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Carbohydrate Changes in Various Organs of Bearing and Nonbearing Pecan Trees[1]

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***Abstract.* Starch and sugars were generally higher in bearing than in nonbearing shoots (wood and bark) of pecan (*Carya illinoensis* (Wang.) Koch). Decreases in shoot starch were reflected by generally simultaneous increases in either shoot or kernel sugars. Shoot starch fell to its lowest level during the liquid stage of ovule development. Leaf starch generally declined as the growing season progressed. Total sugar levels rose in the kernel, shuck, and shell during fruit enlargement and declined during kernel growth. Mature kernels had less sugar than mature shucks. Ovule sugar was highest during the liquid stage and decreased to very low levels at maturity. Ovule starch generally increased as kernels matured.**

Irregular bearing is a major problem affecting pecan production and marketing. It occurs in most important cultivars and may be closely associated with carbohydrate levels (25, 27, 29). Carbohydrate accumulation during the previous growing season could possibly determine if a tree will bear fruit (23), a contention supported by studies of foliage retention (5, 25, 29) and leaf area per fruit (22). Other major factors contributing to irregular bearing by stressing carbohydrate reserves are late fruit maturity, asymetric distribution of fruit growth (dry weight), and stress induced by kernel filling and oil accumulation (24). More information is needed to understand the role of carbohydrates in fruit development and irregular bearing in pecan. The objective of this study was to monitor carbohydrate changes in developing fruit and vegetative organs from irregular bearing trees.

Materials and Methods

Sixty-year-old 'Moneymaker' pecan trees, characteristically an alternate bearer, were sampled weekly from June 9 to October 20, 1980. Twenty current-season branches were randomly collected in late morning from each of 5 trees with a heavy fruit load ("on") and from 5 trees absent of fruit ("off"). Branches from "on" trees consisted of shoot, leaves, and fruit, while branches from "off" trees consisted of shoot and leaves. The 100 "on" shoots and 100 "off" shoots were combined into 2 separate samples. "On" and "off" trees in 1980 were "off" and "on," respectively in 1979. Fifty fruits from each of the 5 bearing trees were also randomly collected and bulked on a weekly basis. All samples were washed immediately in cold water and packed in ice for transfer to the laboratory for storage at −20°C. Before lyophilization, branches were separated into leaves and shoots (wood and bark). Fruits were separated into shuck (involucre), shell (ovary wall and packing material), and kernel (seed coat, embryo, cotyledons, and endosperm) (18). After drying they were ground in a Wiley mill (40-mesh), thoroughly mixed, and stored at room temperature in sealed jars until analyzed.

Sugar and starch were determined by extracting 1 g of material with 85 ml of 80% ethanol in a soxhlet for 24 hr (4). Residues were frozen with their cellulose soxhlet thimbles for subsequent starch analysis. Ethanol soluble carbohydrates were diluted to 100 ml with 80% ethanol and aliquots reacted with anthrone and sulfuric acid (31) and assayed spectrophotometrically for glucose-equivalents based on the method of McCready et al. (17) as modified by Dowler and King (6) for woody tissue. A 0.1 ml aliquot of the ethanolic extract was placed in a test tube with 1 ml of distilled water and 0.5 ml of 2% anthrone in ethyl acetate (14), and 6 ml of 35.6 N H_2SO_4 slowly layered into the solution, mixed, and placed in a 90°C waterbath for 15 min. The solution was cooled to room temperature and optical density recorded at 625 nm with a Bausch and Lomb Spectronic-20 spectrophotometer. Values were compared to a standard curve prepared from glucose.

