

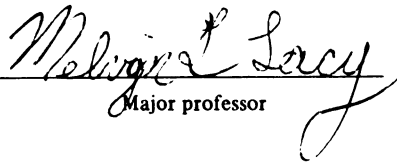




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EFFECT OF STRESSES ON GROWTH AND
YIELD OF ASPARAGUS (APARAGUS OFFICINALIS L.)

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EFFECT OF STRESSES ON GROWTH AND
YIELD OF ASPARAGUS (ASPARAGUS OFFICINALIS L.)

By

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ABSTRACT

EFFECT OF STRESSES ON GROWTH AND
YIELD OF ASPARAGUS (ASPARAGUS OFFICINALIS L.)

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One-year-old asparagus plants held for two months under a constant 16 hour photoperiod and temperatures of 20°-28°C in the greenhouse, differentiated buds and storage roots throughout the growing season. Growth of two-year-old asparagus crowns in the field was unaffected by length of harvest duration. Average weight of stalks produced per crown by the end of the season was also unaffected by length of harvest, although timing and rates of spear and fern production were affected by weather conditions and length of harvest. Extreme variability in growth and development among plants was observed.

In the first year of harvest, marketable yields from four-year-old asparagus plants increased throughout the six weeks of harvest. In the second year of harvest, marketable yields remained about the same during the first six weeks of harvest, then decreased drastically thereafter. Percent marketable spears and average weight of spears declined throughout harvest in both years. In general, two years of yield data were insufficient to determine the effect of length of harvest on long term yields of asparagus.

Soluble storage carbohydrate levels of asparagus plants decreased

during harvest and after harvest as spears and stalks were produced. Carbohydrate levels increased after stalks had matured, and plants regained original levels of carbohydrate by mid- to late summer. Extending the harvest period resulted in increased time and severity of carbohydrate depletion, although all plants, regardless of length of harvest, possessed comparable levels of storage carbohydrate by the end of the season. Asparagus plants replenished the supply of carbohydrate in established storage roots before producing new storage roots.

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 and 10% glucose and had a molecular weight of approximately 3200 (18 molecules of fructose and 2 molecules of glucose).

Treatment of asparagus plants with the herbicides, simazine, linuron, or terbacil, either inoculated with Fusarium oxysporum f. sp. asparagi and F. moniliforme or uninoculated, resulted in increased crown survival and increased total yields. Treatment with field rates of simazine resulted in higher total yields per crown than with other herbicides; marketable yields were not affected. Soil populations of F. oxysporum and F. moniliforme were not significantly different in any of the treatments by the end of the second season after inoculation.

To Roland and Bobby

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INTRODUCTION

Commercial asparagus production in Michigan is a multimillion dollar industry (30). Michigan presently ranks third among states in asparagus production, behind California and Washington (30), and acreage planted to asparagus continues to increase (19). Despite increased production due to increased acreage, yields of asparagus have been in a general decline since 1970 (19), causing great consternation within the asparagus industry. Although the exact reasons for the decline in yields are not known, stress factors—cultural, biological, and environmental—are undoubtedly involved. The general scope and purpose of these investigations was to examine the effect of stresses on the growth and yield of asparagus.

In order to understand the effects of stresses on the growth and yield of asparagus it is important to have an understanding of those factors—genetic, physiological, and environmental—which affect its development. It is generally accepted that the growth and marketable yield of asparagus is closely related to reserve carbohydrate materials produced and stored during the fern period of the preceeding year. It is also agreed that marketable yields are directly related to bud size and spear size. Many stresses may be imposed on asparagus plants which affect levels of storage carbohydrate and/or bud and spear size. These include: spring freezes, extended harvest periods, drought, weed competition, and damage from insects, disease, tillage, and herbicides. Many of these stresses can be ameliorated to a greater or lesser extent. Damage from

weeds, insects, and disease can be minimized through the use of pesticides and resistant asparagus varieties. Tillage damage can be eliminated with zero-tillage cultural practices. Even spring freezing and drought can be ameliorated to some extent through the use of irrigation. The one stress which cannot be avoided is harvesting of asparagus. It is necessary, therefore, to have a precise understanding of how length of harvest affects levels of storage carbohydrate and bud and spear size in order to optimize yields without adversely affecting plant vigor. The major thrust of this research was to investigate the effect of length of harvest on yields and seasonal fluctuations of storage carbohydrates. Investigations were also initiated to observe the effect of length of harvest on growth and development of asparagus plants, to elucidate the structure and composition of storage carbohydrates, and to examine the effect of different herbicide treatments on yields of asparagus and their interaction with asparagus root rot fungi.

LITERATURE REVIEW

I. Growth and Bud Formation in Asparagus

Individual asparagus plants, even of the same cultivar, differ considerably in their growth patterns (35). This variability is due to the fact that asparagus is dioecious; plants of the same variety are not isogenetic. Male crowns, on the average, are larger than female crowns and are of greater longevity (35). Male plants produce fewer but larger spears (35,12). Sawada (25) calculated that mature male plants produced 28% more carbohydrate, on a daily basis, than female plants.

An understanding of bud formation and subsequent spear formation is prerequisite to understanding growth and development of asparagus. Buds are produced in terminal clusters, and the clusters operate independently (23). Buds produced one season give rise to spears the next season (35, 3). Bud size diminishes from larger buds near the stalks to smaller buds near the periphery of the crown (35,23). Spear size is directly related to bud size (3,23), although Tiedjens (35) observed that large buds did occasionally give rise to small spears. Rajzer (23) studied the relationship of bud dominance and size to spear size. He observed that the primary bud (the first to break dormancy) produced the largest spear in the cluster, that the secondary bud produced the second largest spear, that the tertiary bud produced the third largest spear, etc. There seemed to be a direct correlation between the size of buds and the size of the stalks which furnished the carbohydrate for their formation (36). The

production of small spears during late harvest, the production of small stalks after harvest, and the subsequent production of small buds seemed to be a result of reduced carbohydrate levels (36). Spear diameter one season was positively correlated with stalk diameter the preceeding season (7).

Depth of plant and/or soil temperatures have an effect on spear number and size. The deeper asparagus crowns were planted (up to 30 cm) the fewer but larger the spears they produced (33). Soil temperatures of between 19° and 24°C produced the largest spears, while 26°C produced maximum yields (9). No studies have been conducted concerning the effect of temperature on bud size.

II. Effect of Length of Harvest on Yields

Several studies have been conducted during the past 50 years concerning the effect of length of harvest on asparagus yields. Jones (12) studied the effect of two lengths of harvest on the yields of male and female 'Palmetto' asparagus plants in California. One set of plots was cut for approximately 9 weeks in 1926 when plants were four years old, 11 weeks in 1927-28, and 12 weeks in 1929-31: another set of plots was cut for approximately 4 weeks in 1925 when plants were three years old, 11 weeks in 1926, 13 weeks in 1927-28, and 14 weeks in 1929-31. While both male and female plants yielded a significantly greater number of spears in the longer harvest plots, only the male plants yielded a significantly greater weight of spears. The average spear weight for plots harvested for the longer period was significantly lower than plots harvested for the shorter period. Although there were no significant differences in number of stalks produced, stalks of plants in the longer

harvest weighed significantly less than those in the shorter harvest.

Haber (11) studied the effect of harvesting for different periods of time on the yields of 'Mary Washington' asparagus plants in Iowa. Plots were harvested for periods of 1, 3, 5, 7, 9, or 11 weeks, beginning when the plants were four years old (1929). In the first year, due to the long harvest, the plots harvested for 11 weeks yielded the greatest number and weight of spears. In 1920, plots harvested for 9 or 11 weeks yielded equal numbers and weights of spears. Plants harvested for 11 weeks declined in vigor from year to year; after five years the majority of plants were dead. From 1930-34, plots harvested for 9 weeks yielded a greater number of spears than any of the other plots; however, only in 1931 did they yield the greatest weight of spears. In 1932 and 1933 plots harvested for 7 or 9 weeks produced approximately equal weights of spears; in 1934 plots harvested for 7 weeks produced the greatest weight of spears. Spear size in plots harvested for 1 or 3 weeks increased from 1929-34 in comparable fashion: spear size in plots harvested for 5 weeks also increased in size, but to a lesser extent. Spear size in plots harvested for 7 weeks remained approximately the same or increased gradually. Spear size in plots harvested for 9 weeks declined.

Williams and Garthwaite (39) studied the effect of three lengths of harvest on the yields of 'Giant Mammoth' asparagus plants in England. Plots were harvested for periods of 6, 8, or 10 weeks, beginning when the plots were five years old (1964), with the exception of the plots harvested for 6 weeks which were first cut when four years old (1963). In the first two years of harvest, plots harvested for 10 weeks produced a significantly greater number of spears than plots harvested for 6 or 8 weeks. In 1966 and 1967 plots harvested for 8 or 10 weeks produced approximately equal

numbers of spears. In 1968 plots harvested for 8 weeks significantly out-yielded plots harvested for 6 or 10 weeks. Weights of spears were not reported. Percentage of large spears in the 6 and 8 week harvest periods was comparable and declined gradually from 1964-68; percentage of large spears in the 10 week harvest period declined drastically. The plots harvested for 6 weeks consistently produced a greater number of early spears than the plots harvested for 8 weeks, which in general, produced a greater number of early spears than the plots harvested for 10 weeks. Monetary return for each of the harvest periods was averaged over the five year period. Taking into account total number of spears, size of spears, and earliness of spears, the plots harvested for 8 weeks yielded a significantly greater monetary return than those harvested for 6 or 10 weeks.

Deonier and Hoffman (5) studied the effect of six lengths of harvest and times of harvest on the yields of 'Mary Washington' asparagus plants in Mississippi. Plots were harvested for periods of 4, 8, or 12 weeks in the spring, 4 or 6 weeks in the fall, or 4 weeks both in the spring and fall; plants were first harvested when four years old (1937). The best marketable yields, averaged over the five year period, were from plots harvested continuously for 8 weeks in the spring. Plots harvested for 12 weeks in the spring yielded well from 1937-39, but by 1940-41 yields had dropped off drastically. Yields from plots harvested for 4 weeks in the spring increased from year to year and by 1941 were only slightly less than those from plots harvested for 8 weeks. Yields from plots harvested only in the fall, or in both spring and fall, were small. Percentage of marketable spears produced in 1941 was greatest in the 4 week spring harvest, followed by the 8 and 12 week spring harvests, and

was least in the fall and spring-fall harvests.

Takatori, et al. (30) studied the effect of several lengths of harvest and times of harvest on the yields of asparagus plants in California. Plots were harvested for 4 weeks (Feb. - March), 8 1/2 weeks (Feb. - April), 13 weeks (Feb. - May), or 17 weeks (Feb. - June) in the spring; 8 1/2 weeks (June - July, July - Aug., Aug. - Sept., or Sept. - Oct.) in the summer or fall; or 4 weeks in the spring and fall (Feb. - April and Aug. - Sept., or Feb. - April and Sept. - Oct). From 1966-68 best overall yields (numbers and weights of marketable spears) were obtained from the plots harvested for 8 1/2 weeks in the spring. Decreases in yields from the plots harvested for 13 or 17 weeks in the spring during 1966 were caused by losses in spear size. During 1967 and 1968 both weights and numbers of spears decreased. The reduction in numbers of spears produced was a result of loss in stand. In 1969, all plots were harvested for 8 1/2 weeks in the spring. Plots previously harvested for 4 weeks in the spring yielded the greatest weight of marketable spears, followed by the plots harvested for 8 1/2 weeks in the spring and the plots harvested for 4 weeks in the spring and fall (Feb. - April and Sept. - Oct). The plots harvested in the fall (Sept. - Oct.) and spring and fall (Feb. - April and Aug. - Sept.) yielded moderately well, while the plots harvested for 13 or 17 weeks in the spring and the plots harvested in the summer yielded very poorly.

Lewis (17) studied the effect of time of initiation of harvest on subsequent yields of 'Mary Washington' asparagus plants in Illinois. Six harvest treatments were carried out:

Treatment Number	1927	1928	Weeks Harvested		1931-33
			1929	1930	
1	0	0	4	6	8
2	0	2	4	8	8
3	0	4	6	8	8
4	2	4	8	8	8
5	4	6	8	8	8
6	6	8	8	8	8

From 1931-33, when all plots were harvested for an 8 week period, treatment two produced both the greatest number and weight of spears, followed closely by treatment one. Treatments three and four yielded approximately equal numbers and weights of spears, while treatments five and six lagged far behind the other treatments. Treatment two produced the highest percentage of large spears and greatest average spear weight, averaged over the seven year period, followed by treatments one and three. Lewis calculated monetary return per acre for each of the treatments, based on total spear weight and spear size, and found that treatment two yielded the greatest monetary return over the seven year period. Lloyd and McCollum (18) continued Lewis' study from 1933-37. They found that the trends discussed above continued through 1937 (all plots were cut for 8 weeks from 1931-37). Treatment two continued to yield the greatest total number and weight of spears as well as greatest number and weight of large spears, followed closely by treatment one.

