Distribution and Diurnal Variation of Nonstructural Carbohydrate in Peanut Grown Under Unshaded and Shaded Conditions[1]

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ABSTRACT

Diurnal variation in carbohydrate content of plant parts has been recognized for many years. Such variation in the peanut (*Arachis hypogaea* L.) plant has received little attention. In order to better understand the dynamics of carbohydrate accumulation and utilization in peanut, a greenhouse study was undertaken to examine the effects of shading on diurnal patterns of carbohydrate distribution in this crop. The cultivar, NC 4, was grown under unaltered greenhouse conditions (control) or under shade cloth (37% of photosynthetic photon flux of control). Plants were harvested 40 and 64 days after planting, at beginning bloom and full-pod. At each harvest date plants were sampled every three hours during a 24-h cycle. The concentrations of reducing and nonreducing sugars and starch were analyzed in leaf, stem, and root plus nodule tissues for all samples. Control plants accumulated about twice the dry matter, nodule mass, and fixed N_2 as shaded. The carbohydrate concentration was greater in tissues of controls than in those of shaded plants. Growth stage affected both carbohydrate concentration in tissues and the diurnal variation within tissues. All plants harvested at the bloom stage showed significant but individual diurnal effects on concentrations of reducing and nonreducing sugars and starch in leaves, and significant and comparable patterns of nonreducing sugars and starch in roots. Plants harvested at the pod stage exhibited few significant diurnal effects; there was substantial plant-to-plant variability at this stage. Results from this study emphasize the importance of considering the time of day and developmental stage when analyzing nonstructural carbohydrates in peanut tissues.

Key Words: *Arachis hypogaea* L., Kjeldahl nitrogen, growth stage.

Diurnal variation in carbohydrate content of legumes is well documented (16). Many investigators who have observed diurnal patterns of carbohydrates in legumes have examined forage species, specifically alfalfa (*Medicago sativa* L.) and clovers (*Trifolium* spp.) (4, 7, 13). These investigations were undertaken primarily to determine the time of day when nutritional content of legume forage was optimal. More recent investigators have been interested in the daily dynamics of carbohydrate concentration with respect to accumulation, translocation and utilization by the whole plant (3, 11, 16, 22). These later scientists were attempting to better understand how plants utilize current and accumulated photosynthate and the extent to which the whole plant depends on leaves versus other organs for energy during darkness. However, the paucity of information about pool sizes and turn-over rates still limits our understanding of these phenomena. The legume species presently receiving the most attention with regard to the study of photosynthate utilization is soybean (*Glycine max* (L.) Merr).

The authors are unaware of previous studies examining diurnal variation of carbohydrate concentration in the peanut (*Arachis hypogaea* L.). In a prior study (5), we examined carbohydrate content of peanut tissues under different light conditions at a specific time of day (three to four hours after the start of the photoperiod). It is likely the results of that study might have differed had the plants been sampled at a different hour.

Given that possibility, the primary objective of this study was to examine the distribution and diurnal changes in concentration of nonstructural carbohydrate (NSC) in peanut tissues at two growth stages. In addition, we wanted to compare two light regimes in the greenhouse. We hoped to establish two general populations, one growing "normally" under adequate irradiance (control) and the other having significantly less growth under irradiance-limiting conditions (shaded). It was expected that control plants would accumulate more NSC than shaded plants and that diurnal changes might follow different patterns under the two light regimes.

Materials and Methods

This research was conducted in a greenhouse at North Carolina State University from June 3 through August 5, 1983.

Seeds of the virginia peanut cultivar NC 4 were pregerminated approximately 36 h. Four seed were planted 2.5 cm deep in a 1:1 mix of vermiculite and sand in 15 cm diameter pots. Before covering the seed, 2.0 mL of yeast extract mannitol (YEM) broth containing about 10^9 organisms per mL of "cowpea" *Bradyrhizobium* strain 32Hl was poured directly over the seed. One week after planting, the inoculation precedure was repeated, pouring the inoculum on the surface of the medium, to insure more than adequate numbers of rhizobia for infection and nodulation to occur. After two weeks the peanuts were thinned to one plant per pot.

