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Factors which influence the levels of soluble carbohydrate reserves of cool season turfgrasses.

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FACTORS WHICH INFLUENCE THE LEVELS OF SOLUBLE CARBOHYDRATE RESERVES OF COOL SEASON TURFGRASSES

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INTRODUCTION

Purpose of Study. With golf course maintenance practices, turfgrasses are subjected to intense management and highly unnatural conditions. For example, both high nitrogen and water levels are maintained throughout the growing season to keep the grasses in the active vegetative stage of growth. Also very frequent close clipping of the turf is a "requirement of the game." Under such artificial conditions, turf managers teeter on a narrow threshold between success and failure. Characteristically, turf managers follow rather definite routines as to fertilizer application, disease control practices and other managerial factors, with little regard for seasonal climatic changes. It is the purpose of this study to examine the following: 1) the seasonal fluctuation of carbohydrate levels on four turfgrasses, 2) the correlation between the levels of carbohydrate reserve and the growth responses. There has been little, if any, study of the carbohydrate levels of turfgrasses grown under intense management. However, a number of studies have been pursued on the carbohydrate regimes of forage grasses as affected by climatic conditions, fertility levels, and other management factors.

Specific Objectives.

1. To determine and compare the seasonal fluctuations of both percent total fructose and percent total soluble carbohydrates of four varieties of turfgrasses at four fertility levels.
2. To determine the carbohydrate reserve distribution in the leaf, stem, rhizome and root tissues of the four turf grass varieties.
3. To observe the effect of soil temperature on carbohydrate reserves.
4. To determine the seasonal fluctuation of nitrogen and potassium in the foliage of the turfgrasses under the four levels of fertility.

REVIEW OF LITERATURE

Carbohydrate Reserve Fractions

Reserves stored in the vegetative organs of biennial, perennial and annual forage plants are essential to the survival and to the production of plant tissue, in particular, during periods when carbohydrate utilization exceeds photosynthetic activities.

The term total "available carbohydrates" may be defined to include all those carbohydrates which are utilized in plant growth and development as a potential source of energy, either directly or indirectly, after having been broken down by enzymes. In most ordinary higher green plants the available carbohydrates include the sugars, fructosans, dextrin and starch; whereas hemicellulose and true cellulose act as structural materials and as such, cannot be further broken down in the same way as the nonstructural carbohydrates (4,55,107,108).

According to De Cugnac (18), two groups of perennial grasses can be distinguished according to the type of reserve carbohydrates stored in the vegetative organs. One group contains the grasses native to tropical latitudes, such as Bermudagrass, Bahiagrass, and Dallisgrass. These grasses are characterized by the accumulation of sucrose and starch as reserve carbohydrates (108,109). The second group contains the "cool season" grasses native to temperate latitudes, such as the blue- and bent-grasses, and ryegrass, timothy and orchardgrass. These grasses are characterized by the accumulation of sucrose and fructosan as reserve carbohydrates (4,69,91,109). Similar conclusions have been confirmed by other investigators (3,18,34,73,83).

Stereochemical Nature of Fructose

Among the higher plants, most members of the families Campanulaceae and Compositae and the subclass Monocotyledonae accumulate fructosan as their reserve carbohydrate (75,95).

The fructosans are described by many investigators (31,75,95) as being levorotatory, amorphous or microcrystaline, of varying solubility in cold water, highly soluble in hot water and insoluble in absolute alcohol. They are non-reducing, readily hydrolyzed by plant enzymes, hydrolyzed to fructose by acids and impart a purple color from the action of hydrochloric acid vapor and which therefore distinguishes them from the polysaccharides not containing fructose. They are categorized into two main divisions, those having a 1-2 linkage or the inulin type and those with a 2-6 linkage or the phlein type. Intermediate stages having both types of linkages are also found (31,73). The fructosans with 2-6 linkages found in grasses, appear to be similar in comparison to the 1-2 linkages found in the Compositae but are thought to occur in chain lengths shorter than 35 units (55,57,75,87). The product levan has been frequently reported in literature (73,75,95) as being identical to the grass fructosans in regards to its main structural features, although not necessarily in its molecular size. Levan is formed by the action of the microorganizm Bacillus mesentericus on sucrose.

