Table 6. ATP content of 42 day-old seed of 4 corn genotypes after various imbibition times.

Imbibition time (hrs)	ATP content (nmoles/seed)			
	bt	*sh2*	*su*	*normal*
0	4.0	12.6	6.0	1.3
2	1.7	13.0	1.7	1.4
4	2.1	1.8	1.8	1.2
8	1.8	3.6	1.8	.8
16	17.2	36.5	12.8	7.5
24	10.6	7.1	4.7	9.3
48	136.0	138.0	21.6	100.0
72	246.0	1200.0	394.0	986.0
96	87.0	194.0	152.0	68.0

adversely affected seed viability and vigor. Seeds that were harvested 42 days post-pollination were larger than those harvested on the 16th day. Large seeds probably contain more endosperm and therefore should have the capacity to produce more ATP initially or at least over a longer period of time than small seeds. However, averaged over all genotypes seeds imbibed for 4 hr had higher ATP concentrations during early maturity (18-20 days). ATP then dropped off at 22 days, and finally increased slightly at maturity (42 days). Thus, larger and more mature seeds did not produce the higher levels of ATP after 4 hr imbibition that may have been expected from the larger endosperms. When imbibition time was increased to 96 hr all lines had a similar pattern of ATP production. Since the ATP content was highest after 72 and 96 hr imbibition for the smallest endosperm genotype, *sh2,* it does not appear that the lack of ATP during early germination was a limiting factor leading to poor vigor in the *sh2* line.

In the present work, no determinations were made on the total adenylate pool (ATP, ADP, AMP) which would give an indication of the energy charge. The turnover rate of ATP may play an important role in distinguishing the vigor characteristics between *normal* and *sh2*. A higher turnover rate of ATP for *normal* as compared to *sh2* may account for the lower ATP levels found in *normal* in this research.

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Effect of Harvest Duration on Yield and on Depletion of Storage Carbohydrates in Asparagus Roots[1]

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Additional index words. oligosaccharides, *Asparagus officinalis*

Abstract. **Harvesting a young planting of asparagus (*Asparagus officinalis* L.) for 4 or 6 weeks the second year after transplanting 1-year-old crowns, followed by harvesting for 8 or 10 weeks the third year, reduced yields significantly the fourth year. Carbohydrate levels in asparagus storage roots decreased during harvest and continued to decrease after harvest during fern production. Carbohydrate levels increased in storage roots after stalks had matured, and were restored to preharvest levels by mid- to late summer. All treatments possessed comparable levels of storage carbohydrates by the end of the season. Asparagus storage carbohydrates were identified as fructose-oligosaccharides, which varied considerably in size, mobility, and percent fructose and glucose. The largest oligosaccharides were composed of ∿ 90% fructose, ∿ 10% glucose; molecular weights did not exceed 4,000.**

Asparagus yields have experienced a general decline in Michigan since 1970, although total production has increased because of expanded area planted (6). The reasons for the decline in yield are not well understood, but are probably due to environmental and biological stresses. The effects of

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these stresses on asparagus vigor have not been extensively studied.

Asparagus plants produce and store carbohydrates during one growing season for overwintering and for initiation of bud, spear, and fern growth the following season (8). Carbohydrate levels in asparagus plants are, presumably, directly related to plant vigor and hence to growth and yields. Previous studies on the effect of length of harvest on subsequent yields of asparagus indicated that a harvest period of 7-9 weeks was most desirable (2, 4, 5, 11, 12). However, such studies took several years to complete, and recommendations were for an average year, without taking into account current season conditions. The purpose of these studies was to investigate the effect of different harvest periods on fluctuations of storage carbohydrates in asparagus plants throughout the growing season, to determine whether carbohydrate levels were a reliable index of subsequent yields, and to determine the nature and structure of the storage carbohydrates(s).

Methods and Materials

A 2-year-old asparagus plot (started with 1-year-old crowns 'Mary Washington') was divided into 1.5 x 7.7 m plots replicated 5 times. Plots were harvested for periods of 0, 2, 4, or 6 weeks, and spears were counted and weighed. Storage roots were sampled for total soluble carbohydrates every 1-2 weeks from April 9 to October 29 by collecting 15-20 cm soil cores containing root segments about 15 cm from the center of the crowns. Two to 3 plants from each plot were sampled on each sampling date and four core samples were taken per crown. Storage root segments were dried at 96°C for 12 hr, then ground in a Wiley Mill. Soluble carbohydrates were extracted from 1 g dried tissue in a Sorvall Onmi-mixer in 100 ml distilled water at 4,400 r.p.m. and filtered. Total carbohydrates were determined by a modified anthrone reagent method (7). One ml of extract solution was mixed with 9 ml of anthrone reagent, placed in a boiling water bath for 15 min., then immediately cooled in an ice bath. Optical density was measured at 620 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. Carbohydrate concentrations were determined using a standard curve derived from sucrose. In the second year, the harvest periods of 0, 2, 4, or 6 weeks were extended by 4 weeks each, so that the plots were harvested for 4, 6, 8, or 10 weeks. In the third year, all plots were harvested for 6 weeks.