Peanuts were grown under unsupplemented greenhouse lighting (control) or under polypropylene shade cloth designed to exclude 63% of sunlight (shade). The temperature of the greenhouse was thermostatically-controlled, and when temperature exceeded 26.7 C, a cooling system became operational. Plants were watered daily, alternating between deionized water and a nitrogen (N)-free nutrient solution (15). Assuming that the potting medium could supply only an insignificant amount of N, these plants received virtually all their N from symbiotic fixation.

Peanut plants were harvested at beginning bloom 40 days after planting (DAP) and at full pod, 64 DAP (2). On both harvest dates, plants were sampled every three h beginning at 0700 EDT and ending at 0400 EDT of the following day. At each sample time plants were separated into leaves plus petioles, stems, roots plus nodules, and fruit (when present). The root system of each plant was washed with tap water. All tissues were placed in a freezer set at -18 C, then freeze dried and stored in the freezer at -18 C until grinding and analysis. Dry weights were taken for all tissues. Each sample was ground in a cyclone sample mill to pass a 1.0 mm screen.

In addition to carbohydrate sampling, plants sampled at 1000, 1300 and 1600 EDT were examined in more detail. Morphological and phenological traits were recorded and leaf area was measured with a

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portable leaf area meter (LICOR LI-3000, Licor Corp., Lincoln, NB.[3]). The number and weight of nodules per plant were also noted.

Sugar and starch fractions of ground tissue-samples were separated using the techniques described by Smith (19). The starch fraction was hydrolyzed to glucose following the same procedure. A quantitative measure of reducing power was made on aliquots of the sugar pool directly, the sugar pool after 1.0 N H_2SO_4 acid hydrolysis, and the hydrolyzed starch fraction, using the procedure of Umbreit *et al.*(21), as modified by Mateles (14). A quantitative estimate of nonreducing sugars was calculated by subtracting reducing-sugar concentration from the total-sugar concentration (sugar pool after acid hydrolysis). Carbohydrate data are reported as g glucose equivalents. It was assumed that non-reducing sugars represent primarily sucrose. The N content of samples was determined by a Kjeldahl digestion, employing a zirconium copper catalyst (6), followed by NH_4^+-N analysis of the digest using the phenol-hypochlorite procedure (20).

This experiment consisted of three replicates in different areas of the greenhouse. Within a replicate, control and shaded plants were grouped separately. The experimental design was a split-split block. The two harvest dates were treated as main blocks. Sample times were handled as sub-blocks within dates, and light treatments were treated as sub-blocks within sample times. Data were analyzed using standard analysis of variance techniques. In particular, the effects of time-of-day on carbohydrate concentrations were analyzed separately for control or shaded treatments on a given day.

Results and Discussion

Sunrise and sunset occured at 0608 and 2033 EDT, respectively, 40 DAP, and at 0626 and 2016, respectively, 64 DAP. As shown by photosynthetic photon flux readings the light conditions were similar for both dates with the exception of intermittent cloud cover (Table 1). Shaded plants received approximately one-third of the photosynthetic photon flux as the control plants.

Table 1. Photosynthetic photon flux levels under control and shaded conditions on two sample dates.