III. Effect of Length of Harvest on Seasonal Fluctuations of Storage

Carbohydrates

One previous study was conducted in North Carolina on the effect of

length of harvest period on fluctuations of storage carbohydrates in asparagus (27). Asparagus plants were periodically sampled and assayed using harvest periods of 4 or 6 weeks. Reducing sugars, non-reducing sugars, starch, and total nitrogen were determined. Large fluctuations in levels of non-reducing sugars were found throughout the season, while levels of reducing sugars and starch fluctuated very little. Concentrations of non-reducing sugars decreased at a moderate rate from beginning to end of harvest, after which concentrations decreased dramatically. Sugar levels reached a minimum about a month after the end of harvest, then increased rapidly for about two months, regaining levels comparable to those of early spring by late summer. Lengthening the harvest period from 4 to 6 weeks caused a greater depletion of carbohydrate reserves, and a longer period for carbohydrate accumulation after the end of harvest was required, than with the shorter harvest. Harvesting for 6 weeks did not appear to permanently affect the carbohydrate composition of the crown. Scott et al. (27) also followed carbohydrate fluctuations in asparagus plants harvested in the fall. They found a similar pattern of cyclic depletion and replenishment of storage carbohydrate, except that the pattern was shifted by about six months. Carbohydrate reserves began to decline in early fall as harvesting began, remained at moderate levels through the winter, and declined further during the spring as fern was being produced. Carbohydrate reserves were replenished by early summer and remained so until harvesting began again in early fall. Plants harvested in the spring were in a state of carbohydrate depletion only about three months out of the year, while plants harvested in the fall were in a state of depletion or semi-depletion about nine months out of the year.

IV. Characterization of Asparagus Storage Carbohydrates

Carbohydrates from asparagus storage roots were first isolated in 1909 (34). When sections of asparagus storage root were treated with 95% ethanol a milky white precipitate was formed. The precipitate was composed, in part, of an inulin-like carbohydrate which was termed asparagose. Upon hydrolysis asparagose yielded 93% fructose and 7% glucose.

A storage carbohydrate was isolated from asparagus in 1937 which was termed asparagosin (26). The asparagosin molecule was estimated to be composed of 13-14 fructose residues. Hydrolysis of the methylated derivative yielded dimethyl-, trimethyl-, and tetramethyl-fructose; the trimethyl-D-fructose was identified as 3, 4, 6- trimethyl-D-fructofuranose. Whether glucose was present in the molecule was not determined.

Sucrose was reported as the storage carbohydrate of asparagus by Scott et al. (27). Since no quantitative data were presented, it appeared that the investigators presumed (without direct evidence) that the non-reducing portion of the carbohydrate present was sucrose.

Shiomi et al. (29) isolated and characterized eight fructo-oligosaccharides from asparagus. Oligosaccharides were exhaustively methylated and hydrolyzed, and methylated derivatives analyzed by gas-liquid chromatography. Identification of constituent monosaccharides and glycosidic bonds were deduced by comparing retention times of the methylated derivatives with retention times of methylated sugars of known composition and structure; mass spectrometry was not used. The eight fructo-oligosaccharides were tentatively identified as $1^F(1-\beta\text{-fructofuranosyl})_1$ sucrose {1-kestose}: $1^F(1-\beta\text{-fructofuranosyl})_2$ sucrose {nystose}: $1^F(1-\beta\text{-fructofuranosyl})_3$ sucrose: $6^G(1-\beta\text{-fructofuranosyl})_1$ sucrose {neokestose}:

$6^G(1-\beta\text{-fructofuranosyl})_2$ sucrose: $6^G(1-\beta\text{-fructofuranosyl})_3$ sucrose: and $1^F(1-\beta\text{-fructofuranosyl})_2-6^G\text{-}\beta\text{-fructofuranosyl}$ sucrose.

V. Effect of Three Herbicides on Spear and Stalk Production, and Crown Survival in Natural and Artificially Infested Soil

Herbicides are known to have a wide variety of effects on soil microorganisms and plants, resulting in both increased and decreased disease incidence and severity (2,13,15). Herbicides may directly affect population levels or activities of pathogens in the soil, affect levels or activities of microorganisms antagonistic to soil-borne pathogens, or somehow affect resistance or predisposition of plants to disease (13).

Fusarium oxysporum f. sp. asparagi Cohen and Heald and Fusarium moniliforme (Sheld.) emend. Snyder and Hans. are the causal agents of the wilt and root rot diseases of asparagus (10). Kaufman (14) found that treatment of soybean-cropped soils with the phenylurea herbicides linuron and diuron caused a decrease in the total number of Fusarium propagules in those soils, but that there was no effect on Fusarium populations in corn-cropped soils. Percich and Lockwood (21) demonstrated that populations of Fusarium in Conover atrazine amended loam soil were increased 4-fold. Population levels and activities of propagules of Fusarium solani, f. sp. pisi and Fusarium roseum f. sp. cerealis 'Culmorum', as well as incidence of pea root rot and corn seedling blight, were enhanced by atrazine.

Simazine-treated plots have occasionally been observed to yield a greater total weight of spears than weeded check plots (Putnam, personal communication). However, Welker and Brogdon (38) did not obtain higher yields from simazine-treated plots than from weeded controls. Dry weight

of asparagus plants from soils treated with simazine at 4.4 kg/ha (for two years) or DMPA at 16.5 kg/ha was significantly greater than from untreated plants or plants treated with a number of other herbicides (24). This was attributed to superior weed control by simazine; however, other investigators (22,38) did not find simazine superior to other herbicides in controlling weeds in asparagus.

METHODS AND MATERIALS

I. Growth and Bud Formation in Asparagus

Ten one-year-old 'Mary Washington' asparagus crowns were planted in wooden boxes (three crowns each in two 107x51x46 cm boxes, 2 crowns each in two 81x41x46 cm boxes), filled to a depth of 28 cm with steamed soil, and placed in the greenhouse. Crowns were placed on the surface of soil in the boxes, then covered with 10-15 cm of expanded silica (Perlite). Plants were held under a constant 16 hour photoperiod. Soil temperatures ranged from 20°-28°C. Periodically, the Perlite was removed to observe growth and development of the crowns, and to record numbers of buds, spears, and new storage roots after which the Perlite was replaced.

A two-year-old asparagus plot at the Botany and Plant Pathology Farm was divided into 1.5 x 18 meter replicated plots arranged in a completely randomized design which were harvested for periods of 0, 3, or 6 weeks, respectively. Periodically (4/14/77 through 9/7/77) three to six complete crowns and storage root systems from each of the plots were removed from the soil. Plants were taken to the laboratory, washed free from soil, and divided into spears and/or stalks, crowns, and storage roots. Plant parts not immediately processed were stored at ~5°C. Crowns, spears, and portions of stalks were weighed, dried at 96°C for approximately 12 hours, and reweighed to determine dry weight. Dry weight of whole stalks was computed by extrapolating percent dry matter of stalk portions to the entire stalk. Observations concerning growth and development of plants were noted.

II.-III. Effect of Length of Harvest on Yields and Seasonal Fluctuations
of Storage Carbohydrates

A three-year-old asparagus plot at the Botany and Plant Pathology Farm was divided into 1.5 x 7.7 meter replicated plots arranged in a randomized complete block design which were harvested for periods of 0, 2, 4, or 6 weeks, respectively. Storage roots from plants in each treatment were sampled periodically from 4/9/77 through 10/29/77 by collecting a 15-20 cm soil core containing root segments approximately 15 cm from the center of the crowns with a 70 cm soil probe. Two to three plants from each plot were sampled on each sampling date and four core samples were taken per plant. Storage root segments were weighed and dried at 96°C for approximately 12 hours, reweighed to determine dry weight, and then ground in a Wiley Mill using a screen with 0.40 mm openings. Particles retained and passed through the sieve were collected for analysis. Soluble storage carbohydrates were extracted from one gram dried ground tissue in a Sorvall Omni-mixer (Ivan Sorvall, Inc., Norwalk, CT) bucket containing 100 ml distilled water for two minutes at 4,400 rpm. Solutions were centrifuged for 15 minutes at 4,000 rpm in a Universal Model U-V International centrifuge (International Equipment Co., Needham, MA) to remove emulsions, then filtered through Whatman glass-fiber filter paper. Extract solutions were diluted 50-100 fold and total carbohydrates determined by a modified anthrone analysis (20). One ml of extract solution was mixed with 9 ml of anthrone reagent (0.2 grams of anthrone dissolved in 100 ml of 95% sulfuric acid), placed in a boiling water bath for 15 minutes, then immediately cooled in an ice bath. After the solutions had warmed to room temperature, optical density was measured at 620 nm with a Bausch and Lomb Spectronic 20 spectrophotometer (Bausch and Lomb,

Rochester, New York). Carbohydrate concentrations were determined using a standard curve derived from sucrose.

In the following year, the harvest period was extended for 4 weeks for each of the plots, resulting in harvest periods of 4, 6, 8, or 10 weeks corresponding to the 0, 2, 4, or 6 week harvest periods of the previous year. Storage roots were not sampled in these plots the second year.

In the two-year-old asparagus plot (page 16), three to six complete crowns and storage root systems from each of the plots were removed periodically from 4/14/77 through 9/7/77 from the soil for analysis of storage carbohydrate levels. An attempt was made to obtain as much of the storage root system as was possible, although small quantities of roots were inevitably lost. Plants were taken to the laboratory, washed free from soil, and divided into spears and/or stalks, crowns, and storage roots. Storage roots were washed and stored at $\sim 5^{\circ}\text{C}$ until further processing. Randomly chosen storage roots were cut into 2.5 - 4.0 cm segments, weighed, dried at 96°C for approximately 12 hours, reweighed to determine dry weight, and then ground in a Wiley Mill as before. Carbohydrate determinations were performed using the anthrone method as previously described, with the exception that fructose was substituted for sucrose in deriving the standard curve.

IV. Characterization of Asparagus Storage Carbohydrates

One half gram of dried ground storage root tissue was extracted with 100 ml of boiling 80% ethanol in a Sorvall Omni-mixer for five minutes at 10,800 rpm. The carbohydrate solution was filtered through Whatman glass-fiber filter paper, taken to dryness in a flash evaporator,

redissolved in distilled water, filtered through a 0.22 μm pore size microfilter (Millipore Corp., Bedford, MA), and again taken to dryness in a flash evaporator. The residue was redissolved in 1-2 ml of distilled water and stored in vials at $\sim 5^{\circ}\text{C}$ until analyzed.

Aliquots of extract solution were streaked on Whatman 3MM chromatography paper and developed in propanol:ethyl acetate:water (7:1:2) (8) for periods of 4, 12, or 24 days using a descending technique. Sucrose and/or raffinose were used as reference sugars for the chromatograms developed for 4 or 12 days. One of the carbohydrate bands eluted from the chromatogram developed for 12 days, and subsequently characterized, was used as a reference sugar for the chromatogram developed for 24 days, by which time sucrose and raffinose had been eluted off the chromatogram. Oligosaccharides were detected by spraying a small longitudinal strip of the chromatogram with ketose sugar-specific NEP indicator (1% w/v α -naphthol in ethanol with 10:1 v/v 80% H_3PO_4) (1), then heating at 100°C for 2 minutes. Oligosaccharides were eluted from the undeveloped portions of chromatograms. Sections containing specific oligosaccharides were cut into small squares and extracted with 50 ml of double distilled water for 30 minutes on a reciprocal shaker (115 cycles/minute). Carbohydrate solutions were filtered through a 0.22 μm pore size microfilter, taken to dryness in a flash evaporator, redissolved in 1 ml of double distilled water, and stored at $\sim 5^{\circ}\text{C}$ until analyzed.

Fructose and glucose were determined via gas-liquid chromatography (31,4,28). Quantities of carbohydrate solution containing 50-100 μg of oligosaccharide were freeze-dried, then hydrolyzed with 1N HCl at 52° - 54°C for one hour in Kimax vials covered with teflon-lined caps. HCl was neutralized with AgCO_3 and the precipitate washed once with 100 μl

acetic anhydride and twice with 1 ml of dry methanol. All fractions were collected and taken to dryness in a vacuum desiccator. Hydrolyzed samples were trimethyl-silylated overnight with 50 μ l of Tri-Sil Z (Pierce Chemical Co., Rockford, IL). Samples were injected into either a Perkin Elmer 900 gas chromatograph equipped with a 3.7 m glass column (3% SE-30 stationary phase, 100-120 mesh Chromosorb Q packing) or Perkin Elmer 910 gas chromatograph equipped with a 3.7 m glass column (3% SP-2100 stationary phase, 100-120 mesh Chromosorb Q packing), both with hydrogen flame ionization detectors. Separation of monosaccharides was accomplished using a temperature-programmed analysis of 140°-200°C (4 minutes at 140°C followed by increases of 2°C min⁻¹) for the 900 model and 120°-180°C (4 minutes at 120°C followed by increases of 2°C min⁻¹) for the 910 model. The flow rate of nitrogen carrier gas was 50 ml/min; injection port and detector temperatures were 260°C. Peak areas were determined electronically with a Spectra-Physics Autolab System IV interfaced with the gas chromatographs. Standards of inulin and sucrose were included with each batch of samples analyzed.