A 1-year-old asparagus plot (previously planted with 1-year-old crowns) was divided into 1.5 x 18.0 m plots replicated 5 times. Plots were harvested for periods of 0, 3, or 6 weeks. Three to 6 plants were removed from the soil every 1-2 weeks from April 14 through September 9, 1977, thoroughly washed, weighed, and storage roots were severed from the rhizome. Randomly chosen storage roots were cut into 2.5-4.0 cm segments, dried at 96°C for 12 hr, ground in a Wiley Mill, and processed as previously described, with the exception that fructose was substituted for sucrose in deriving the standard curve.

A 0.5 g sample of dried ground storage root tissue was extracted with 100 ml boiling 80% ethanol in a Sorvall Omnimixer at 10,800 r.p.m. for 5 min. The solution was filtered, evaporated to dryness, and redissolved in 1-2 ml distilled water. Aliquots of solution were streaked on Whatman 3MM chromatography paper and developed in 7 propanol:1 ethyl acetate:2 water (3) for periods of 4, 12, or 24 days. Oligosaccharides were detected by applying N.E.P. ketose-specific indicator and heating at 100°C for 2 min (1). Oligosaccharides were extracted from chromatograms with double distilled water, filtered, then hydrolized with 1 N HCl at 52-54°C for 1 hr. The HCl was neutralized with dry $AgCO_3$ until the precipitate darkened, and then the precipate was washed with 100 µl acetic anhydride and 2 ml dry methanol, and evaporated to dryness in a vacuum desiccator. Samples were trimethylsilylated overnight with 50 µl of Tri-Sil Z and analyzed with either a Perkin Elmer 900 gas chromatograph equipped with a 3.7 m 3% SE-30 glass column or a Perkin Elmer 910 equipped with a 3.7 m SP-2100 glass column (10). Temperature programming was 140°-200° on the P.E. 900 and 120°-180° on the P.E. 910 (4 min at 120° or 140° followed by increases of 2° min^{-1}). Flow rate was 50 ml N/min; injection port and detector temperatures were 260°. Standards of inulin and sucrose were run with each batch.

Molecular weight was estimated using Bio-Gel P-4 and P-6 400 mesh exclusion beads, packed in separate 1 cm diameter columns to bed heights of 23 cm. Flow rate was 3.2 ml/hr; the void volume was 3.6 ml as determined with blue dextran. Fractions were analyzed for carbohydrate by a modified anthrone technique as described above.

Results

Harvesting asparagus plots for 4 or 6 weeks in the second year after transplanting crowns, and for 8 or 10 weeks the following year, reduced yields significantly the fourth year after transplanting (Table 1). Percentage of marketable size spears (> one cm in diameter 12 cm from the tip) was lowest in plots harvested for the longest period of time. Storage carbohydrates (% of dry weight) decreased gradually through the harvest periods (April-May), (Fig. 1). One to 2 weeks following harvest, carbohydrate levels decreased rapidly as shoots were

Table 1. Effect of length of harvest period on 'Mary Washington' asparagus yield in subsequent years.

Length of harvest (weeks)			1978 yields		
1976[z]	1977	1978	No. marketable[y] spears/ha x 10^3	Yield of marketable spears (kg/ha)	Marketable spears (%)
0	4	6	155 a	3120 a	65 a
2	6	6	130 a	2640 a	65 a
4	8	6	99 b	1955 b	58 ab
6	10	6	84 b	1706 b	55 b

[z]Plots were first harvested 2 years after transplanting 1-year-old crowns.

[y]Spears > 1 cm in diameter.

[x]Mean separation within columns by Duncan's multiple range test, 5% level.

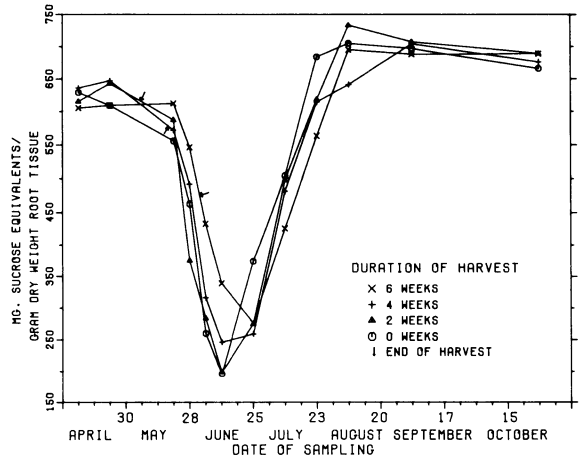

Fig. 1. Amounts of soluble carbohydrates in storage roots of a 2-year-old asparagus planting harvested for 0, 2, 4, or 6 weeks.

