

## The Influence of Season and Temperature on Carbohydrates in Avocado Shoots<sup>1</sup>

J. RODRIGUES<sup>2</sup> and G. F. RYAN  
*University of California, Los Angeles*

Studies of the seasonal fluctuation in carbohydrates in various deciduous, subtropical, and tropical fruit trees have shown that similar changes occur in such trees as apple, avocado and citrus, despite important physiological differences between the species (4, 5, 10, 11, 18). The studies have indicated that fluctuations in carbohydrates are influenced by seasonal environmental factors and by the growth activity of the plants.

Investigations with several plants have shown there is a definite relationship between sugar-starch equilibrium and low temperature. Potatoes subjected to low temperature accumulate sucrose (1). A decrease in starch along with a simultaneous increase in sugars has been reported in grapefruit trees under low temperature conditions (18). Levitt (13) has reviewed numerous papers dealing with this subject.

The present investigation was conducted to determine seasonal fluctuations in carbohydrates in young avocado shoots and the influence of temperature on these fluctuations. One part of the study was concerned with carbohydrate changes in scion wood stored at low temperatures. Possible relationships between carbohydrate accumulation and grafting success have been considered in a separate report (17).

### MATERIALS AND METHODS

The Guatemalan avocado variety Hass was used for the experiments reported here.

Shoot growth in avocado can in general be classified into two growth flushes. Spring growth starts soon after the peak of blossoming and may continue into midsummer in young trees. The autumn growth generally starts in late summer and in the climate of southern California, continues into winter.

In order to study changes occurring in twigs of these growth cycles, samples for analysis were collected at 15-day intervals from both flushes from the time the twigs were about 7-8 weeks old (16-17 per cent dry matter) until they were about a year old (38-40 per cent dry matter).

For experiments dealing with low temperature-carbohydrate relationships, autumn, shoot growth was collected in February, 1957, when the twigs were rich in reserve carbohydrates. The shoots were stored in 0.0015 inch polyethylene bags at 40°F. Carbohydrate determinations were made at regular intervals until September, 1957.

Autumn growth of 1957, high in carbohydrates, was collected, in December, 1957, and

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<sup>2</sup>Present address: Peelibetta Estate, Ammatti, Coorg, India.

stored in polyethylene bags. The shoots were subjected to temperatures of 50°, 40°, and 35°F for 32 days. Determinations of starch and sugar were made at intervals of 48 hours for 16 days. The wood was again analyzed at the end of the storage period (32 days) to determine *the* extent of starch conversion under low temperature conditions.

Young avocado trees were subjected to low temperatures of 48°F during the day and 34°F at night for 9 days under artificial light, and changes in starch and sugar were determined. The controls *in* this experiment were also under artificial light and were subjected to 78°F during the day and 65°F at night. The light was supplied by daylight fluorescent tubes supplemented with incandescent bulbs (1 watt incandescent to 4 watts fluorescent), giving an intensity of about 600 f.c. at the tops of the trees.

For all analytical studies, 4 to 6 shoots, excluding about an inch of the terminal growth, were collected and cut into pieces about one half an. in length. From this material two 4-4½, gram samples were taken for extraction. After extraction for 45 minutes in hot 80% ethanol (2), the residue was washed, dried, ground, and saved for starch determination.

The combined extract and washings, which were used for sugar determinations, were concentrated, cleared with Ba(OH)<sub>2</sub> and ZnSO<sub>4</sub> (20), filtered, evaporated, and made up to 10 ml with water. Duplicate 30 µl aliquots were analyzed quantitatively by paper chromatography, using butanol-1, acetic acid and water (4:1:5) as solvent in the one-dimensional descending method.

The sugars were located and identified by development of a parallel strip with aniline hydrogen oxalate (9). Glucose and fructose appear as brown spots and maltose as a faint yellow spot with this reagent. The glucose and fructose spots on the main chromatogram were then developed with triphenyltetrazolium reagent, eluted with 95% ethanol, and the density of the color determined at 500 mµ (22). The area containing sucrose was located by means of R<sub>f</sub> value in relation to the location of the developed spots of reducing sugars on the parallel strip. This area was eluted with ethanol, the color was developed with diphenylamine reagent and the density determined at 640 mµ (21).

Mannoheptitol (perseitol) was separated with butanol-1, acetic acid, water (4:1:5), and butanol-1, ethanol, water (4:1:5) (7) and identified with brom cresol purple reagent (3).

Starch was determined by incubating the dried powdered residue with diastase and then analyzing for glucose as described above. The procedure used was in general that of Shoffer and Somogyi as modified by Heinze and Murneek (8).

