatal components associated with the thylakoid reactions. Whole-plant growth is more strongly associated with development of first true leaf than photosynthetic efficiency of those leaves in the peanut crop.

Acknowledgments

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