	July 12		August 5	
Hour	Control	Shaded	Control	Shaded
	μ E m^{-2} s^{-1}			
0700	327	103	253	80
1000	950	330	830	270
1300	1620	530	-	-
1600	530	170	770	240
1900	180	50	120	35

Plant growth and the distribution of carbohydrate. Control and shaded conditions in the greenhouse supported two generally distinct populations of plants (Table 2). Shaded peanut exhibited signs of etiolation, with longer stems than controls. Control plants developed more leaves, but total leaf area was significantly, though marginally, reduced by shade only at 40 DAP (Table 2). The specific leaf area of shaded plants was greater than that for control plants. Dry matter accumulation was lower for shaded plants. Flowering began about 30 DAP for control plants and was delayed about four days by shade. Pegging and fruit production were also delayed under shade. At 64 DAP, shaded peanuts had 14% of the immature-fruit weight of the unshaded peanuts. More nodules developed on the control plants and, as with other tissues, nodule dry weight was lower for shaded plants. In addition, more N_2 fixation occurred in control plants, compared to those under shade. The effects of shading on plant growth in this study are similar to those reported by others who had begun shading treatments shortly after emergence (12).

Table 2. Mean peanut plant traits at two growth stages.

	Bloom stage (40 DAP)		Pod stage (64 DAP)	
Plant trait	Control	Shaded	Control	Shaded
Length of main stem (cm)	13.0	30.0**	7.9	50.0**
No. of leaves	38	26**	82	53**
Leaf area (cm^2)	480.0	404.9*	1390.3	1131.2
Specific leaf area (cm^2 $leaf^{-1}$)	12.6	15.2**	17.4	20.9*
Leaf weight (g $plant^{-1}$)	3.47	2.07**	9.00	5.36**
Stem weight (g $plant^{-1}$)	1.96	1.34**	7.32	3.86**
Root weight (g $plant^{-1}$)	1.86	0.65**	3.81	1.00**
Fruit weight (g $plant^{-1}$)	—	—	2.61	0.36**
Nodule weight (mg $plant^{-1}$)	148.2	59.1**	295.5	126.4**
Nodule no. per plant	162	62**	360	144**
N fixed (mg N $plant^{-1}$)	112.2	51.7**	553.5	232.8**

*, ** indicate a significant difference at $P < 0.05$ and 0.01, respectively, between shaded and control plants on a particular harvest date.

The accumulation of NSC was greater in control than in shaded peanut (Table 3). At 40 DAP, concentrations of all carbohydrate fractions measured, except leaf reducing-sugars and stem nonreducing-sugars, were significantly higher in control plants. Unlike most carbohydrate pools at that date, the concentration of leaf reducing-sugar was greater in shaded plants. At 64 DAP, concentrations of non-reducing sugars in leaves, starch in stems, and all carbohydrate pools in roots were significantly greater in unshaded versus shaded plants. All other carbohydrate pools in leaves, stems, and fruit did not differ in concentration between control and shaded peanut at this later sampling.

The major pool of NSC in all peanut plants occurred as leaf starch (Table 4). This observation agrees with other investigations that have shown that peanut accumulates relatively high levels of starch in leaves (5, 8, 9).

Table 3. Concetrations of carbohydrate pools in peanut averaged across all times for treatments and development stage.

Tissue	Fraction	Bloom stage (40 DAP) Control	Bloom stage (40 DAP) Shaded	Pod stage (64 DAP) Control	Pod stage (64 DAP) Shaded
		- g glucose equivalents kg^{-1} -			
Leaves	reducing sugars	14.5	17.9**	18.8	18.0
	nonreducing sugars	6.1	4.1**	11.4	7.5**
	starch	219.3	120.0**	121.2	109.5
Stems	reducing sugars	11.2	9.8*	13.6	13.3
	nonreducing sugars	12.2	10.6	16.1	13.4
	starch	116.3	33.7**	126.0	24.3**
Roots	reducing sugars	13.5	8.2**	9.9	5.4**
	nonreducing sugars	58.9	22.7**	32.3	9.1*
	starch	52.7	20.8**	69.8	7.9**
Fruit	reducing sugars	—	—	119.6	118.2
	nonreducing sugars	—	—	84.8	73.8
	starch	—	—	79.8	71.7

*, ** indicate a significant difference at $P < 0.05$ and 0.01, respectively, between shaded and control plants at a particular stage.