Schluback (84) postulated that northern adapted grasses contain a series of condensation products of fructose and that the fructosans form a homologous series of polymers, with the degree of polymerization varying with the grass species. Palmer (71) concluded that the fructosans of Italian ryegrass and orchardgrass roots contain about fourteen fructofuranose units in a chain, although the molecular weights determined by physical methods (ultracentrifuge) shows molecules containing about thirty units which may therefore imply branch chain structures. Laidlaw and Reid (45) found that the fructosans in perennial ryegrass have chain lengths of twenty-five to thirty fructose units. Percival (73) found that the fructosans of orchardgrass contained 25-30 fructose units and that they are linked by a 2-6 linked fructofuranose unit, and terminated by a glucose unit. Likewise, in the study of the Jerusalem artichoke (Helianthus tuberosus L.) a commercial source of fructose, Edelman and Jefford (23) found that the tuber contained a whole series of fructosans, each member of which has the general formula of Glu - Fru - fru_n and n may be any number from (sucrose) to about 35 fructose units.

Percival (73) postulated that the natural fructosans are built up from sucrose by the attachment of fructofuranose units to C_1 to give the inulin type or to C_6 to give the grass-fructosan (phlein) type structure. The stereochemical nature of the two structures is different, the former being composed of fructofuranose units arranged one above the other like a pile of plates or leaves of a book and the other as an elongated molecule in which the units lie end to end in a linear pattern. The stereochemical nature of the two structures explains the relatively insolubility of inulin in water as compared with the soluble and readily diffusible phlein-type fructosans (31,75).

Methods of Analysis

Fractional separation of the fructosan series is difficult since they differ principally in the number of fructose residues. The size of the chain or the condensation units can be obtained by varying the concentration of ethanol from 95 to 0 percent in the extraction procedure. Smith and Grotelueschen (87) found that low concentrations of ethanol yield steadily increasing percentages of carbohydrates indicating a series of fructosan of increasing chain length. A concentration of 85 percent ethanol was found to be more suitable to extract the free sugar in those species containing predominantly long chain fructosans. With species containing predominantly short chain fructosans 90 percent ethanol was found to be most suitable.

The extracting of "total available carbohydrates" has been accomplished by the use of a series of solvents varying from water (4,68,102) to varying concentrations of acids (38,89) to various preparations of amylase (30,75,80,95,106). Grotelueschen and Smith (29) determined the fractions present in cold and hot water, varying concentrations of acids, and takadiastase extracts, by the use of thin-layer paper chromatography. Water was found to be a suitable extractant only when fructosans predominate, since starch is not readily soluble in cold water. Amylase preparations such as takadiastase, have been used for the hydrolysis of starch. The extraction with the .2N H_2SO_4 acid strength indicated that fructosan containing tissue need not be hydrolyzed with nearly as strong an acid strength as starch containing tissue. The .2N extraction was found to only partially hydrolyze the starch. Extractions would therefore be more of a problem as the starch content increases. In addition, direct acid extraction is very difficult in the presence of hemicellulose whether the polysaccharides are mainly starch or fructosans since there was some evidence found of the presence of arabinose, xylose and mannose, which are constituents of hemicellulose.

Smith, <u>et</u> <u>al</u>. (89) in an extraction study of total available carbohydrates from grass tissue (fructosan storage) and legume tissue (starch storage), found the acids values averaged only 0.4% higher for timothy and only 0.5% higher for the alfalfa than with the enzyme method. The water extractions gave lower value than the enzyme method in both timothy and alfalfa. The differences in values were due probably to insoluble carbohydrates, possibly starch, that were not being removed with water but were removed under the conditions of the enzyme method.

The .2N H_2SO_4 extraction procedure involves much less time than the enzyme method, and the carbohydrate values obtained are similar. Acid extraction requires about 8 hours for a complete analysis as compared with 12 hours working time plus 44 hours incubation for the enzyme method (29,89).

Localization of Carbohydrate Reserves

Fructosans have been shown to occur in all parts of the plant - roots, stem, and leaves. The amount found in any one part of the plant has been shown to be related to season, effect of defoliation, influences of fertilizers and grazing methods (15,21,26,31,34,55,59,62,74,91). This is discussed in a review by Weinmann (108). It has been shown by Waite and Boyd (102,103) that the stems of growing plants commonly contain much higher levels of fructose polymers than does the leaf portion of the same stem. Sprague and Sullivan (91), working with orchardgrass, have shown that in general the fructosans are largely concentrated in the stem and lower portion of the plant in rather high amounts with as much as 36 percent of the lower portion of the stem being composed of fructosans. Similar conclusion regarding the concentration of fructans in basal stem tissue was observed in cool season grasses by other investigators in the field (15,21, 85,91,102,108).