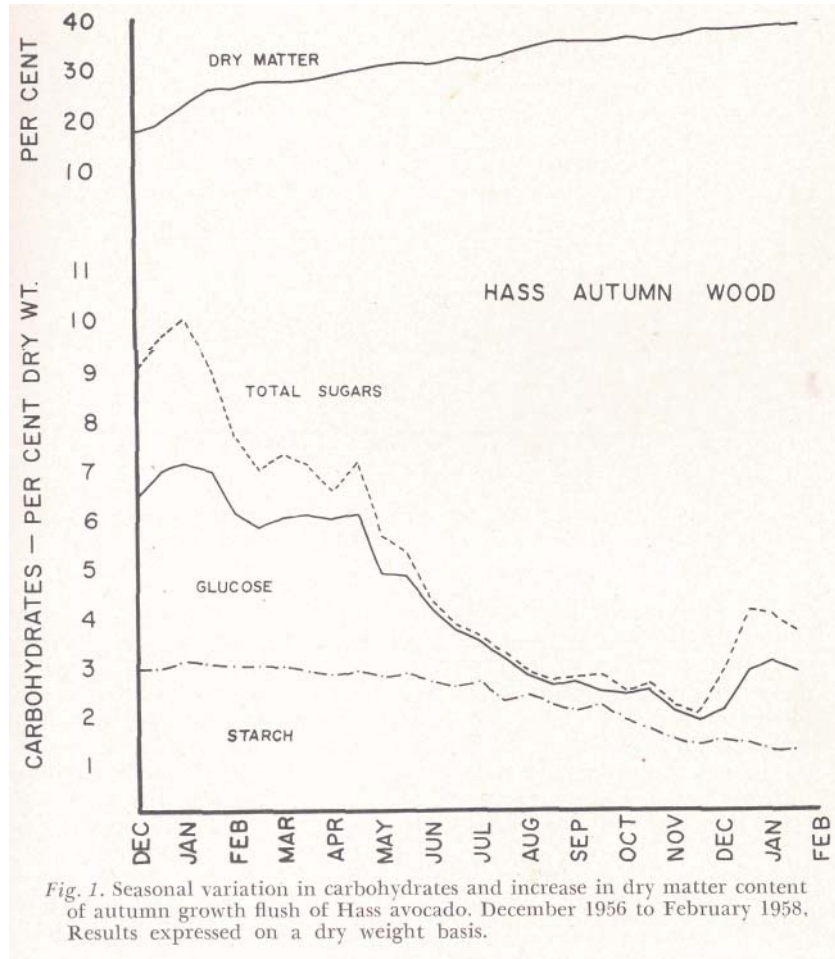
## RESULTS AND DISCUSSION

*Seasonal changes in carbohydrates:* Data pertaining to seasonal variation in carbohydrates are shown in Figs. 1 and 2. The autumn wood of 1956 (Fig. 1) accumulated large quantities of sugar and starch the first winter. A decrease in reserve carbohydrates began in January and continued until autumn. In November to December, when the wood was about a year old, a rapid increase occurred in total sugars while starch remained low. Total sugars did not reach the high level of a year earlier.

In the course of shoot growth there was a gradual increase in dry matter from 16-17 per cent to 38-40 per cent. The results expressed as per cent of fresh weight are shown in

Fig. 3.

Fig. 1, with the results expressed on a dry weight basis, shows a gradual decrease in starch from winter 1956-1957 to winter 1957-1958. When expressed as per cent fresh weight (Fig. 3) the results show an increase in starch until May, followed in July by the start of a decrease to a low point in January.



In Fig. 2, data from analyses started in December on wood of the previous spring flush are shown along with results of analyses started the following spring on new growth and continued through December to January. Together the two sets of curves present the carbohydrate fluctuation for an entire year. Spring growth (Fig. 2) showed a high level of starch and sugar in May. Increase in dry matter coincided with a decrease in total carbohydrates on a dry weight basis. A sharp increase in total sugars occurred in December-January, while starch remained low. Results obtained the previous year with 1956 spring growth had shown that this winter accumulation was followed by a depletion of sugars in the spring. In the 1957 spring growth, as in autumn growth, depletion of starch occurred in winter. This was followed by an increase in April-May.