Total galactose was determined with a Galactostat Reagent Set (Worthington Biochemical Corp., Freehold, N.J.).

Molecular weight was estimated using gel exclusion chromatography (37). Bio-Gel P-4 and P-6 400 mesh beads (Bio-Rad Laboratories, Richmond, CA) were packed in 1 cm diameter columns to a bed height of 23 cm. With a head of 80 cm, flow rate was 3.2 ml/hour and the void volume was 3.6 ml as determined with blue dextran. One mg of storage carbohydrate was applied to the top of the columns in 0.1 ml of distilled water. Fractions collected were analyzed for total carbohydrate using the anthrone method, as previously described.

V. Effect of Three Herbicides on Spear and Stalk Production, and Crown Survival in Natural and Artificially Infested Soil

One-year-old 'Mary Washington' crowns were planted at the Botany and Plant Pathology Farm. Prior to covering the crowns, half of the plots were inoculated in the row with 50 grams/25 foot row colonized wheat seed inoculum containing both Fusarium oxysporum f. sp. asparagi and F. moniliforme. Simazine (4.4 kg/ha), linuron (2.2 kg/ha), or terbacil (2.2 kg/ha) were applied in the spring prior to spear emergence in 1975, 1976, and 1977 using a CO₂-powered small plot sprayer (AZ Field Test Service, Accord, N.Y.). One set of plots was left untreated and was weeded by hand or with a rototiller as needed. Crown survival and stalk production were recorded in October, 1976. In the spring of 1977, all plots were harvested for four weeks. Crown survival and stalk production were recorded in September, 1977.

Soil samples were taken on October 5, 1976 and July 25, 1977, by obtaining three 2.5×10^{-13} cm soil cores per plot from between crowns within the row. Population levels were determined using the selective medium and plating techniques of Komada (16). The basal medium contained (per liter) 1 gram K₂HPO₄, 0.5 grams KCl, 0.5 grams MgSO₄ · 7H₂O, 0.01 grams Fe-Na-EDTA, 2.0 grams L-asparagine, 20.0 grams D-galactase, and 15.0 grams of agar. The antimicrobial supplement consisted of (per liter) 1.0 grams of PCNB, 0.5 grams Oxygall, 1.0 gram Na₂B₄O₇ · 10H₂O, and 0.25 grams of chloramphenicol. All materials were mixed and autoclaved for one hour to sterilize and melt the agar, cooled to 50°C in a water bath, the pH adjusted to 3.8-4.0 with a 10% solution of phosphoric acid, then poured into sterile disposable Petri dishes.

Soil suspensions were prepared by adding 10 grams of soil to 90 ml

of 0.1% sterile water agar, followed by shaking for 15 minutes on a reciprocal shaker (170 cycles/minute). Further dilutions were made with 0.1% sterile water agar, and 0.5 ml of soil suspension diluted 1000-fold was pipetted onto each of 5 pre-poured plates. After incubation for 8-9 days, colony counts of F. oxysporum and F. moniliiforme were made on the basis of colony morphology and pigmentation, and microscopic observations of microconidiophores. Soil moisture was determined by drying 10 grams of soil in tared aluminum soil canisters at 100°C for 24 hours, reweighing and calculating % moisture.

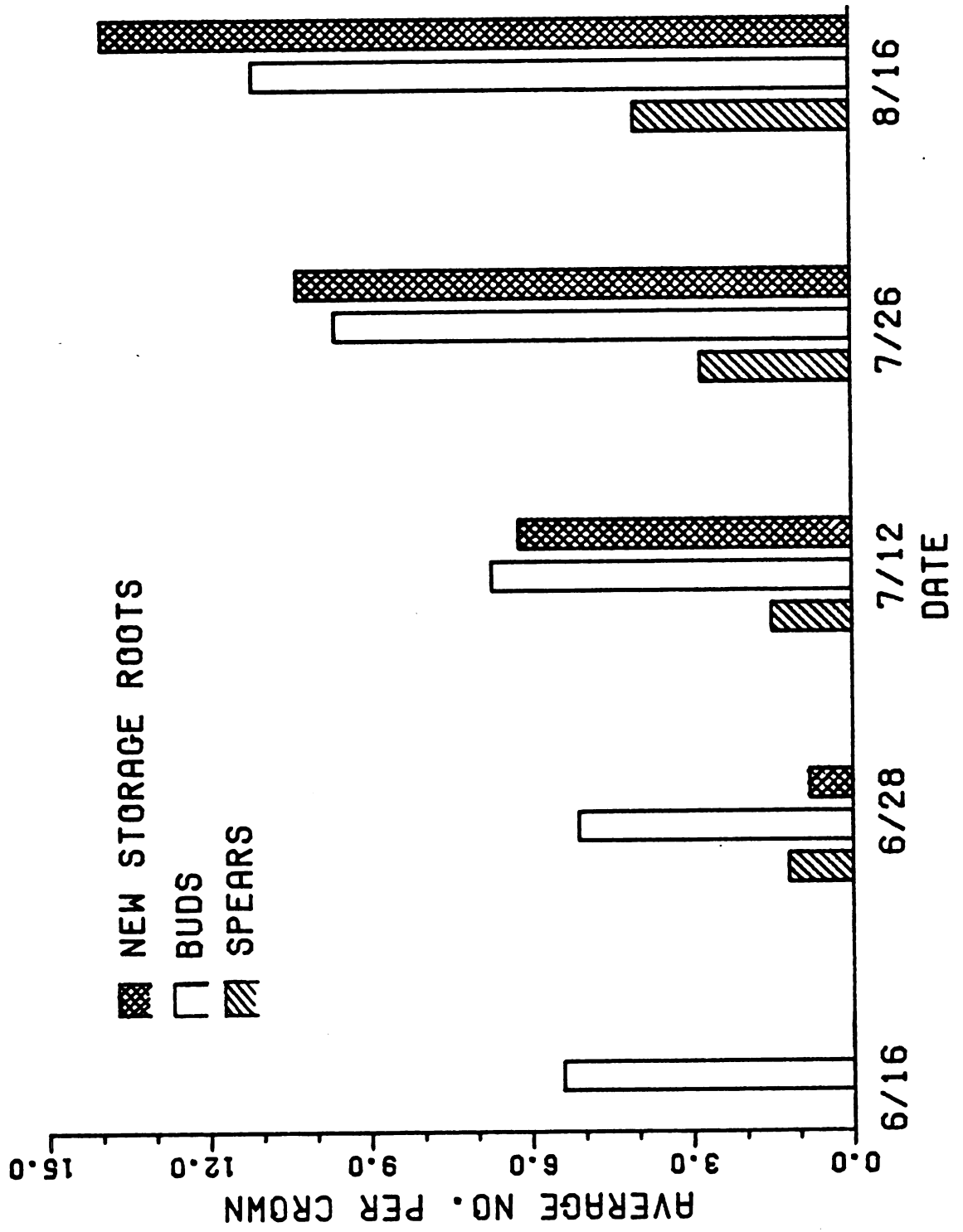
RESULTS

I. Growth and Bud Formation in Asparagus

Spear growth was initiated 2-3 days after planting on crowns planted in boxes in the greenhouse and covered with Perlite. Spears were produced throughout the growing season (Figure 1). New buds and storage roots appeared within two weeks after planting of crowns and also were found throughout the growing season. The average number of buds per crown approximately doubled from mid-June through mid-August. Five plants flowered during the course of the season and were male; five plants did not flower and were presumed to be female. Overall growth and development varied considerably from crown to crown.

In the field, dry weight of crowns from plots harvested for periods of 0, 3, or 6 weeks approximately doubled through the course of the season, although crown size fluctuated considerably from sampling date to sampling date. In the unharvested plots, stalks grew and matured quickly; average dry weight of all stalks on each crown reached a maximum of approximately 34-36 grams by mid-July. In the plots harvested for 3 weeks, stalks also grew and matured quickly after the end of harvest (5/9/77); average dry weight of all stalks on each crown reached a maximum of approximately 34-36 grams by early July. In the plots harvested for 6 weeks, stalks grew more slowly than in the other harvest periods after the end of harvest (5/30/77) (two weeks of cool temperatures in mid-July undoubtedly affected growth rates of spears). However, there was an average of 32

Figure 1.--Production of spears, buds, and new storage roots by one-year-old plants held under a constant 16-hour photoperiod and soil temperature of 20°-28°C.



grams (dry weight) of stalks on each crown by the last sampling date (9/7/77). A light freeze in early May, approximately one week after harvesting began, killed a number of spears in the unharvested plots.

II. Effect of Length of Harvest on Yields

During the first year of harvest (1976), yields from all three plots were similar on any given harvest date (Figure 2). Total and marketable spear production (numbers and weights) in plots harvested for 4 or 6 weeks increased through the third and fourth weeks of harvest surpassing spear production during the first two weeks of harvest (Table 1). Total and marketable numbers of spears produced during the last two weeks of harvest were approximately double that of the middle two weeks of harvest in plots harvested for 6 weeks; total and marketable weights of spears also increased sharply. Percent marketable spears decreased in all plots throughout the harvest periods (Table 1). The average weight of spears increased during the third and fourth weeks of harvest in plots harvested for 4 or 6 weeks, then declined during the last two weeks of harvest in plots harvested for 6 weeks.

During the second harvest season (1977), plots harvested for 10 weeks (harvested for 6 weeks the preceeding year) out-yielded all other plots on any given harvest date (Figure 3). The total number of spears produced during the fifth and sixth weeks of harvest was almost equal to the total number of spears produced during the first four weeks of harvest in plots harvested for 6, 8, or 10 weeks; total weight of spears also increased sharply (Table 2). Total numbers and weights of spears produced decreased dramatically after the sixth week of harvest in plots harvested for 8 or 10 weeks. Percent marketable spears and the average weight of spears

Figure 2.--Marketable yields from asparagus plots harvested for 2, 4, or 6 weeks.

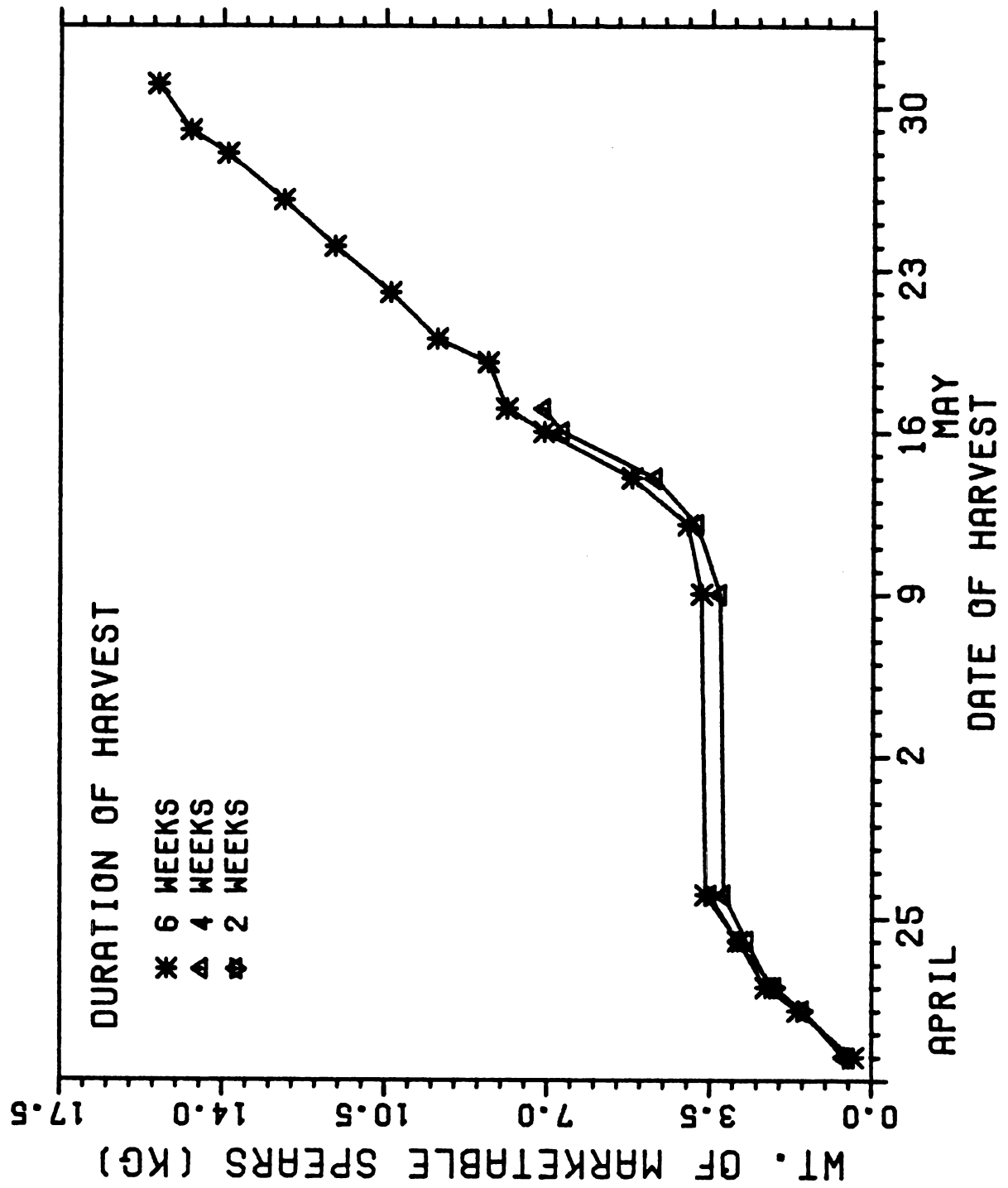


Table 1.--Initial yields from asparagus plots harvested for 2, 4, or 6 weeks.