Starch was assayed by digesting the tissue residues from ethanolic extraction with amyloglucosidase (19). Each of the dried residues, with the cellulose soxhlet thimbles that were cut into small pieces, was placed in a 25 × 150 mm test tube with 30 ml of distilled water and boiled for 15 min. After cooling, 3 ml of 0.2 M sodium acetate (pH 5.0) and 250 mg of amyloglucosidase in 2 ml of the sodium acetate buffer was added and incubated for 24 hr at room temperature. Samples were then filtered with Whatman No. 2 filter paper and brought up to 50 ml with distilled water. Released glucose was assayed as described above. Each 1 g sample was analyzed 4 times for both sugar and starch. The average of the 4 replicates was used in subsequent calculations. No group of sub-sample readings differed by more than 1%. Preliminary evaluation of soxhlet extraction and enzyme digestion of one gram of thoroughly mixed sample materials gave reproductibility to within 5%.

Results and Discussion

Total carbohydrates were generally higher in "on" than in "off" shoots (Fig. 1-A). Although means over 20 weeks for "on" and "off" shoots were equal, "on" shoots generally had higher starch levels until mid-September (Fig. 1-B). This disagrees with observations in apple (10, 12, 13) and pistachio (3) where nonbearing spurs had a higher starch content than those bearing fruit but confirms a previous estimate of starch levels from microscopic studies of pecan (7). During most of the period of rapid embryo growth (Table 1), starch was lower in bearing than nonbearing shoots (Fig. 1-B), which is attributed to stress induced by kernel filling. This rise in starch in nonbearing shoots relative to bearing is substantiated by estimates made by counting starch granules (7). Sugars were generally higher in "on" shoots (Fig. 1-C), which agrees with determinations made in bearing apple spurs (12).

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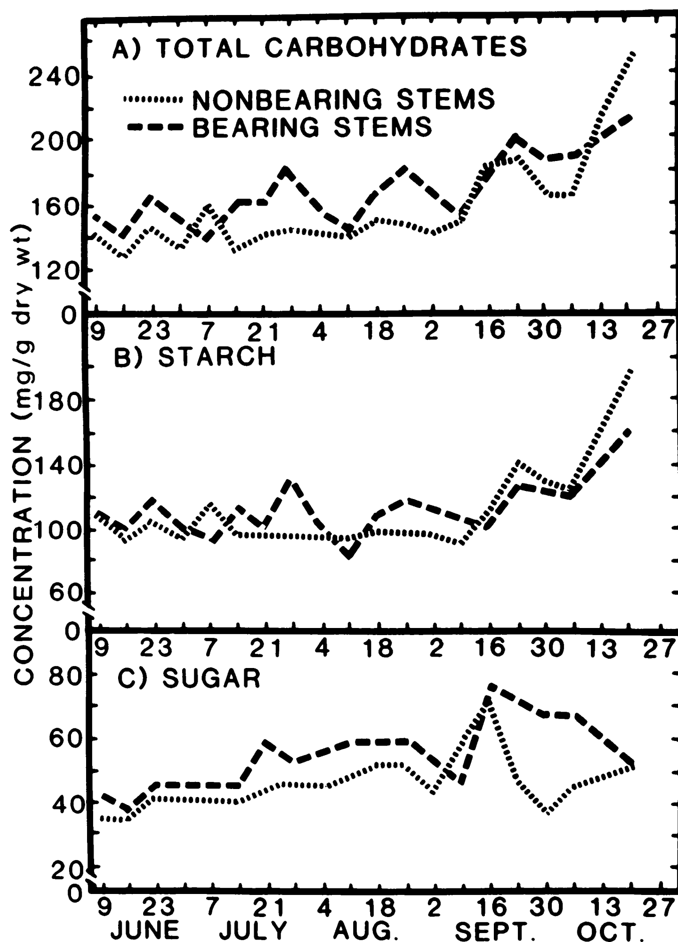

Fig. 1. Concentration (glucose-equivalents) of (A) total carbohydrates, (B) starch, and (C) soluble sugars in shoots (wood and bark) from bearing and nonbearing pecan trees.