Huber (10) has shown that the rate of sucrose synthesis in leaves, as measured by the activity of sucrose phosphate synthetase (SPS), is negatively correlated with leaf starch accumulation. Compared to other species, peanut has relatively low activities of SPS in its leaves. The low levels of leaf nonreducing sugars observed in this study are probably a consequence of low SPS activities. Carbon partitioning between starch and sucrose appear to be genetically controlled, but the environment can have a modifying effect (8, 10). Shading may have modified C-partitioning in this experiment. In comparison to 40 DAP, at 64 DAP, when control plants were pegging and producing fruit, leaf starch made up a smaller percentage of the plant's total NSC (Table 4). As fruit became a prominent sink in controls, there was a greater demand for current photosynthate and less starch accumulated in their leaves. Shaded plants developed less fruit than control and the percentage of NSC in the fruit was less. In this case the bulk of NSC was maintained as leaf starch. Unlike concentrations of leaf starch, concentrations of reducing and nonreducing sugars in leaves and stems of control and shaded plants were higher at the later sampling date (Table 3). The higher sugar concentrations at 64 DAP were not all significantly greater ($P<0.05$) than concentrations at 40 DAP, but the trend was consistent.

Table 4. Means for the content of carbohydrate fractions of peanut tissues as a percent of the total nonstructural carbohydrate (TNSC) content per plant.

Tissue	Fraction	Bloom stage (40 DAP) Control	Bloom stage (40 DAP) Shaded	Pod stage (64 DAP) Control	Pod stage (64 DAP) Shaded
		—— % of TNSC $plant^{-1}$ ——			
Leaves	reducing sugars	3.8	9.7**	4.8	10.2**
	nonreducing sugars	1.6	2.3**	3.0	4.2**
	starch	57.2	60.0**	31.1	52.2**
Stems	reducing sugars	1.7	3.6**	2.8	5.7**
	nonreducing sugars	1.8	3.9**	3.3	5.7**
	starch	16.9	11.8**	25.4	8.6**
Roots	reducing sugars	1.8	1.4**	1.0	0.6**
	nonreducing sugars	7.9	3.8**	3.1	1.0**
	starch	7.3	3.5**	7.0	0.9**
Fruit	reducing sugars	—	—	7.7	4.4**
	nonreducing sugars	—	—	4.8	3.7
	starch	—	—	6.0	2.9**

*, ** indicate a significant difference at $P < 0.05$ and 0.01, respectively, between shaded and control plants at a particular date.

In addition to starch in leaves, that in stems and roots represented a relatively sizable pool of NSC, especially in control plants (Table 4.) Unlike other sugar pools, whose concentrations were usually less than 20 g glucose equivalents kg^{-1}, the mean concentrations of nonreducing sugars in roots of unshaded plants were higher (Table 3). Root nonreducing-sugars are likely an important, readily-available source of carbohydrate. Reducing and non-reducing sugar pools in roots of plants under both light regimes had significantly lower concentrations at the full-pod stage than at the earlier sampling date (Table 3). In general, the percentage of TNSC occurring in roots was reduced at the pod stage, compared to the bloom stage (Table 4). Either utilization of carbohydrate in roots was faster or less carbohydrate was translocated to the roots at the later stage, particularly in the shade-grown plants.

Diurnal trends at beginning-bloom. No diurnal changes in tissue weights were detectable in this experiment on either of the harvest dates. Plant-to-plant variation made it impossible to detect any real-weight change over the times sampled.