Harvest Period	Total Weeks Harvested	Total Number Spears	Number Marketable Spears ^a	Percent Marketable Spears	Total		Average		Weight Marketable Spears (kg/ha)
					Weight Spears (kg/ha)	Spears	Weight Spear (gm/spear)	Spears	
4/19-5/8	2	309	164	53.0	415		15.7		300
	4	263	144	54.7	362		16.1		270
	6	265	162	61.1	386		17.0		311
5/9-5/17	4	336	160	47.6	481		16.7		337
	6	310	170	54.8	487		18.4		363
5/19-5/31	6	658	333	50.6	864		15.3		646
4/19-5/31	2	309	164	53.0	415		15.7		300
	4	599	304	50.7	842		16.4		607
	6	1,233	665	53.9	1,738		16.4		1,320

^aSpears > 1 cm in diameter 12.5 cm from the tip were classed as marketable.

Figure 3.--Marketable yields from asparagus plots harvested for 4, 6, 8, or 10 weeks. Plots were harvested for 0, 2, 4, or 6 weeks the previous year.

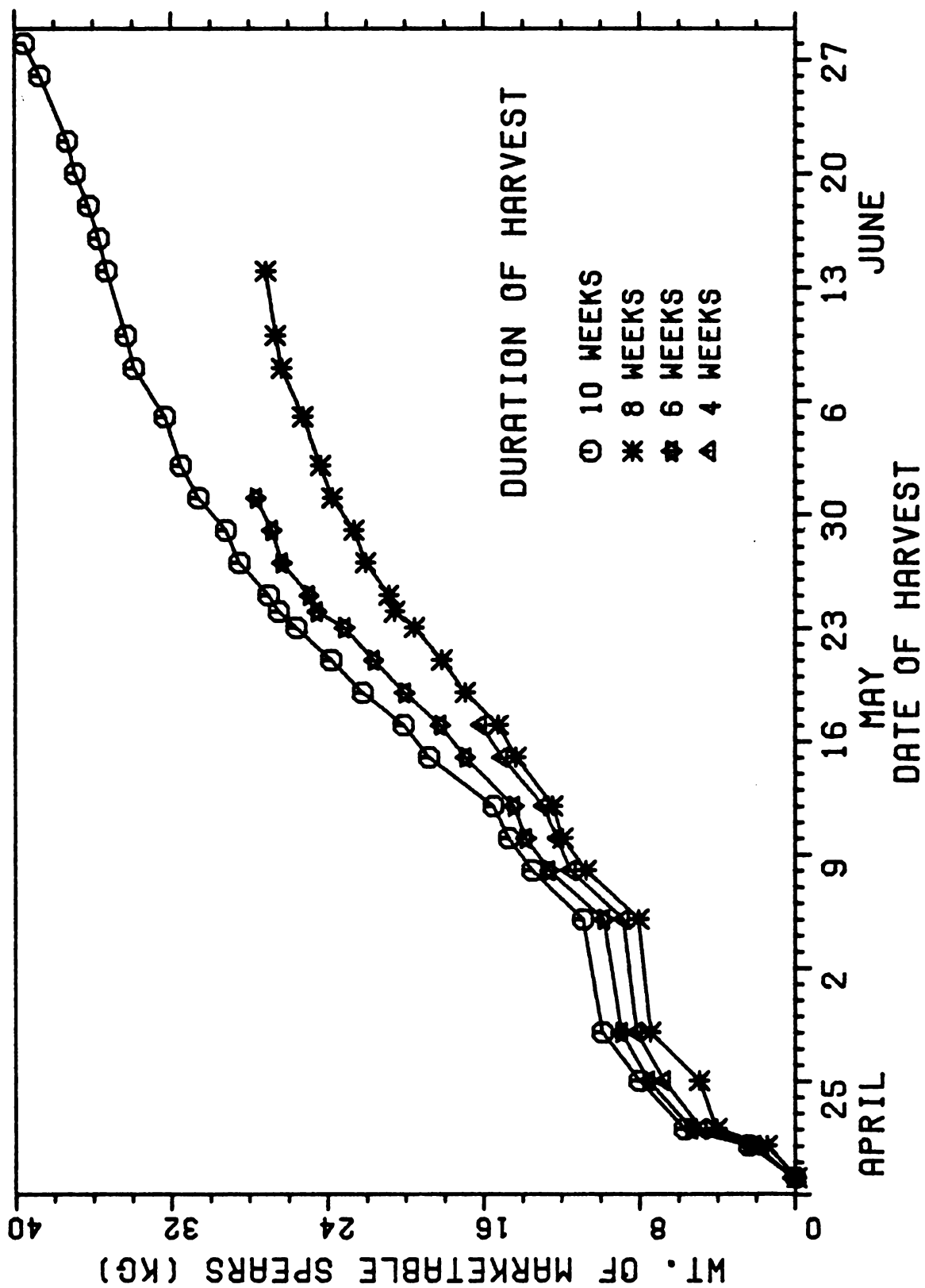


Table 2.--Yields from asparagus plots harvested for 0, 2, 4, or 6 weeks the previous year.

Harvest Period	Total Weeks Harvested	Total Number Spears	Number Marketable Spears ^a	Percent Marketable Spears	Total Weight Spears		Average Weight Spears		Weight Marketable Spears	
					(kg/ha)		(gm/spear)		(kg/ha)	
4/18-5/16	4	1,296	685	52.8	1,930	17.4	1,394			
	6	1,326	781	58.9	2,061	18.2	1,579			
	8	1,324	681	51.4	1,898	16.7	1,322			
	10	1,367	798	58.4	2,244	19.2	1,723			
5/18-5/30	6	1,223	369	30.2	1,557	14.9	805			
	8	1,146	347	30.3	1,442	14.7	722			
	10	1,141	410	35.9	1,541	15.8	896			
6/1-6/13	8	664	158	23.8	698	12.3	291			
	10	616	203	32.9	720	13.6	403			
6/15-6/27	10	695	183	26.3	827	13.9	358			
4/18-6/27	4	1,296	685	52.8	1,930	17.4	1,394			
	6	2,549	1,150	45.1	3,619	16.6	2,383			
	8	3,134	1,186	37.8	4,040	15.1	2,333			
	10	3,819	1,594	41.7	5,501	16.3	3,381			

^aSpears > 1 cm in diameter 12.5 cm from the tip were classed as marketable.

decreased in all plots throughout harvest. Numbers and weights of marketable spears remained about the same in all plots harvested through the first six weeks of harvest, then decreased dramatically thereafter (Table 2). Percentage of marketable spears produced by plots harvested for 10 weeks fluctuated between 65 and 75% during the first three weeks of harvest, decreased rapidly during the fourth week of harvest, then fluctuated between 25 and 40% through the duration of harvest (Figure 4).

Total and marketable spear production (numbers and weights) during the second year of harvest, 4 years after planting, was approximately double that of the first year during any given time period.

III. Effect of Length of Harvest on Seasonal Fluctuations of Storage Carbohydrates

In 1976, percent storage carbohydrate in four-year-old plants decreased gradually through the harvest periods (April-May) and slightly beyond (Figure 5). One to two weeks after the end of harvest, carbohydrate levels decreased at a more rapid rate (as ferns were produced) than during harvest, reaching a low ebb in early June. Then carbohydrate levels increased rapidly, reaching peak levels in mid- to late August. Thereafter, carbohydrate levels gradually decreased. Unfortunately, the integrity of the different harvest periods was disrupted due to a freeze in mid-April, approximately one week after harvesting began, which lasted for approximately two weeks. The full effects of this cold period on storage carbohydrate production are not known. Only between the 6 week harvest and the other three harvest periods could statistically significant differences in percent storage carbohydrate be demonstrated (Table 3), and then, only during that part of the season when carbohydrate levels

Figure 4.--Percentage of marketable spears produced by asparagus plants
harvested over a 10 week period.

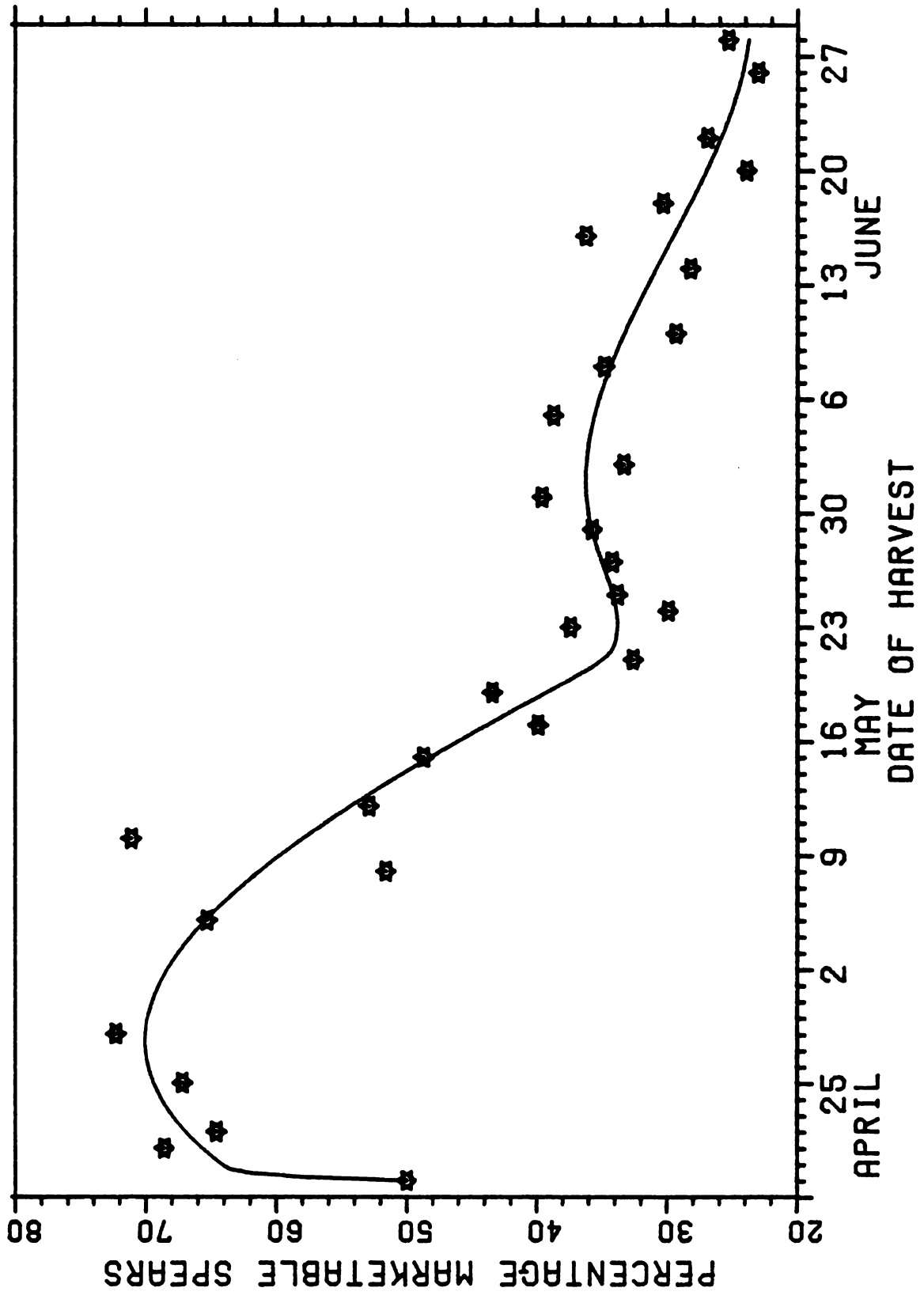


Figure 5.--Effect of length of harvest on levels of soluble storage carbohydrate (1976).