The drop in starch in "on" shoots on July 21 (Fig 1-B) corresponded with a sharp rise in soluble sugars on July 21 (Fig. 1-C). Starch concentrations peaked again on July 28 and sharply dropped in early August without causing a corresponding sharp change in soluble sugars. This suggests translocation to other organs as evidenced by observed increases in soluble sugars in fruit organs. This occurred when the vacuole of the expanding ovule was filling with a liquid material containing sugars ('water' stage) (Table 1). After a decrease in stem carbohydrates in early September, there was a sharp increase (Fig. 1A-C) in both "on" and "off" shoots which suggests an environmental induction. Following this change, sugar concentration was greater and starch had a tendency to be less in "on" than in "off" shoots. This is attributed to the continual filling of the ovule up until October 20.

The distinction of carbohydrates in leaves was not clear. Total carbohydrates fluctuated greatly in both "on" and "off" leaves (Fig. 2-A). Starch concentration in leaves from both "on" and "off" trees generally declined throughout the season (Fig. 2-B). Starch had a tendency to be higher in "off" leaves.

During the rapid fruit enlargement period, total carbohydrates had a tendency to be greater in shells than in shucks (Fig. 3-A). During this period, shells contained more soluble sugars and less starch than shucks (Fig. 3-B, C). Starch and sugars decreased in both shucks and shells during the shell hardening stage. Levels in shells drop rapidly during this time. Starch was always higher in shucks than shells and the decreased in shuck starch was minimal. Shuck sugars were much higher than those in the shell once shell hardening was initiated and higher than that of the ovule after embryo growth began. This drop in ovule sugars in early September corresponds with the reported time of accelerated oil synthesis in the embryo (26). Ovule carbohydrates were very high during the liquid stage and was mostly soluble sugars. These sugar observations are supported by previous observations in other pecan cultivars (26). At maturity, ovule starch was at its highest level (9%) and sugars at their lowest (1%).

The sharp drop in shoot starch in "on" trees below that of "off" trees in late July and early August appears to represents a period of stress. Amling and Marcus (1) reported that morphological pistillate flower primordia differentiation can be induced to occur in early August but not earlier. The coincidence of decreased starch, earliest floral differentiation date, rapidly expanding liquid filled ovule, and high ovule sugar level, suggests that this may possibly be the time that biochemical changes are occurring that could affect the next crop. This possibility is supported by studies in apple showing that rapid starch depletion coincides with the appearance of high amounts of gibberellins in developing seeds (16, 21). Gibberellin and auxin have been shown to activate starch degradation (10, 15, 20), and both are translocated from seed (8, 9, 11).

The observation that defoliation of pecan in August or September reduces total carbohydrates in shoot tissue until late spring (28, 29), and prevented the development of pistillate flowers (27), suggests that carbohydrate levels play a critical role in ir-

Table 1. Fruit development of 'Moneymaker' pecan.

Sample date	Developmental stage	Mean fruit size: Length (cm)	Mean fruit size: Diam (cm)
May 25	Fruit set	0.9	0.4
June 2	Initial fruit growth	1.0	0.4
9	Rapid fruit enlargement begins	1.1	0.5
Aug. 4	Rapid fruit enlargement ends	3.0	1.8
11	Liquid endosperm	3.0	1.9
18	Shell hardening and liquid endosperm	3.0	2.0
25	Shell hardening, liquid endosperm, embryo and cotyledon growth begins	3.0	2.0
Sept. 2	Rapid embryo and cotyledon growth, liquid endosperm ends	3.0	2.0
Oct. 13	Decreasing embryo and cotyledon growth	3.0	2.0
20	Fruit maturity	3.0	2.0