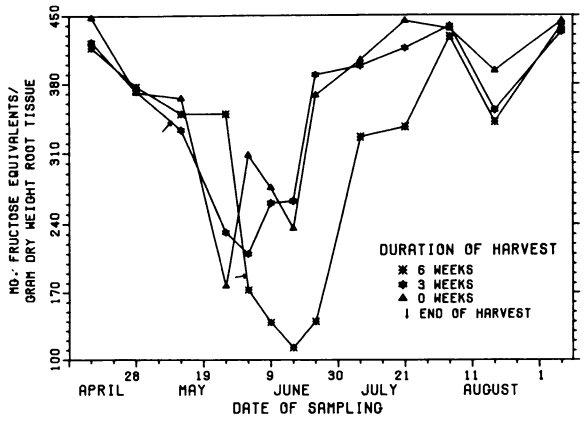

Fig. 2. Amounts of soluble carbohydrates in storage roots of a 1-year-old asparagus planting harvested for 0, 3, or 6 weeks.

produced, reaching a minimum in early June. Then carbohydrate levels increased rapidly, reaching peak levels in mid- to late-August. Thereafter, carbohydrate levels gradually decreased. The integrity of the different harvest periods was disrupted due to several nights of freezing temperatures for 2 weeks in April 1976, during the initial harvest period. The full effects of this cold period on storage carbohydrate production or utilization are not known. Significant differences in percent storage carbohydrate could only be demonstrated between the 6 week harvest and the other 3 harvest periods during that part of the season when carbohydrate levels were declining rapidly. Carbohydrate levels in plants harvested for 6 weeks were consistently higher for 3 weeks after harvest and reached a minimum 1 to 2 weeks later than carbohydrate levels in plants which were harvested for 0, 2, or 4 weeks, indicating that more reserve carbohydrate was utilized in producing full size ferns than in producing 17-25 cm spears which were snapped off before they could fully elongate.

In the second experiment, where entire crowns were dug and sampled, percent storage carbohydrate decreased through harvest and after harvest during shoot production, then increased rapidly, reaching peak levels in mid- to late-summer (Fig. 2). Percent storage carbohydrate of plants harvested for 0 or 3 weeks leveled off in late May to early June, then increased and peaked in late July to early August, while percent storage carbohydrate of plants harvested for 6 weeks leveled off 2 to 3 weeks later than plants harvested for 0 or 3 weeks.

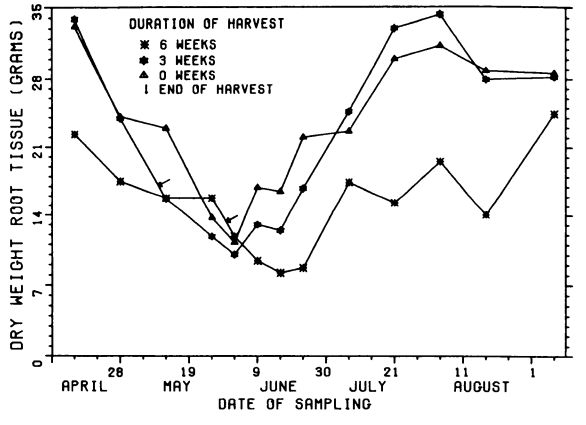

Fig. 3. Effect of length of harvest on storage root dry weight.

Table 2. Ratios of fructose:glucose in hydrolyzates of oligosaccharides eluted from chromatograms developed for 4, 12, or 24 days.

Band eluted from chromatogram	Glucose[z] (%)	Estimated ratio of fructose:glucose
1	33	2:1
2	26	3:1
3	22	4:1
4A	25	–[x]
4B	26	–
5A	20	–
5B	21	–
6[y]	15	–
7	15	–
8	15	–
9	14	–
10	10	9:1

[z]Loss of fructose during acid hydrolysis necessitated multiplying levels of percent fructose by a constant derived from loss of fructose from a molecule (sucrose and/or inulin) of known composition.

[y]Bands 6, 7, 8, and 10 were eluted from both 12 and 24 day developed chromatograms. Values are the average of both determinations.

[x]Ratios of glucose:fructose not estimated due to suspected oligosaccharide heterogeneity and fructose lability.

Total storage root dry weight from plants harvested for 6 weeks was significantly less from mid-June to early September than for plants harvested for 0 or 3 weeks (Fig. 3).