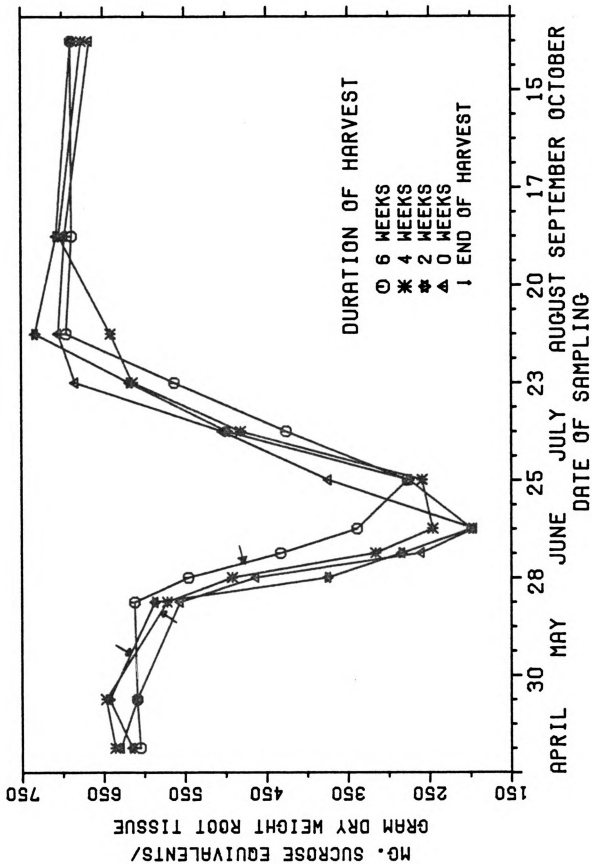


Table 3.--Effect of length of harvest on levels of soluble storage carbohydrate and percent storage root dry matter (1976).

Sampling Date	Length of Harvest (weeks)	Total Carbohydrate ^a (% of dry weight)	Percent Dry Matter
4/9	0	62.9 A ^b	21.3 A
	2	61.5 A	21.9 A
(4/18 - harvest initiated)	4	63.6 A	22.1 A
	6	60.5 A	22.0 A
4/23	0	60.9 A	21.0 AB
	2	64.3 A	22.9 B
	4	64.7 A	21.6 AB
	6	60.9 A	20.2 A
5/21	0	55.6 A	17.6 A
	2	58.7 A	17.8 A
	4	57.2 A	17.1 A
	6	61.2 A	19.0 A
5/28	0	46.3 AB	15.6 A
	2	37.5 A	14.7 A
(5/31 - harvest terminated)	4	49.2 A	14.6 A
	6	54.6 B	17.9 B
6/4	0	26.0 A	12.1 A
	2	28.4 A	12.2 A
	4	31.6 A	12.7 A
	6	43.3 B	14.5 B
6/11	0	19.6 A	11.9 AB
	2	19.9 A	11.4 A
	4	24.6 A	11.5 A
	6	33.9 A	14.0 B
6/25	0	37.4 A	14.6 A
	2	27.5 A	12.1 A
	4	25.9 A	13.9 A
	6	27.7 A	13.1 A
7/9	0	50.4 A	15.2 A
	2	49.8 A	16.8 A
	4	48.2 A	14.8 A
	6	42.6 A	14.8 A
7/23	0	68.4 A	22.2 B
	2	61.8 A	18.8 AB
	4	61.4 A	17.4 A
	6	56.3 A	19.0 AB

Table 3.--(cont.)

Sampling Date	Length of Harvest (weeks)	Total Carbohydrate ^a (% of dry weight)	Percent Dry Matter
8/6	0	70.5 A	21.4 A
	2	73.3 A	22.1 A
	4	64.1 A	21.2 A
	6	69.5 A	22.5 A
9/3	0	69.7 A	25.8 A
	2	70.7 A	24.7 A
	4	70.4 A	24.5 A
	6	68.8 A	25.1 A
10/29	0	66.6 A	24.8 A
	2	68.9 A	25.7 A
	4	67.6 A	25.5 A
	6	68.9 A	25.8 A

^aCarbohydrate was measured by the anthrone method and is expressed as sucrose equivalents.

were declining rapidly (early June). Carbohydrate levels in plants harvested for 6 weeks were consistently higher during harvest and bottomed out one to two weeks later than carbohydrate levels in plants which were harvested for 0, 2, or 4 weeks. Fluctuations in percent storage root dry matter closely followed fluctuations in percent storage carbohydrate (Table 3).

In 1977, fluctuations in percent storage carbohydrate in three-year-old asparagus crowns were nearly identical to those of the preceeding year (Figure 6). In general, carbohydrate levels of plants in all three harvest periods decreased through harvest and after harvest during fern production, then increased rapidly, reaching peak levels in mid- to late summer. Percent storage carbohydrate of plants harvested for 0 or 3 weeks bottomed out in late May to early June and peaked in late July to early August, while percent storage carbohydrate of plants harvested for 6 weeks bottomed out two to three weeks later than plants harvested for 0 or 3 weeks (mid-June) and did not regain levels of carbohydrate comparable to plants harvested for 0 or 3 weeks until early August. Fluctuations in total carbohydrate per storage root system (Figure 7) and dry weight of storage roots (Figure 8) were similar in direction and magnitude to fluctuations in percent storage carbohydrate (Figure 6), reflecting the pattern of storage carbohydrate utilization by asparagus crowns. Plants harvested for 6 weeks experienced a more severe depletion of total carbohydrate per storage root system and a greater loss in dry weight of storage roots than plants harvested for 0 or 3 weeks, and also required a considerably longer period of time to replenish their supply of storage carbohydrate and dry root matter. With the exception of the first week in June, there were no statistically significant differences

Figure 6.--Effect of length of harvest on levels of soluble storage carbohydrate (1977).

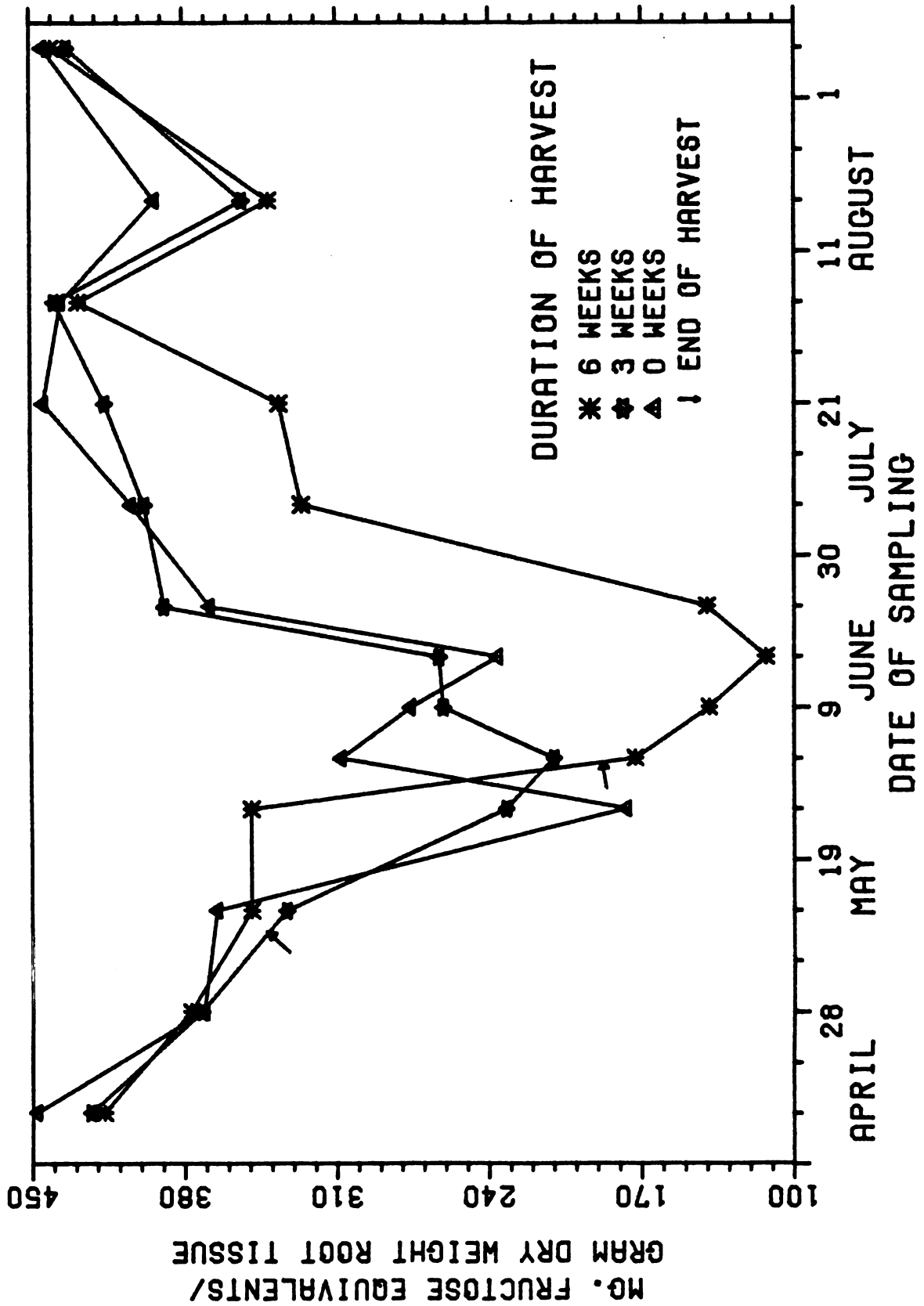


Figure 7.--Effect of length of harvest on storage root growth.