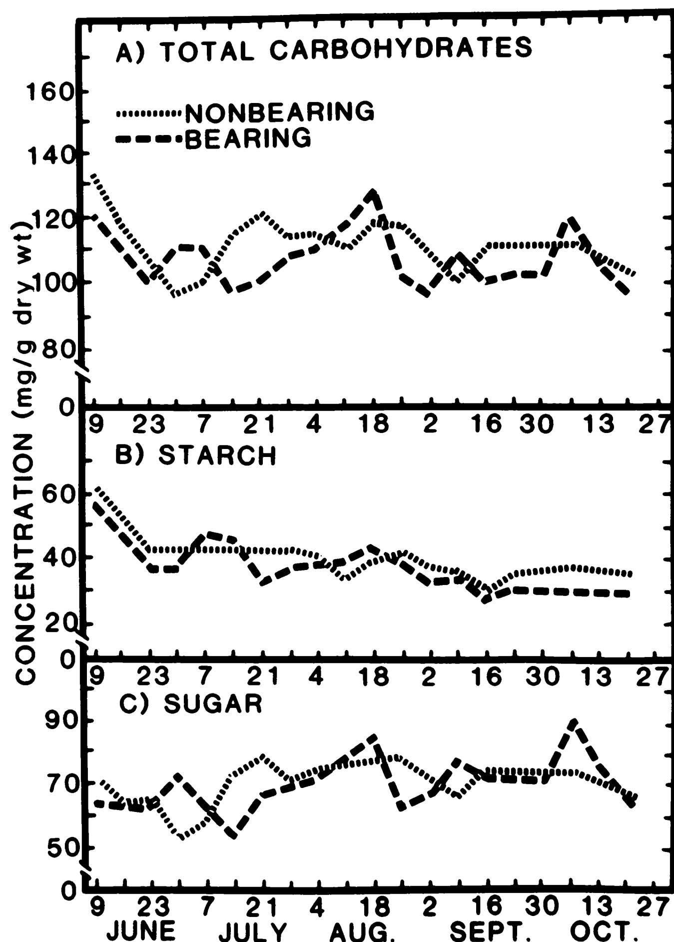

Fig. 2. Concentration (glucose-equivalents) of (A) total carbohydrates, (B) starch, and (C) soluble sugars in leaves from bearing and nonbearing pecan trees.

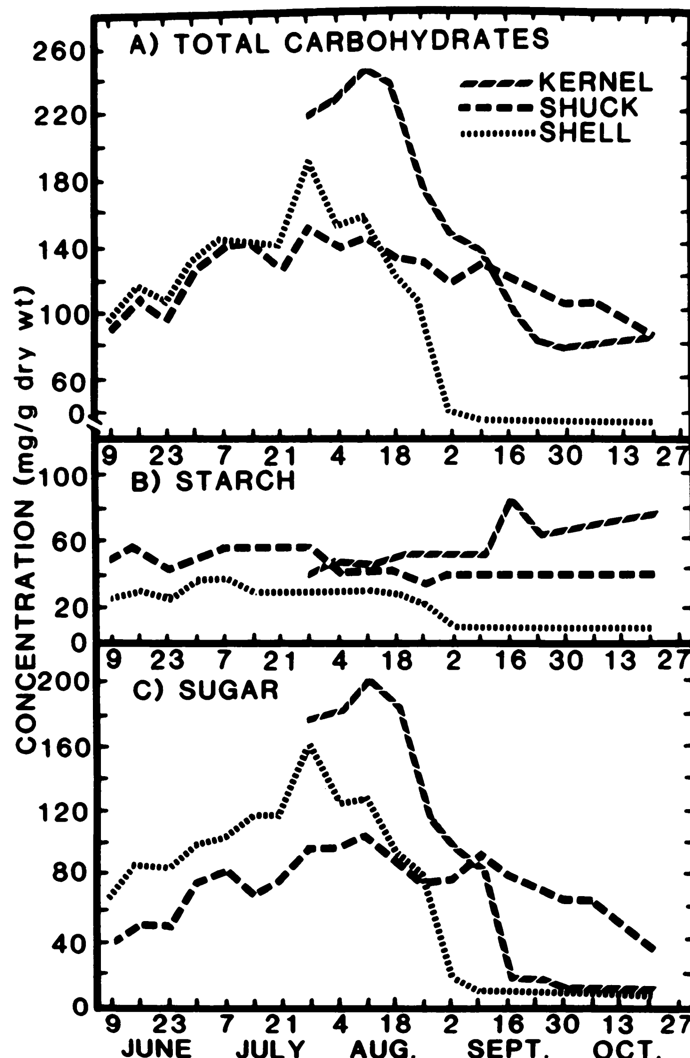

Fig. 3. Carbohydrate concentration (glucose-equivalents) in kernel (seed coat, embryo, cotyledons, and endosperm), shuck (involucre), and shell (ovary wall and packing materials) during growth and maturation of the pecan fruit.