Reducing sugar pools in leaves showed significant (*a*

= 0.02) time differences under both light regimes. In shaded leaves reducing sugars were lowest early in the day and greatest before darkness, about 70% more concentrated at 1900 compared to 0700 (Fig. 1b). In unshaded leaves reducing sugars were relatively constant except for low levels early in the photoperiod and late in the afternoon. Nonreducing sugars exhibited significant diurnal variation under shaded and control conditions (a = 0.06 and 0.01, respectively). Under both light regimes, levels of leaf nonreducing-sugars were greatest during the day and approximately half the day-levels during darkness (Fig. 1c). Because nonreducing sugars represent translocatable sugars, higher concentrations of nonreducing sugars during the day might be indicative of greater translocation of carbohydrate away from the leaf at that time (17). Possibly, greater export of carbon from leaves of controls, compared to shade plants, is indicated by higher nonreducing sugar concentrations in leaves of the former. The most concentrated of all NSC fractions, leaf starch showed significant fluctuation in control peanut (α = 0.01) but less statistically definitive fluctuation in shaded peanut (α = 0.0.1). Leaf starch increased throughout daylight hours, peaked at 1600 and 1900, and then declined at night in both light treatments (Fig. 1a). From 0700 until late in the day, concentration of leaf starch rose from 69 to 167 g glucose equivalents kg^{-1} in shade peanuts and from 183 to 255 in control peanuts. Whereas Kerr (11), using specific leaves, observed utilization of 91% of accumulated soybean leaf starch after 12 h of darkness, only 28% and 36% of starch in control and shaded peanut leaf biomass, respectively, was utilized after about 8 h of darkness in this study. Leaf starch appeared to be an important source of carbon during darkness, but it was not nearly all utilized.

Carbohydrate pools in peanut stems showed no significant differences among times-of-day sampled. Movement of sugars in and out of stems must have been maintained at a fairly stable rate. In addition, stem starch, which represented a substantial proportion of the plants' total NSC content, did not fluctuate significantly. Stem starch may represent a reserve not necessarily catabolized under "normal" daily conditions.

Although no significant differences among times of sampling were indicated for reducing sugars in roots, there were differences indicated for concentrations of root nonreducing-sugars and starch (40 DAP). For root nonreducing sugar 40 DAP a = 0.07 and 0.01 for control and shade plants, respectively, while the probability levels associated with the starch pools were a = 0.04 for control plants and a = 0.04 for shaded plants. Peak concentrations of those two pools were observed at 1300 or 1600 and lowest levels were observed early in the day (Fig. 2). Root starch was very stable from 1900 to 0400. Although root constituents fluctuated significantly more than stem starch, no clear pattern of turnover was evident in the root-carbohydrate pools. The root pools, representing a sizable carbohydrate supply, also may not be fully utilized on a daily basis. It is a limitation of this study that plants were not sampled at the end of the dark period, i.e. after 24 h. It is possible that more substantial change in pools that otherwise appeared stable would have been observed.