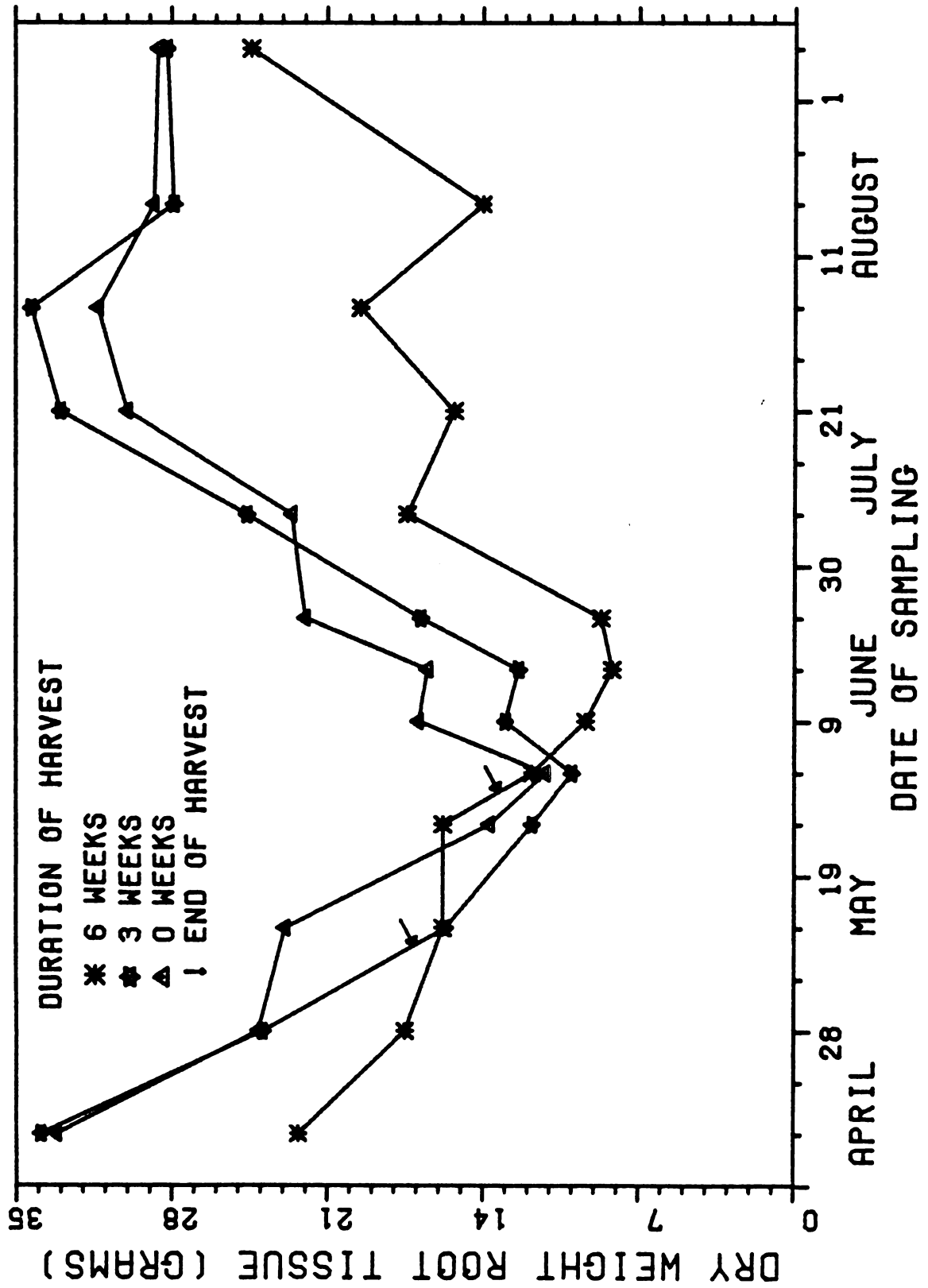
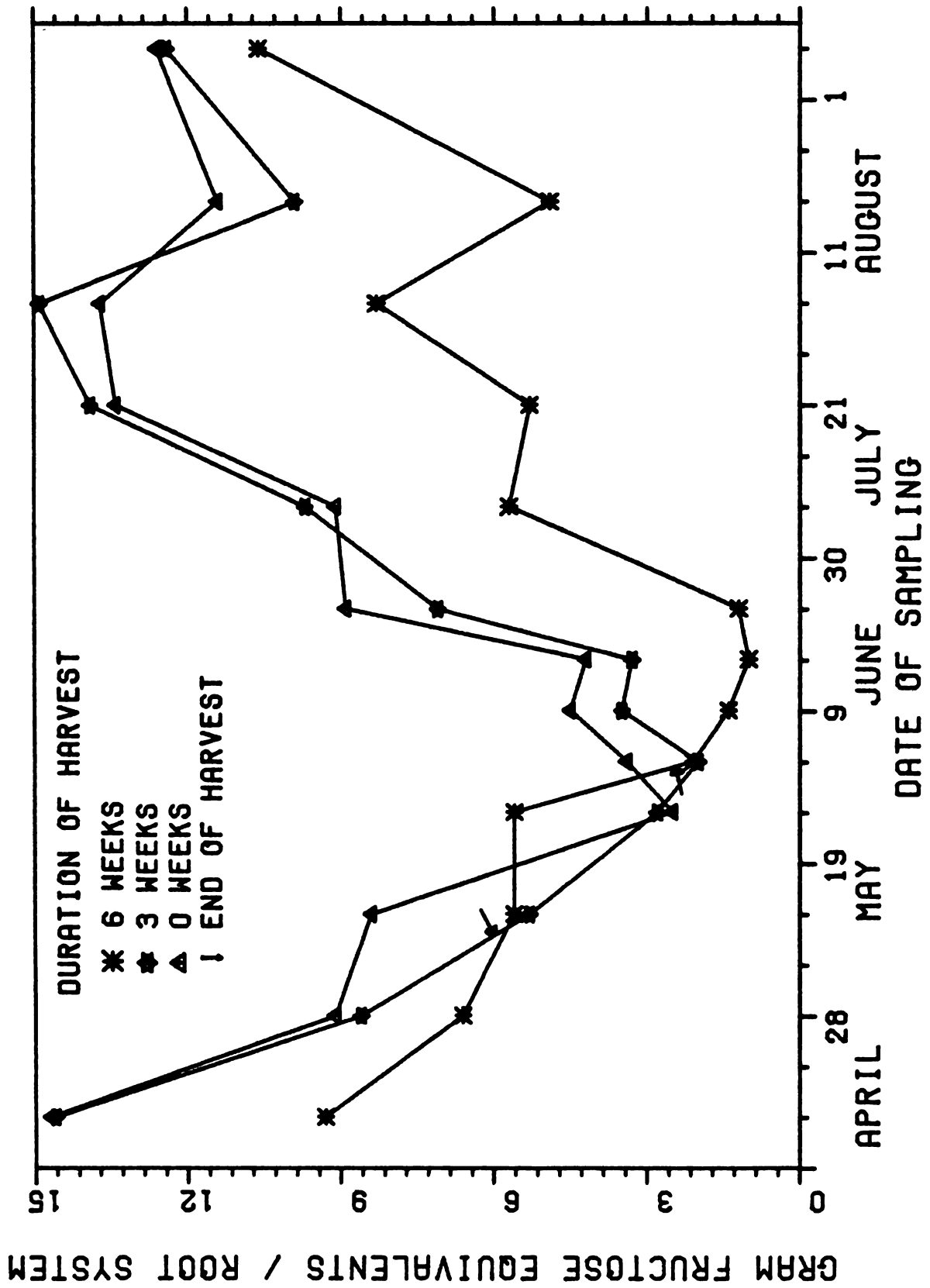


Figure 8.--Effect of length of harvest on total soluble carbohydrate per storage root system.



in percent storage carbohydrate, total carbohydrate per storage root system, or dry weight of storage roots between the 0 or 3 week harvest periods (Table 4). There were dramatic differences between the 0 or 3 week harvest periods and the 6 week harvest period. Plots harvested for 6 weeks had significantly less percent storage carbohydrate from early June through mid-July, significantly less total carbohydrate per storage root system from early June through late August, and significantly less dry weight of storage roots from mid-June through late August, but there were no significant differences thereafter. Values for percent storage carbohydrate, total carbohydrate per storage root system, and dry weight of storage roots were approximately the same for all three harvest periods at the first (4/14/77) and last (9/7/77) sampling dates. Fluctuations in percent storage root dry matter closely followed fluctuations in percent storage carbohydrate (Table 4).

IV. Characterization of Asparagus Storage Carbohydrates

Asparagus storage carbohydrates were readily soluble in 80% ethanol. Re-extraction of tissue with distilled water revealed only trace quantities of soluble carbohydrate remaining.

Paper chromatography revealed a plethora of oligosaccharides varying in size and mobility, all containing ketose sugars (Figure 9). Twelve distinct oligosaccharide bands were eluted from one or more of the chromatograms: two bands (nos. 1 and 2) could be distinguished on the chromatogram developed for 4 days, eight bands (nos. 3, 4, 5, 6, 7, 8, 9, and 10) on the chromatogram developed for 12 days, and eight bands (nos. 4A, 4B, 5A, 5B, 6, 7, 8, and 10) on the chromatogram developed for 24 days. Bands 4 and 5 on the chromatogram developed for 12 days separated

Table 4.--Effect of length of harvest on levels of soluble storage carbohydrate, total carbohydrate per storage root system, dry weight of storage roots, and percent storage root dry matter (1977).

Sampling Date	Length of Harvest (weeks)	Total Carbohydrate ^a (% of dry weight)	Total Carbohydrate per Storage Root System (g.)	Dry Weight Root Tissue (g.)	Percent Dry Matter
4/14 (4/18 - harvest initiated)	0	44.8 A ^b	14.7 A	33.2 A	25.9 A
	3	42.3 A	14.6 A	33.9 A	25.9 A
	6	41.7 A	9.3 A	22.3 A	24.1 A
4/28	0	37.1 A	9.1 A	24.1 A	22.2 A
	3	37.2 A	8.6 A	23.9 A	21.5 A
	6	37.7 A	6.6 A	17.5 A	20.6 A
5/12	0	36.5 A	8.4 A	22.9 A	21.4 A
	3	33.3 A	5.3 A	15.7 A	19.4 A
	6	34.9 A	5.6 A	15.8 A	20.5 A
5/26 (5/30 - harvest terminated)	0	17.7 A	2.5 A	13.7 A	16.2 A
	3	23.2 A	2.8 A	11.8 A	16.3 A
	6	34.9 A	5.6 A	15.8 A	20.5 A
6/2	0	30.8 B	3.4 B	11.2 A	15.0 A
	3	21.0 A	2.0 A	10.0 A	13.6 A
	6	17.3 A	2.1 A	11.8 A	12.9 A
6/9	0	27.6 B	4.5 B	16.9 A	20.0 B
	3	26.1 B	3.5 B	13.0 A	18.8 B
	6	13.9 A	1.4 A	9.4 A	12.6 A
6/16	0	23.6 B	4.2 B	16.5 B	22.3 C
	3	26.3 B	3.3 AB	12.4 AB	19.4 B
	6	11.3 A	1.0 A	8.2 A	12.8 A

Table 4.--(cont.)

Sampling Date	Length of Harvest (weeks)	Total Carbohydrate ^a (% of dry weight)	Total Carbohydrate per Storage Root System (g.)	Dry Weight Root Tissue (g.)	Percent Dry Matter
6/23	0	36.8 B	8.9 B	22.0 B	22.1 B
	3	38.9 B	7.1 B	16.8 B	20.3 B
	6	14.0 A	1.2 A	8.7 A	12.2 A
7/7	0	40.4 B	9.1 B	22.6 A	19.8 A
	3	39.8 B	9.7 B	24.6 A	20.4 A
	6	32.6 A	5.7 A	17.4 A	16.4 A
7/21	0	44.4 A	13.4 B	30.0 B	23.3 A
	3	41.6 A	13.9 B	33.0 B	22.0 A
	6	33.6 A	5.3 A	15.3 A	20.0 A
8/5	0	43.6 A	13.7 B	31.3 B	22.0 A
	3	43.9 A	15.1 B	34.3 B	19.0 A
	6	42.8 A	8.3 A	19.5 A	21.2 A
8/16	0	39.3 A	11.4 B	28.8 B	21.8 A
	3	35.3 A	9.9 B	27.9 B	20.7 A
	6	34.1 A	4.9 A	14.0 A	17.9 A
9/7	0	44.4 A	12.6 A	28.6 A	23.2 A
	3	43.3 A	12.4 A	28.2 A	21.7 A
	6	44.0 A	10.6 A	24.4 A	20.6 A

^aCarbohydrate was measured by the anthrone method and is expressed as fructose equivalents.

^bValues followed by the same letter do not differ significantly ($P = .05$) by Duncan's multiple range test.

Figure 9.--Representation of paper chromatograms developed for 4, 12, or 24 days, drawn to 0.5 scale lengthwise. Numbers identify bands of carbohydrate eluted from undeveloped portions of the chromatograms for analysis.

4 DAYS

12 DAYS

24 DAYS

Raffinose

Sucrose

4A

4B

band "4"

3

1

5A

5B

4

2

Raffinose

5

6

7

8

9

10

Inulin

6

7

8

10

Inulin

into two distinct bands (4A and 4B, 5A and 5B) when time of development was extended to 24 days. Bands 6, 7, 8, and 10 were eluted from chromatograms developed for both 12 and 24 days. Band no. 9 was not eluted from the chromatogram developed for 24 days due to its heterogeneous nature.

Gas chromatography of hydrolyzates revealed that the eluted oligosaccharides were composed predominantly of fructose and, to a lesser extent, glucose (Table 5). Ratios of fructose to glucose ranged from 2:1 (Figure 10) for the oligosaccharide with the greatest mobility to 9:1 (Figure 11) for the oligosaccharide with the least mobility (no movement from the origin). Unfortunately, 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.

Only trace quantities of galactose were detected.

Gel exclusion chromatography indicated the molecular weight of the largest oligosaccharides to be between 3,000 and 4,000 (Table 6). The 0.7 ml fraction collected immediately after the void volume contained approximately 10% of the applied material.

V. Effect of Three Herbicides on Spear and Stalk Production and Crown Survival in Natural and Artificially Infested Soil

The inoculated plots receiving no herbicide contained significantly fewer crowns by October, 1976 than the uninoculated non-herbicide-treated plots or the herbicide-treated plots, inoculated or uninoculated (Table 7). Non-herbicide-treated plots produced significantly fewer stalks per plot than the herbicide-treated plots. By September, 1977, the untreated plots inoculated or uninoculated, contained significantly fewer crowns

Table 5.--Ratios of fructose:glucose in hydrolyzates of oligosaccharides eluted from chromatograms developed for 4, 12, or 24 days.

Band Eluted from Chromatogram	Percent Glucose ^a	Estimated Ratio of Fructose:Glucose
1	33	2:1
2	26	3:1
3	22	4:1
4A	25	- ^c
4B	26	-
5A	20	-
5B	21	-
6 ^b	15	-
7	15	-
8	15	-
9	14	-
10	10	9:1

^aLoss 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.

^bBands 6, 7, 8, and 10 were eluted from both 12 and 24 day developed chromatograms. Values are the average of both determinations.

^cRatios of glucose:fructose not estimated due to suspected oligosaccharide heterogeneity and fructose lability.