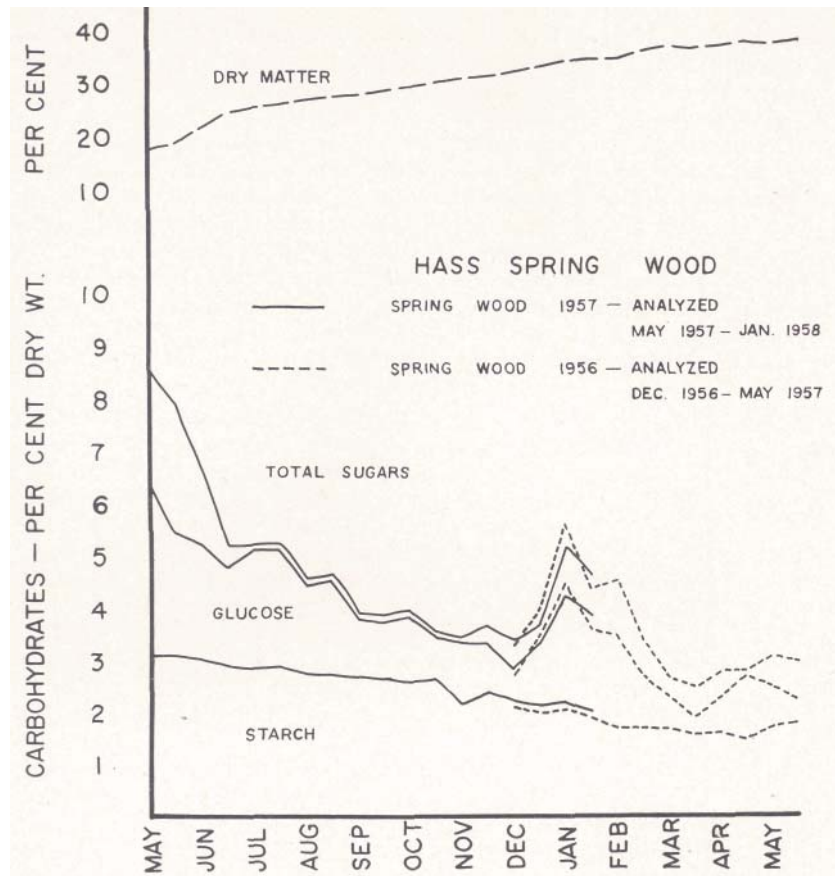
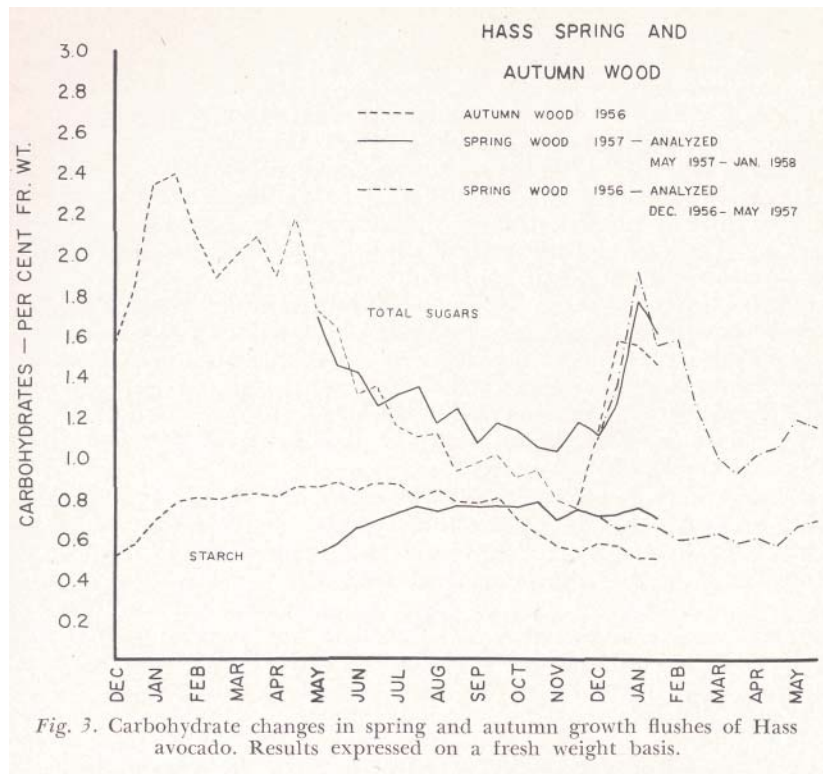


Fig. 2. Seasonal variation in carbohydrates and increase in dry matter content of spring growth flush of Hass avocado. Results expressed on a dry weight basis.