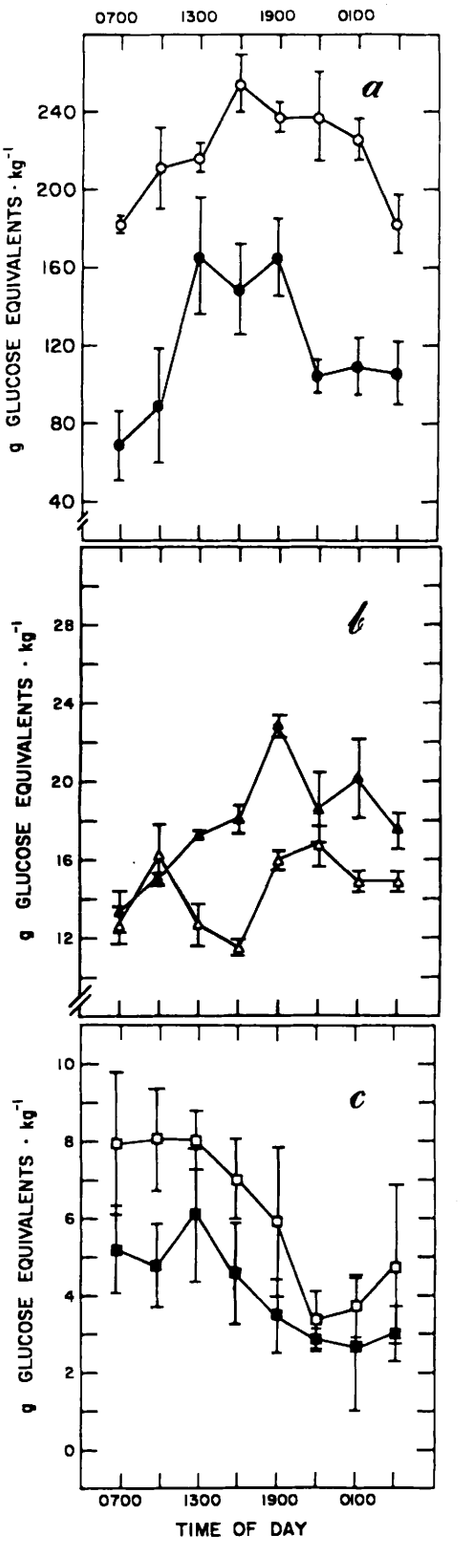
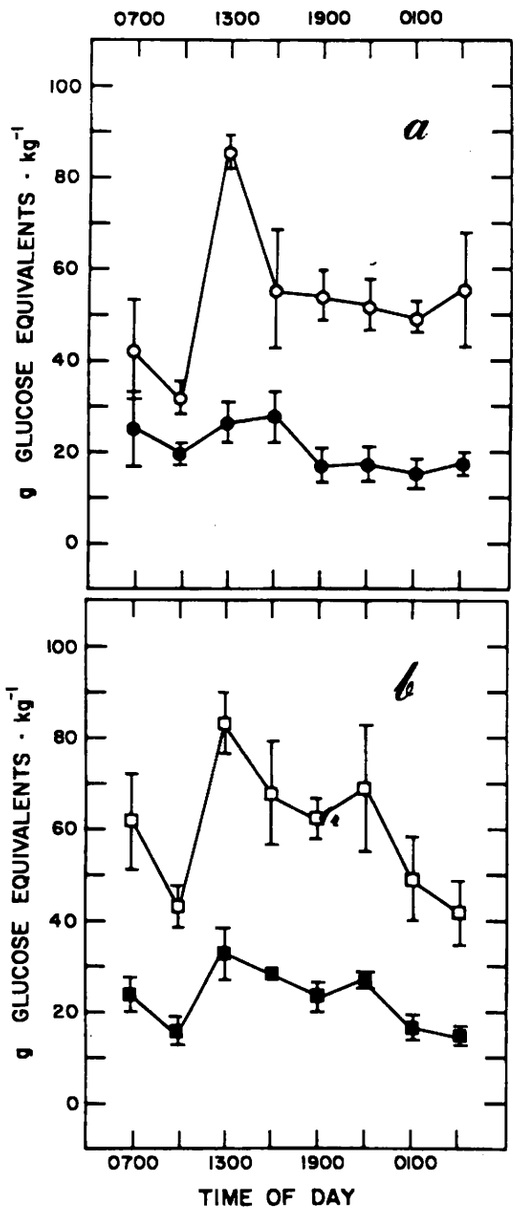


Fig. 1. Diurnal concentrations of peanut leaf carbohydrates at blooming (40 DAP). Treatments are (△), control; (▲), shaded. Bars indicate standard diviation (S.D.) from the means of three samples.