Figure 10.--Gas chromatogram of trimethylsilyl derivatives of oligosaccharide hydrolyzates (band #1, Figure 9). Peaks are: 1. fructose; 2. fructose; 3. glucose; 4. fructose; 5. unknown; and 6. glucose.

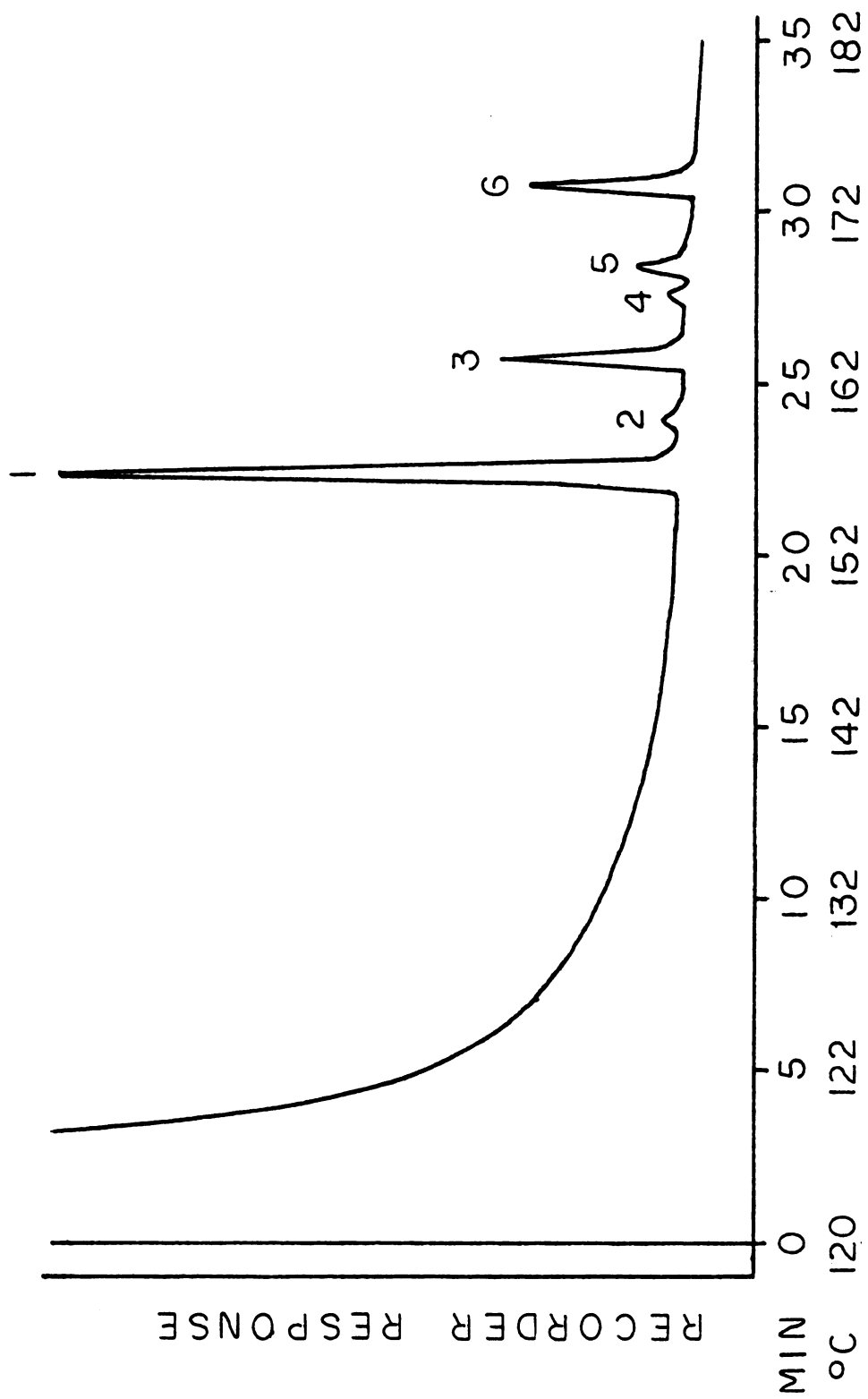


Figure 11.--Gas chromatogram of trimethylsilyl derivatives of oligosaccharide hydrolyzates (band #10, Figure 9). Peaks are: 1. fructose; 2. fructose; 3. glucose; 4. fructose; 5. unknown; and 6. glucose.

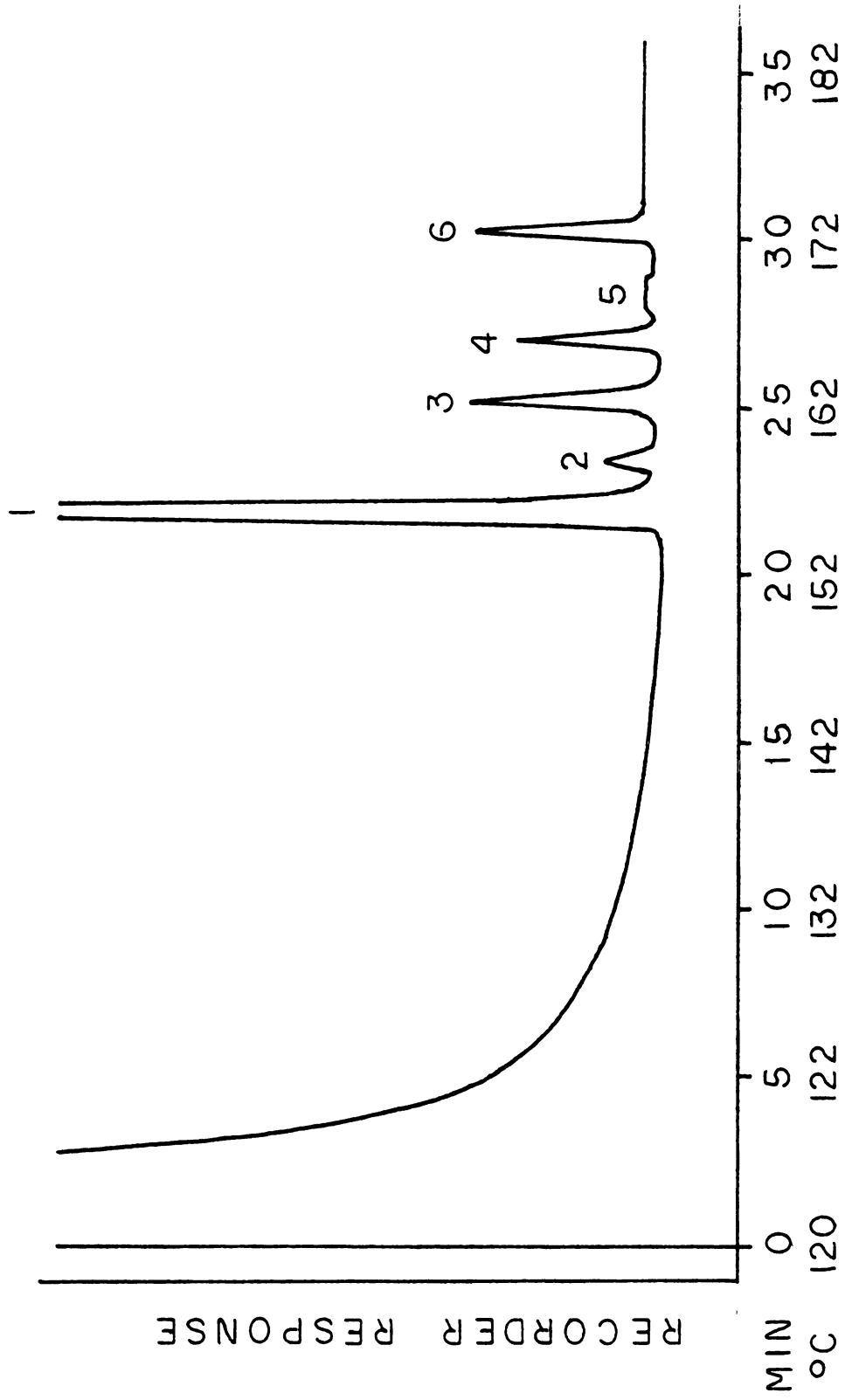


Table 6.--Molecular weight determination of oligosaccharides extracted from asparagus storage roots.

Fraction Collected ^a	<u>Exclusion Limit</u>	
	<u>4000</u>	<u>6000</u>
	<u>Optical Density^b</u>	
3 ml	0 ^d	0
0.7 ml ^c	0	0
0.7 ml	0.275	0

^aOne mg of material in 0.1 ml of distilled water was applied to the top of the column.

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

^cApproximates the void volume.

^dEach value is the average of two determinations.

Table 7.--Effect of three herbicides on crown survival and stalk production in natural and Fusarium infested soil.

Treatment	Rate (kg/ha)	Inoculated	Surviving Crowns per Plot ^a		Total Number of Stalks per Plot		Stalks per Crown
			1976	1977	1976	1977	
Simazine	4.4	Yes	18.4 B ^c	17.8 B	105.2 B	228.8 B	12.82 B
		No	19.0 B	18.0 B	106.8 B	229.2 B	12.75 B
Linuron	2.2	Yes	18.4 B	18.0 B	106.4 B	214.6 B	11.92 B
		No	20.0 B	18.8 B	108.4 B	233.0 B	12.40 B
Terbacil	2.2	Yes	19.6 B	18.8 B	114.4 B	231.4 B	12.33 B
		No	19.0 B	18.0 B	116.6 B	245.2 B	13.75 B
Untreated		Yes	16.0 A	15.6 A	65.8 A	153.8 A	9.90 A
		No	18.8 B	17.2 AB	81.2 A	150.0 A	8.85 A

^aEach plot originally contained 20 crowns.

^bEach inoculated plot received 50 grams of a mixture of colonized wheat seed inoculum containing F. oxysporum f. sp. asparagi and F. moniliforme.

^cValues followed by the same letter do not differ significantly (P = .05) by Duncan's multiple range test.

(Tables 7, 10) and produced significantly fewer stalks per plot and stalks per crown than the herbicide-treated plots, inoculated or uninoculated (Table 7).

Yields were computed both on a plot (Table 8) and a plant basis (Table 9). Untreated plots yielded significantly fewer total numbers and less total weight of spears than herbicide-treated plots (Table 10). There was a significant interaction between inoculum level and herbicide treatment affecting marketable spear production (Table 10). The inoculated untreated plots yielded significantly fewer numbers and less weight of marketable spears than the inoculated herbicide-treated plots, while the uninoculated untreated plots yielded significantly higher numbers and greater weight of marketable spears than the uninoculated herbicide-treated plots. There were no significant differences between non-herbicide and herbicide-treated plots on a per plant basis (Tables 9, 10); however, plots treated with simazine (4.4 kg/ha) yielded significantly greater numbers and weights of total spears than plots treated with linuron (2.2 kg/ha) or terbacil (2.2 kg/ha) (Table 10). Simazine treatment did not affect yields of marketable spears.

There were no significant differences between soil populations of Fusarium oxysporum and F. moniliforme in any of the treatments on either of the sampling dates (Table 11). The majority of isolates were F. oxysporum.

Table 8.--Effect of three herbicides on yields of asparagus per plot in natural and Fusarium infested soil.

Treatment	Rate (kg/ha)	Inoculated	Total		Percent Marketable Spears	Total		Weight Marketable Spears (gm)
			Number Spears	Number Marketable Spears		Weight Spears (gm)	Weight Spears (gm)	
Simazine	4.4	Yes ^a	179.0 B ^b	46.6 A	26.1 A	2,184.8 A	1,056.5 A	
		No	170.8 B	43.6 A	25.4 A	2,028.9 A	958.9 A	
Linuron	2.2	Yes	162.2 B	48.2 A	29.8 A	2,022.9 A	1,046.2 A	
		No	166.8 B	45.6 A	27.9 A	1,980.1 A	938.8 A	
Terbacil	2.2	Yes	163.2 B	45.4 A	28.3 A	2,097.7 A	982.5 A	
		No	167.0 B	41.6 A	24.4 A	2,128.6 A	937.3 A	
Untreated		Yes	132.4 A	35.2 A	27.3 A	1,631.1 A	763.9 A	
		No	153.8 AB	48.0 A	31.2 A	1,971.3 A	1,092.0 A	

^aEach plot received 50 grams of a mixture of colonized wheat seed inoculum containing F. oxysporum f. sp. asparagi and F. moniliforme.

^bValues followed by the same letter do not differ significantly (P = .05) by Duncan's multiple range test.

Table 9.--Effect of three herbicides on yields of asparagus per crown in natural and Fusarium infested soil.