Comparison of spring and autumn wood (Fig. 3) shows that there were interesting similarities between these two flushes, though there were significant differences in total carbohydrates. The young autumn and spring growths were both rich in carbohydrates in winter and spring. A similar decrease in total sugars in summer and a marked increase in late autumn or early winter occurred in both growth flushes. The decrease in starch occurred earlier in autumn growth than in spring growth.



Young shoots are generally rich in carbohydrates even though the leaves in these shoots are not fully developed for maximum photosynthesis. This would indicate that translocation of reserve food material from older to younger shoots may occur. Depletion of carbohydrates in autumn wood during spring and summer coincides with blossoming and shoot growth. Similarly, in autumn the decrease in carbohydrates in spring wood occurs during autumn shoot growth.

Changes in sugar show similar trends whether the results are expressed as per cent of fresh weight or as per cent of dry weight. However, with starch, marked differences are apparent when results are expressed as per cent of fresh weight rather than as per cent of dry weight. This illustrates the limitations of analytical methods in attempts to relate changes in content of constituents to growth activities.

The major reserve carbohydrates in avocado were found to be glucose, fructose, sucrose and starch. Occurrence of mannoheptulose in avocado leaves has been shown by Nordal and Bensen (15). Though the occurrence of mannoheptulose is known, chromatographic separation of this sugar was not possible since in the various solvents used the Rf value of this sugar is identical with that of glucose. Nordal and Bensen (15) successfully separated mannoheptulose in borate buffer solvent. Similar trials in the present investigation failed to reproduce their results. The data presented here for glucose may therefore represent a mixture of glucose and mannoheptulose. However, tests with diphenylamine reagent (20) indicated only traces of this seven carbon keto sugar in avocado shoots.

Occurrence of large quantities of mannoheptitol (perseitol) in avocado apparently indicates that some relationship exists between the formation of mannoheptitol and mannoheptulose content. However, the exact role of this sugar alcohol in plant metabolism is not yet known.

*Low temperature and reserve carbohydrates:* Table 1 shows the changes occurring in wood under refrigeration. Almost complete depletion of starch occurred during low temperature storage. Coincident with the decrease in starch there was an increase in glucose and sucrose, with glucose predominating. There was an increase in total sugars until starch was depleted and then a gradual decrease. Traces of maltose were noticed after one month of cold storage. Analyses made in later months did not reveal any traces of this disaccharide.

Table 1.—Effects of low temperature (40°F) storage on carbohydrates in Hass avocado wood, cut and stored in February, 1957.

Storage period (months)	Per cent, dry weight basis			
	Glucose	Fructose	Sucrose	Starch
0.....	6.85	1.15	1.25	3.05
1.....	8.66	Trace	1.64	0.91
2.....	8.50	0.35	1.52	0.42
3.....	7.10	Trace	0.65	Trace
4.....	7.40	Trace	Trace	Trace
5.....	7.35	Trace	Trace	Trace
6.....	7.40	Trace	Trace	Trace
7.....	6.95	Trace	Trace	Trace

Starch depletion and sugar accumulation showed the same trend at 35° and 50°F as at 40°F (Table 2), but the rate of change differed with temperature. Decrease in starch and increase in sugar were most rapid at 35 °F.

Table 2.—Effects of low temperature storage on carbohydrates in Hass avocado wood, cut and stored in December, 1958.

Storage period (days)	Starch (per cent dry weight)			Total sugars (per cent dry weight)		
	Storage at			Storage at		
	35°F	40°F	50°F	35°F	40°F	50°F
0.....	2.95	2.95	2.95	9.00	9.00	9.00
2.....	3.05	2.85	3.05	9.24	8.65	8.91
4.....	3.15	2.89	3.10	9.35	8.55	8.67
6.....	3.05	2.75	2.95	9.47	8.79	9.21
8.....	2.95	2.91	2.89	9.55	9.22	9.24
10.....	2.85	2.81	2.91	9.37	9.59	8.95
12.....	2.64	2.69	2.81	9.43	9.45	9.04
14.....	2.30	2.52	2.71	9.51	9.61	8.96
16.....	2.00	2.31	2.65	9.90	9.73	9.21
32.....	0.54	1.20	2.05	11.28	10.31	9.56

Table 3.—Low temperature effects on carbohydrates in young Hass avocado trees.

Time	Starch (per cent dry weight)		Total sugars (per cent dry weight)	
	78°F max. 65°F min.	48°F max. 34°F min.	78°F max. 65°F min.	48°F max. 34°F min.
	0 days.....	3.40	2.90	3.02
3 days.....	3.21	2.34	3.12	4.80
6 days.....	2.90	1.84	2.83	4.84
9 days.....	2.65	0.67	2.70	5.48

L.S.D. at 5% level for starch 1.10.  
L.S.D. at 5% level for sugar 0.77.