a) Leaf starch
b) Leaf reducing sugars
c) Leaf nonreducing sugars

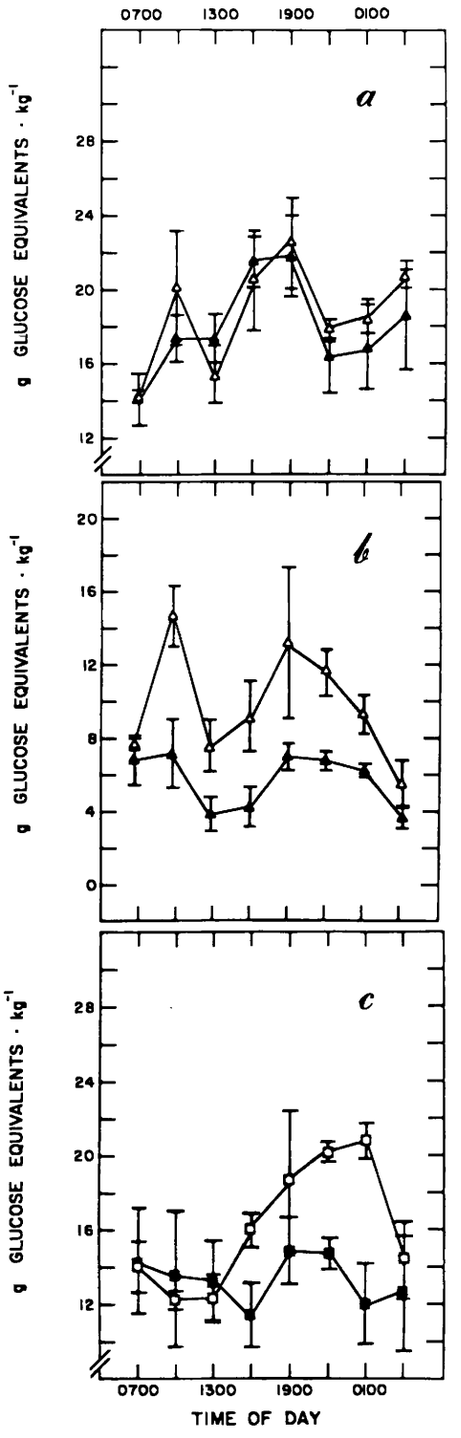
Fig. 2. Diurnal concentrations of peanut root carbohydrates at bloom stage (40 DAP). Treatments are (Δ), control; (▲), shaded. Bars indicate standard deviation (S.D.) from the means of three samples.
a) Root starch
b) Root nonreducing sugars



Diurnal trends at full-pod stage. At 64 DAP, statistically significant diurnal variation was observed for the reducing sugars of the leaves ($a = 0.04$) and root ($a = 0.03$, and the nonreducing sugars of the stem ($a = 0.02$) of the control plants (Fig. 3).

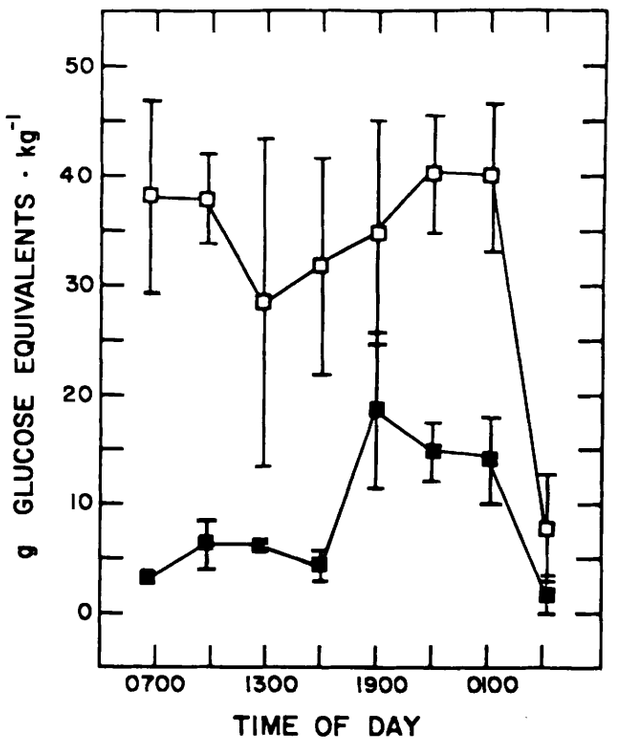
The concentrations of leaf reducing-sugars in unshaded plants did not follow an obvious trend (Fig. 3a). Samples at 0700 and 1300 had lower concentrations than samples from other times. Likewise, the levels of root reducing-sugars were lowest at 0700, 1300 and 0400, being almost two times greater at other sample times (Fig. 3b). The nonreducing sugar content of control plant stems followed an unusual trend at 64 DAP (Fig. 3c). This pool increased in concentration from 12 g glucose equivalents kg^{-1} at 1300 to almost 21 at 0100. The concentration then declined sharply by 0400.