regular bearing. They may be influencing morphological differentiation and development of pistillate flower primordia in early spring (24) while other factors may be controlling initiation prior to early August. An understanding of flowering in pecan may be dependent upon factors in addition to carbohydrates. Efforts must be made to determine the role of hormones and nutrient elements, and how they interact with carbohydrates.

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Gene Dose Effects on Cane Thorn Density and Cotyledonary Gland Number in Tetraploid Blackberries[1]

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Abstract. **Fourteen tetraploid seedling populations of blackberry (*Rubus* sp.), representing quadruplex, (*TTTT*), triplex (*TTTt*), duplex (*TTtt*), simplex (*Tttt*), and nulliplex (*tttt*) genotypes for the major gene conferring thorniness, were evaluated for segregation of cane thorn density and cotyledonary gland number. Comparisons of seedling distribution curves, means and variances of segregating and non-segregating populations did not show a gene dose effect on gland and thorn occurrence. Inheritance of cotyledonary glands and cane thorns in blackberry was qualitative with the density of glands and thorns apparently controlled by several modifying genes.**

Thornlessness in cultivated blackberries (*Rubus* subgenus *Eubatus*) is a desired goal of most blackberry breeding programs. Although the existence of a thornless character in *Rubus* was reported as early as 1629 (11), only recently have commercial cultivars been developed through controlled breeding. Blackberry breeders in the 19th and early 20th centuries often obtained only thorny seedlings in advanced generations of thornless parents. It is now known that most of the thornless sports available were periclinal chimeras in which the gametes were derived from genetically thorny tissue (3).

The most valuable source of genetic thornlessness at the tetraploid level has been the British cultivar 'Merton Thornless'. It is the progenitor of several commercial thornless cultivars including 'Thornfree', 'Smoothstem', 'Black Satin', and 'Dirksen Thornless' (1, 13). Thornlessness of 'Merton Thornless' and its derivatives is conditioned by a single gene with the thornless condition expressed in the homozygous recessive state (12). Crosses of thornless with a homozygous dominant thorny cultivar produce a 35:1 thorny to thornless ratio in the F_2 generation.

Selection for thornless blackberries in segregating populations was made much more efficient by the discovery of Crane and Darlington (2) that thorny seedlings possessed glands along the edges of the cotyledons while thornless seedlings had glabrous cotyledons. Thus, thorny segregates can be eliminated shortly after germination.

In the F_2 generation of a cross of a homozygous thorny clone (*TTTT*) and a thornless clone (*tttt*), 5 genotypes should be produced in the ratio 1 *TTTT*: 8 *TTTt*: 18 *TTtt*: 8 *Tttt*: 1 *tttt*, assuming random chromosome segregation. While the homozygous recessive genotype can be readily identified by the lack of cotyledonary glands, no method is known to separate the various thorny genotypes except by a test cross. Identification and use of simplex and duplex individuals would enhance breeding progress since their use in breeding would give a greater proportion of thornless segregates than the conventional 35:1 ratio obtained from using a quadruplex parent.

Scott et al. (12) observed that thorny segregates obtained from F_2 populations of 'Merton Thornless' crossed with thorny genotypes varied in degree of thorniness. We have observed that cotyledons of thorny segregates vary in number of marginal glands. The purpose of this study was to test the hypothesis that cane thorn density and cotyledonary gland number are associated characters and that both vary in frequency due to an additive gene dose effect at the locus for thorniness.

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