Waite and Boyd (102,103) postulated that there must be a rapid synthesis of the more complex sugars leading to the formation of a fructosan as the carbohydrate reserve. Although it is not clear what governs the equilibrium between the various sugars (simple vs. complex sugars), it was suggested that a limiting osmotic pressure may be one such factor. The formation of the higher sugars would be a simple way of preventing the osmotic pressure from rising beyond a certain limit while allowing for the formation of a reserve. An interpretation of the magnitude of the reserve and the part of the plant in which the reserve is stored is important since this affects the development of the grass and its power of recovery after defoliation. It can be concluded from the work of Waite and Boyd (103) that the fructosans can be formed in the leaf but that they do not accumulate there to any great extent. It is presumed that most of the carbohydrates produced in excess of the plant's immediate needs are passed to the stem and stored there as fructosans.

An examination of the available carbohydrate fraction in the stem bases of several northern grasses revealed that fructosan comprised from 63 to 77% of the total available carbohydrates in the stem bases of orchardgrass, reed canarygrass, Kentucky bluegrass, and timothy. An exception to the high percentage was found in bromegrass which contained 14% fructosans and 71% sucrose in its stem bases. Tall fescue was found to be intermediate in storing considerable amounts of both sucrose and fructosans (68).

Even though fructosan was not the principal carbohydrate stored in the stem bases of bromegrass it fits the classification established by De Cugnac (18) that grasses native to the temperate zone contain fructosans.

Seasonal and Diurnal Fluctuation of Carbohydrate Reserves

Many investigators in the field, working with cool season grasses have found seasonal variations in carbohydrate levels (15,21,42,55,87,102). For example, Waite (102,103) working with ryegrass, found a high water-soluble carbohydrate content in the spring (up to about 25%) and a low one in the summer (2 - 5%) and a somewhat increased content in autumn. Fluctuation in the individual curves for fructosans in fescues, cocksfoot, and timothy showed two peaks; the first in May and the second in July - August. Ryegrass exhibited a single peak in June.

A study of the diurnal variations of carbohydrates in the aerial parts of the ryegrass revealed that the simple carbohydrates were most affected. The sucrose content rose to a maximum in the late afternoon, the hexoses fell to their lowest value about the same time, and the fructosans varied irregularly (102).

It can be concluded from the above discussion and upon a consideration of the following factors; fertilization, cutting, temperature, moisture, and other maintenance practices: that the fluctuation in carbohydrate level is dependent upon the particular plant species in question as well as upon the influence of one or more of the contributing environmental factors.

Effect of Potassium on Carbohydrate Reserves

A correlation exists between agronomic practices and food reserves as they affect productivity under various soil environments, and as they affect the ecological relationships of such plants under field conditions. Kraus and Kraybill (44) stated that "a plant at any particular time represents the results of all environmental forces acting upon it and is either in a state of equilibrium with such forces or is in a state of becoming so adjusted." The adjustment of plants to conditions which are established by such cultural and maintenance treatments such as grazing, cutting, fertilization, and irrigation is highly complex and must be approached from a study of both internal and external relations (26).

The potassium level of a cell is found to be associated with photosynthesis and respiration and to the metabolic process of ion uptake. Depending upon the stage in plant development, the addition of potassium brings a rapid increase in photosynthesis and respiration and to the metabolic process of ion uptake (59).

Potassium fertilization has been investigated in terms of its role in carbohydrate metabolism. Eaton (22) working with sunflower plants, showed that potassium deficiences decreased the fructosan content. Russel (83) found similar results with barley. Wall (104) reported that the high carbohydrate content induced with a potassium deficiency was caused by some interference with protein synthesis. Loustalot _et al_. (50), working with nitrogen and potassium relations in tung, showed that low potassium was associated with a decrease in the rate of photosynthesis and growth, and a concomitant increase in reducing sugars plus a decrease in non-reducing sugars. He suggested that the trend indicated that potassium plays a role in condensing the hexoses into sugar polymers.

The main effect of potassium deficiency on the nitrogen fractions is the accumulation of ammonia and amides in leaves and stems. Hoagland (36) suggested that the increase in carbon dioxide production is associated with the rapid breakdown of sugars and synthesis of amides in barley roots. These results correspond with other investigators (22,14, 78,83,104).

One explanation for this accumulation is that potassium is needed for the condensation of amino acids to proteins. Under conditions of low potassium, protein is not formed, carbohydrate levels increase and there is an accumulation of non-protein nitrogen compounds (78). The other explanation considers the involvement of potassium in the maintenance of the protoplasmic complex. In the absence of potassium the protoplasm breaks down and the proteins are hydrolyzed resulting in the accumulation of non-protein compounds (22). Teel (99) postulated that it is feasible that both of these mechanisms are operative depending on the species of plant.