Treatment	Rate (kg/ha)	Inoculated	Total Number Spears	Number Marketable Spears	Total Weight Spears (gm)	Weight Marketable Spears (gm)
Simazine	4.4	Yes ^a	9.84 A ^b	2.54 A	119.8 A	57.5 A
		No	9.51 A	2.44 A	112.9 A	53.4 A
Linuron	2.2	Yes	9.16 A	2.70 A	113.8 A	58.5 A
		No	8.43 A	2.31 A	100.1 A	48.0 A
Terbacil	2.2	Yes	8.51 A	2.36 A	99.9 A	51.0 A
		No	8.90 A	2.21 A	103.7 A	49.8 A
Untreated		Yes	8.31 A	2.19 A	101.7 A	47.2 A
		No	8.41 A	2.63 A	107.7 A	59.6 A

^a Each plot received 50 grams of a mixture of colonized wheat seed inoculum containing F. oxysporum f. sp. asparagi and F. monoliforme.

^b Values followed by the same letter do not differ significantly (P = .05) by Duncan's multiple range test.

Table 10.--Orthogonal comparisons of the effects of three herbicides on crown survival, asparagus yields per plot, and asparagus yields per crown in natural and Fusarium infested soil.

Source	d.f.	<u>MS₁</u>	<u>MS₂</u>	<u>MS₃</u>	<u>MS₄</u>	<u>MS₅</u>	<u>MS₆</u>	<u>MS₇</u>
Rep	4	0.96	279.0	154,408	84.65	25,312	0.81	164.8
Treatment	3							
(Untreated vs. treated)	(1)	25.20** ^a	4,712.5**	557,385* ^b	95.4	25,910	3.40	101.7
(Simazine vs. Linuron & Terbacil)	(1)	1.35	680.1	16,361	0.06	6,625	6.19*	963.2*
(Residual)	(1)	0.15	256.3	37,460	0.06	757	0.10	6.46
Inoculation	1	2.02	291.6	18,576	7.22	3,787	0.10	72.3
Treatment X Inoculation	3							
(Untreated vs. Treated X Inoculation)	(1)	4.40	853.3	294,109	476.0*	317,621*	0.13	251.2
(Residual)	(2)	1.17	1.30	34,550	29.81	5,072	0.81	258.4
Error	28	1.73	395.3	120,823	93.53	64,346	1.45	199.2

MS₁ = Surviving crowns per Plot (1977). See Table 7 for means.

MS₂ = Total Number of Spears per Plot. See Table 8 for means.

MS₃ = Total Weight of Spears per Plot. See Table 8 for means.

MS₄ = Number of Marketable Spears per Plot. See Table 8 for means.

MS₅ = Weight of Marketable Spears per Plot. See Table 8 for means.

MS₆ = Total Number of Spears per Crown. See Table 9 for means.

MS₇ = Total Weight of Spears per Crown. See Table 9 for means.

^aStatistically significant (P = .01).

^bStatistically significant (P = .05).

Table 11.--Effect of three herbicides on soil populations of F. oxysporum and F. monoliforme in natural and artificially infested soil.

Treatment	Rate (kg/ha)	Inoculated	Number of Propagules/Gram of Soil	
			10/5/76	7/25/77 ^d
Simazine	4.4	Yes ^a	5,516 ^b A ^c	6,708 A
		No	5,464 A	6,684 A
Linuron	2.2	Yes	4,896 A	9,140 A
		No	5,744 A	5,290 A
Terbacil	2.2	Yes	5,922 A	8,310 A
		No	5,276 A	8,670 A
Untreated		Yes	6,544 A	6,864 A
		No	5,844 A	8,460 A

^aEach inoculated plot received 50 grams of a mixture of colonized wheat seed inoculum containing F. oxysporum f. sp. asparagi and F. monoliforme.

^bEach value is the average of two determinations.

^cValues followed by the same letter do not differ statistically (P = .05) by Duncan's multiple range test.

^dThere were no statistically significant differences using Orthogonal Comparisons.

DISCUSSION

The ultimate goal of a length of harvest study is the determination of the optimum harvest period(s) which will result in maximum yields and profits over the lifetime of an asparagus planting. In order to maximize yields, a compromise must be made between maximizing spear numbers by extending harvest, and maximizing spear size by shortening harvest. All spears less than one cm in diameter are discarded as culls, and yet each spear represents an expenditure of energy by the plant. While growers wish to obtain maximum yields, it is also recognized that larger spears are produced earlier in the season and that average spear size and plant vigor decrease as the harvest season progresses. This was most eloquently demonstrated in the study of Williams and Garwaithe (39). Plots harvested for 10 weeks yielded the greatest number of spears while plots harvested for 6 weeks yielded the highest percentage of marketable and early spears, but plots harvested for 8 weeks were the most profitable because they yielded both a reasonable quantity of spears and a relatively high quality of spear, averaged over a 5 year period. On the basis of several studies (5,11,32,39) a spring harvest of 7-9 weeks is recommended for obtaining maximum yields and profits from asparagus plantings over an extended period of time. Harvesting of asparagus before the 3rd year after planting is not recommended.

Two years of yield data were insufficient in arriving at conclusions concerning the effect of length of harvest on subsequent yields of

asparagus, although it is apparent that harvesting of a four-year-old field should not be continued beyond 6 weeks. Yields of marketable spears decreased dramatically after the 6th week.

Results concerning the effect of length of harvest on seasonal fluctuations of storage carbohydrates agreed closely with those of Scott et al. (27). Production of spears, and subsequently stalks, by asparagus plants required the utilization of carbohydrates produced by the fern and stored in the roots the preceeding year. In the spring, spears were produced at the expense of these stored carbohydrates. Not until the pre-fern stage did stalks begin to photosynthesize more carbohydrate than they respired (6), and not until stalks were almost mature did they produce sufficient carbohydrate to translocate to the roots to replenish the diminished supply. The effect of extending harvest was to lengthen the time and 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. Measurement of percent carbohydrate alone can be misleading. Asparagus plants replenished their supply of storage carbohydrate in established storage roots before investing heavily in new storage root growth. There continued to be significant differences in total carbohydrate per storage root system between plants harvested for 0 or 3 weeks and plants harvested for 6 weeks up to six weeks after there ceased to be significant differences in percent storage carbohydrate.

The absence of significant differences between carbohydrate levels in plants harvested for 0 or 6 weeks in 1976 was probably due to low temperatures beginning one week after harvesting began, which lasted for two weeks and resulted in several killing frosts. Spears produced by

plants in unharvested plots were killed and as a result, the carbohydrate which had been invested in spear growth was lost. The absence of significant differences in percent storage carbohydrate, total carbohydrate per storage root system, and dry weight of storage roots between plants harvested for 0, 3, or 6 weeks by early fall (9/7/77), and absence of differences in carbohydrate levels and storage root dry weight between plants sampled in early spring and those sampled in early fall could be due, in part, to the fact that it was impossible to retrieve entire storage root systems intact from the soil.

Although it is generally agreed that storage carbohydrate levels are directly related to plant vigor, i.e. extensive harvesting leads to carbohydrate exhaustion which in turn leads to plant death, it is not known how carbohydrate levels affect quantity or quality of asparagus yields. Takatori et al. (32) reported that plants harvested for 13 or 17 weeks produced smaller spears in the second harvest season and both smaller and fewer spears in the third and fourth harvest seasons; and that loss in yields was due to a loss in stand. Haber (11) found that plants harvested for 1, 3, or 5 weeks produced spears of increasing size from year to year, and that plants harvested for 7 weeks produced spears of equal size, while plants harvested for 9 or 11 weeks produced spears which decreased in size from year to year and had a high mortality rate. Plant death in both studies was inevitably the result of carbohydrate exhaustion; however, the effect of length of harvest on bud size and subsequently spear size, involved genetic, physiological and environmental factors. Primary buds produced the largest spears while secondary, tertiary, etc. buds produced progressively smaller spears (35,23). The size of buds was related to the size of the stalks which supplied the

carbohydrate for their formation (36). A positive correlation was found between the diameter of spears one season and the diameter of stalks the preceeding season (7). Plants harvested for shorter periods of time produced consistently larger spears because larger primary and secondary buds gave rise to larger spears and subsequently larger stalks, which in turn gave rise to larger buds for the succeeding year's growth. As length of harvest was extended, plants produced progressively smaller spears because smaller tertiary and quaternary buds gave rise to smaller spears and also, as a result of carbohydrate depletion, smaller stalks, which in turn gave rise to smaller buds for the succeeding year's growth.

Soil temperatures are known to affect size and number of spears (9). It seems probable then that soil temperatures also affect size and number of buds. Although uncut plants produced buds continuously, timing of bud formation under field conditions is not known. If bud formation occurred predominantly after the end of harvest, then plants harvested for shorter periods of time would produce larger buds due to cooler soil temperatures, whereas plants harvested for longer periods of time would produce smaller buds to warmer soil temperatures.

There can be no question that asparagus storage carbohydrates are fructans of variable size. Gas chromatography revealed that oligosaccharides were composed predominantly of fructose and to a lesser extent glucose, although ratios varied considerably. Several other investigators have reported isolating fructans from asparagus storage roots (34, 37, 29). The report by Scott et al. (27) that asparagus storage carbohydrate was sucrose was in error. Since the investigators did not state their method(s) of analysis, it appears that they presumed the soluble nonreducing sugars which they isolated from asparagus roots to be sucrose.

Asparagus oligosaccharides possess an inulin-like structure: molecules of fructose are polymerized to β ,1-6 fructosyl-glucopyranose, either through the 1-carbon of fructose or the 6-carbon of glucose (29). The largest oligosaccharides were composed of approximately 90% fructose and 10% glucose and had a molecular weight of approximately 3200, consisting of 18 molecules of fructose and 2 molecules of glucose. There exists not one but a great many asparagus storage carbohydrates. Paper chromatography revealed a plethora of oligosaccharides ranging in size from sucrose up to oligosaccharides which did not move from the origin on the chromatograms. The first carbohydrate fraction collected from the Bio-Gel P-4 column contained only about 10% of the total material applied. Since these carbohydrates were extracted from the storage roots of crowns which were dormant, it would appear that asparagus plants normally possess oligosaccharides of varying size and composition throughout the year.

Although certain herbicides stimulated levels and activities of Fusarium spp. in the soil, resulting in increased disease incidence in the field (21), this did not occur in asparagus plots with any of the three herbicides tested. Herbicide treatment was conducive to increased crown survival in both natural and Fusarium-infested soil. It was impossible to ascertain the exact nature of the herbicide effect. Data were not available on initial populations levels of F. oxysporum and F. moniliforme, for crown survival for the fall of 1975, or for weed infestations in plots not treated with herbicides. Although attempts were made to keep non-herbicide treated plots free from weeds, a weed problem existed in these plots the latter part of 1976 and 1977. If increased crown survival was the result of a plant- and/or pathogen-herbicide interaction several explanations are possible: the herbicides may have

been inhibitory or toxic to Fusarium in the soil, or they may have been stimulatory to microorganisms antagonistic to Fusarium, resulting in decreased disease incidence. The herbicides may have been taken up by asparagus plants resulting in increased disease resistance, either by stimulating plant resistance mechanism, or by acting fungistically, inhibiting the growth of invading fungi.

Decreased stalk production per crown (1977) by plants in untreated plots was probably the result of a weed problem in these plots and accidental chopping down of stalks while rototilling. Untreated control plots (containing significant fewer crowns) yielded significantly fewer total spears and weight of spears than the herbicide treated plots. The interaction of inoculum level with herbicide treatment with regard to numbers and weight of marketable spears is difficult to explain. Why herbicide-treated inoculated plants would yield significantly greater numbers and weights of marketable spears than herbicide-treated uninoculated plots, and why non-herbicide-treated uninoculated plots would yield significantly greater numbers and weights of marketable spears than non-herbicide-treated inoculated plots is not known. More data are needed in order to adequately assess the situation.

Simazine appears to possess growth regulating properties on asparagus. Simazine-treated plots yielded significantly higher numbers and greater weights of total spears than plots treated with linuron or terbacil. Apparently, secondary buds were being forced to break dormancy sooner than normally would occur. Sandhu and Grieg (24) reported that simazine (4.4 kg/ha) caused a significant increase in plant dry weight compared to a weeded check and several other herbicides in two years' time. Dr. A. R. Putnam (personal communication) has observed an apparent increase

in yields from simazine-treated plots relative to hand-weeded controls, but could not rule out the possibility of a decrease in yields in control plots due to presence of weeds. In the present study, simazine caused an increase in yields relative to linuron and terbacil, all of which gave comparable weed control. Welker and Brogdon (38) did not observe an effect of simazine on yields, however, the investigators had many treatments and few replications in their experiment and analyzed their data using Duncan's multiple range test. The author also was unable to demonstrate significant differences using Duncan's multiple range test. Only through the use of orthoganol comparisons, a more powerful statistical technique, could significant differences be demonstrated. Other factors—soil type, organic matter content, rainfall, cultivar type, etc.—could also play a role in determining the simazine effect. The effects of extended use of simazine on asparagus yields are not known.

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