Paper chromatography revealed a number of oligosaccharides varying in size and mobility, all containing ketose sugars. Twelve distinct oligosaccharide bands were eluted from one or more of the chromatograms. Gas chromatography of hydrolyzates revealed that the eluted oligosaccharides were composed predominantly of fructose and, to a lesser extent, glucose (Table 2). Ratios of fructose to glucose ranged from 2:1 for the oligosaccharide with the greatest mobility to 9:1 for the oligosaccharide(s) with the least mobility (no movement from the origin). Exact ratios for most of the oligosaccharides were impossible to estimate due to the heterogeneity of oligosaccharides within bands and the lability of fructose during acid hydrolysis. Gel exclusion chromatography indicated that the molecular weight of the largest oligosaccharides did not exceed 4,000 (Table 3).

Discussion

Results concerning the effect of length of harvest on seasonal fluctuations of storage carobhydrates are in close agreement

Table 3. Molecular weight determination of oligosaccharides extracted from asparagus storage roots.

Fraction[z]	Amount (ml)	Optical density[y] Exclusion limit 4000 (Bio-Gel P-4)	Optical density[y] Exclusion limit 6000 (Bio-Gel P-6)
1	3	0[w]	0
2	0.7[x]	0	0
3	0.7	0.275	0

[z]1 mg of material in 0.1 ml of distilled water was applied to the top of the column.

[y]Carbohydrates were detected by the anthrone method. Optical density of solutions was read at 620 nm in a Bausch and Lomb Spectronic 20 spectrophotometer.

[x]Approximates the void volume.

[w]Each value is the average of 2 determinations.

with those of Scott et al. (8) obtained in South Carolina, although he incorrectly identified the storage carbohydrate as sucrose. Carbohydrates in storage roots were reduced during harvest and were depleted even more severely during the initial fern production period. Storage carbohydrates were replenished after the fern had matured and had begun to produce sufficient carbohydrate for translocation and assimilation. The effect of extending harvest was to increase the severity of carbohydrate depletion, and to decrease the number of days available through the rest of the season for the production of storage carbohydrates for the succeeding year's growth.

In the second experiment, there was a 6 week period (late July to early September) during which there were no significant differences in percent storage carbohydrate, yet plants harvested for 6 weeks had significantly less total root dry matter than plants harvested for 0 or 3 weeks. It appeared that storage root formation was inhibited by the longer harvest period without the percent storage carbohydrate being significantly affected. Our results indicated that harvest should not begin until 2 complete years following transplanting have passed, and should not exceed 7-9 weeks after the planting is well established.

Asparagus storage carbohydrates are fructo-oligosaccharides, contrary to the report by Scott et al. (8). That fructo-oligosaccharides have existed in asparagus roots has been known since 1909; however, due to problems with methodology, estimates of composition and size have remained tentative. Gas chromatography and gel exclusion chromatography in this study clearly indicated that parent oligosaccharides consisted of $\sim$ 10% glucose, and $\sim$ 90% fructose, with molecular weights not exceeding 4,000. Shiomi et al. (9) performed structural analyses of fructo-trisacchardies through pentasaccharides from asparagus roots; however, structural analyses of the larger oligosaccharides have yet to be attempted.

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An Anatomical and Morphological Study of Abscission in Highbush Blueberry Fruit[1]

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Additional index words. Vaccinium corymbosum, fruit ripening, harvesting

Abstract. **Berry/pedicel abscission zone formation in cultivated highbush blueberry (*Vaccinium corymbosum* L.) during fruit ripening appeared at the distal portion of the transition zone (disc) separating the berry and the pedicel. The abscission zone was first evident as a compressed zone of cells at the periphery of the berry/pedicel junction in the immature green stage of berry development. Cell separation initially appeared during the green-pink berry stage concomitant with berry coloration. Separation was characterized primarily by cell wall rupture. No separation layer was formed through either the berry epidermis or the vascular bundles. The number of berries without attached pedicels separating from the cluster under applied force was closely related to the stage of ripening of the fruit. Stresses imparted during ripening by rupturing internal tissues are evident in definite morphological changes appearing on the surface of the fruit.**

Though research is being conducted on the chemical promotion of blueberry fruit abscission (5), no reports on the nature of fruit separation layer ontogeny during the development and maturation of this fruit have appeared, and only a few on the abscission of other crops have appeared (2, 9, 10). This investigation was undertaken to elucidate the anatomical and morphological changes associated with abscission zone ontogeny in developing highbush blueberry fruit.

Materials and Methods

Morphological study. The point of fruit detachment was determined on mature plants of: 'Blueray', 'Bluecrop', 'Herbert', 'Darrow', 'Coville', and 'Lateblue'. Twenty fruit in each of 5 color stages (described below under *Anatomical study*) were selected and detached by manually applying an even pull-force to the berry in a direction approximately parallel to

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