Fig. 3. Diurnal concentration carbohydrates in peanuts at full podding (64 DAP). Treatments are (Δ), control; (▲), shaded. Bars indicate standard deviation (S.D.) from the means of three samples.
a) Leaf reducing sugars
b) Root reducing sugars
c) Stem nonreducing sugars



The only carbohydrate fraction of shaded plants at 64 DAP significantly affected by sampling time was root nonreducing-sugar ($a = 0.05$; Fig. 4). This pool followed a trend similar to nonreducing sugars in control stems with concentrations reaching maximum levels during night samplings but declining again by 0400. Although nonreducing sugars in control roots were not affected by time of sampling, the average concentration of this pool at 0400 dropped drastically from a fairly constant level at all other times (Fig. 4).

Fig. 4. Diurnal concentrations of root nonreducing sugars at full-pod stage (64 DAP). Treatments are (▲), control; (△), shaded. Bars indicate standard deviation (S.D.) from the means of three samples.



A major problem encountered at the full-pod stage was that, within a light treatment, there was considerable among-plant variation. Whole-plant and tissue weights, leaf areas and fruiting at this stage were widely variable. Likewise, the concentrations of carbohydrate pools deviated greatly from means at a given time. Deviation was especially evident in leaf starch pools. Although lower in magnitude, similar variation occurred for stem and root starch. Those carbohydrate fractions were little affected by time-of-day sampled but, apparently, more by particular plant characteristics. It is known that peanut exhibits marked random plant-to-plant variation under relatively uniform conditions (1). This is especially true for fruiting characteristics. It is likely that the random variation in carbohydrate pools observed for peanut in this study resulted from physiological variation among plants exhibiting morphological and phenological differences. Plants with different fruit loads would utilize photosynthate differently, and this variable usage of the plant's energy resources could affect NSC pools throughout the plant.

When a significant diurnal effect did occur for certain pools at the full-pod stage, it was difficult to ascertain the exact biological significance of the changing carbohydrate concentrations (Fig. 3 and 4). It was surprising, for example, to see stem nonreducing-sugars in control peanut reach peak concentrations during darkness (Fig. 3c). Does this indicate increased translocation of sucrose through stems in darkness or was it the result of a temporarily greater accumulation of sucrose? One of the more definitive observations at the last harvest was that nonreducing sugars in roots reached lowest levels under both light regimes at 0400. This suggests that this pool served as a carbon source for utilization by the plants during darkness. Here again, a sampling just before dawn might have been informative.

Conclusions

It is clear from this study that not only do the environment and stage of development affect the NSC pools from day-to-day but they also modify the diurnal patterns. Reducing photosynthesis by shading peanut plants resulted in lower concentrations of carbohydrates in tissues of those plants, compared to tissues of control plants. In addition, when diurnal variation of carbohydrate fractions occurred, the magnitude of change was usually less in shaded plants. It was easier to identify diurnal patterns when peanuts were younger and less variable. Difficulty was encountered as plants grew larger and inter-plant difference became more marked. Any experimental technique that could reduce plant-to-plant variability in studies such as this would greatly improve accuracy. Although difficult to accomplish, a nondestructive sampling of the same plant would be informative. These results do show the importance of examining diurnal changes in NSC content at different stages of development because trends are likely to change drastically. Young, vegetative peanut certainly utilizes photosynthate differently than does the older peanut with developing fruit.

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