Low temperature influenced similar changes in young avocado trees. Table 3 shows a marked decrease in starch and a simultaneous increase in sugars. No maltose was

found when intact trees were subjected to low temperature.

An increase in sugar and a decrease in starch during winter is attributed to a "cold reaction" (10). Sharpies and Burkhart (18), studied carbohydrate changes in grapefruit, and consider that the critical temperature at which starch begins to be converted to sugars is approximately 55 °F. Results in the present studies indicate that conversion of starch to sugar may occur at temperatures of 50°F or lower. In young avocado trees the rate of reaction was markedly accentuated compared with the response of stored shoots. McCready (14) has shown that in potatoes, conversion of starch to sucrose is inhibited in the absence of oxygen. If a similar system exists in avocado, one could postulate that low oxygen supply or high concentration of CO<sub>2</sub> when the wood is stored in polyethylene bags may reduce the rate of reaction. Moreover, the conditions under which the wood was subjected to low temperature were different from those of intact plants.

Starch is degraded by phospholytic cleavage by phosphorylase in potatoes. Extensive studies have shown that the phosphorylase enzyme is equally active at all temperatures studied. Breakdown of starch in potatoes by this enzyme at low temperature is believed to be due to the disappearance of an inhibitor which is normally present (15).

Starch can also be degraded by hydrolytic cleavage by amylases, β-amylase being able to convert short branched amylopectin to maltose. However, evidence indicates that potatoes have low amylase activity and that the enzyme is inactive *in vitro* at 0°C (1).

In avocado there is as yet no evidence to show the mechanism of starch breakdown. It is possible that a system similar to that in potatoes may also be present in avocado. Even if such a system exists, the changes occurring at low temperature cannot be fully explained, particularly the traces of maltose observed in refrigerated wood. Kursanov and Pavlinova (12) consider that the nearest precursors of sucrose in plants are not phosphorylated sugars nor glucose or fructose but compounds with 1,4 cc glucoside linkage such as maltose, dextrans and starch. This would perhaps explain the presence of maltose in stored avocado wood. Absence of maltose in trees subjected to low temperature may be due to a higher rate of reaction in which maltose is converted to hexose sugars or sucrose at a rate higher than in stored wood.

The product of starch breakdown in avocado is primarily glucose as contrasted with sucrose in potatoes, though slight accumulation of sucrose does occur during cold storage of avocado wood. Evidence at hand does not permit any definite conclusion as to the nature of this reaction, but the possibility exists of one or more enzyme systems being involved. Under these conditions conversion of starch to sugar probably constitutes an upset in the normal mechanism of starch-sugar equilibrium.

Winter changes under field conditions may be explained by assuming that similar chemical reactions may occur at high and low temperatures, but the rate of reaction may differ according to the temperature. When seasonal changes in carbohydrates are expressed as per cent of fresh weight, an increase in sugar and a slight decrease in starch is noticed in winter. In summer there is an increase in starch and decrease in sugar. The decrease in sugar could be explained as being due to the most readily available reserve material being utilized during blossoming and shoot growth. Complete depletion is avoided by production of fresh reserves. A process of this nature may

maintain the starch at a fairly constant level.

#### SUMMARY

Young autumn shoots of the avocado variety Hass were generally high in sugars during the winter. A decrease began in January and continued until autumn. In November and December, when the wood was about a year old, a rapid increase in sugars occurred. Changes in sugar showed the same trend whether expressed on a dry weight or a fresh weight basis. Starch showed a gradual, decrease from winter 195(5-57 to winter 1957-58 when expressed on a dry weight basis, but on a fresh weight basis it showed an increase until May followed later in the summer by a decrease.

Young spring wood was rich in sugar in May. On a dry weight basis there was a decrease in both sugar and starch coincident with shoot maturity; on a fresh weight basis there appeared to be a decrease in sugar and an increase in starch in summer.

When, avocado shoots were held at low temperatures, breakdown of starch coincided with increase in glucose. The rate of breakdown of starch was influenced by the storage temperature. Over the range of 35-50°F, the rate of decrease in starch and increase in sugar .was greatest at 35°F and least at 50°F. Traces of maltose were observed at the end of the first month of storage but not in later analyses.

Young avocado trees subjected to low temperature showed a marked decrease in starch and a simultaneous increase in sugar. Degradation of almost all the starch occurred in nine days.

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