Increases in foliage production due to the heavy application of nitrogen have been reported by many workers as having placed a greater demand on the potassium supply of the soil and the maintenance of adequate potassium throughout the growing season (51).

Effect of Nitrogen on Carbohydrate Reserves

Many investigators (14,16,21,54,55,56,80,90,95,96) have shown that high nitrogen stimulated growth and decreases carbohydrates.

Prine and Burton (76) observed that nitrogen promoted increases in stem lengths, plant heights, length of the longest leaf blade per stem, and the number of internodes per stem but decreases were observed in the leaf percentages. Archbold (3) pointed out that during the vegetative development of barley, when the leaf/stem ratio is greater than one, the sugar content of the leaves steadily increases and then falls with onset of stem elongation. It was suggested that nitrogen fertilization delays the onset of stem elongation and hence results in the build up of reducing sugar in the leaves with fertilization. Oswalt (69) made similar observations and concluded that nitrogen apparently stimulates shoot growth at the expense of root growth. Graber (27) concluded that the stimulation of top growth by nitrogen fertilization continually depletes the reserve carbohydrate sources of the plant and that this would suggest that a plant has an optimal level of nitrogen fertilization for maintaining normal carbohydrate balance. The specific action of fertilizers on top and root growth was illustrated in experiments carried out at Roadmaster, England. Nitrogen increased leaf growth and reduced translocation of carbohydrates from leaves to roots (80).

Soluble carbohydrates are used in the protein formation and therefore nitrogen is required for this. The protein formation increases with the nitrogen uptake of the plant. Since the nitrogen supply also affects growth, increased nitrogen application has been found to reduce the carbohydrate content, by protein as well as tissue formation (74).

Deleterious effects of frequent clipping under high nitrogen have been examined by many investigators (15,20,21, 26,27,33,34,35,42,53,68,77). High nitrogen levels in conjunction with frequent clipping under optimum growing conditions leads to rapid vegetative renewal. This draws on the reserve carbohydrate supply and thus eventually depletes the reserves to a critical level. Experiments have shown that the closer the grass is cut to the ground, the greater is the loss of reserves (12,24,33). The decreased photosynthetic surface in conjunction with less shade and higher ground temperature places undue stress upon the reserves and discourages young growth. Sprague et al. (91) working with orchardgrass in the greenhouse, noted that the reserve carbohydrates were depleted for two or three weeks after defoliation under both the high and low rates of nitrogen treatments. Similar observations were by Graber and Ream (27).

The stimulus of high nitrogen has been related to reduced root depth and to root growth stoppage following defoliation. Graber (26) has observed the interaction of high nitrogen and clipping in a reduction of subterranean growth. Cutting does not always cause a sudden drop in reserves as was the case with the application of nitrogen. Heavy applications of nitrogen were found to induce depressions in the fructosan content of the herbage but did not influence the sucrose content to any considerable extent. A similar condition was observed in the roots but proportinally there was more sucrose and less fructosan in comparison to the herbage.

Effect of Nitrogen and Potassium on Carbohydrate Reserves

Increasing the amounts of compound fertilizers in conjunction with frequent clipping was found to result in herbage low in soluble carbohydrates (11,51,75).

Macleod (51) examined the nitrogen-potassium interaction in a number of grass species and observed that potassium fertilization facilitated the storage of carbohydrate reserves at higher rates of nitrogen and that high rates of potassium without nitrogen fertilization were detrimental to the storage of carbohydrate reserves. The yield and percent total available carbohydrates were correlated significantly with one another in one or more of the grass species. Oswalt (69) found that a potassium deficiency developed in high nitrogen plots late in the growing season and that this deficiency appeared to be correlated with a low food reserve.

The combination of high levels of nitrogen and potassium produced a turf that was less resistant to high temperatures than turf grown on high nitrogen and low phosphorus. The combination of high potassium with high nitrogen increased the resistance of turf over that grown on high nitrogen and low potassium (53). The effect of minerals on the growth and retardation of bluegrasses was examined by Pellet and Roberts (72). Under a favorable temperature for growth a high level of nitrogen caused an increase in foliar production accompanied by a low percentage dry weight. The turf produced on low nitrogen was more resistant to high temperatures than turf grown on high nitrogen. The growth responses were believed to be related to reserve carbohydrate levels with the plant. Macleod (51) concluded that both nitrogen and potassium were required by plants to store carbohydrates and nitrogenous reserves, and the supply of nitrogen and potassium should not be limited during the hardening and wintering stages.

Effect of Temperature on Growth and Carbohydrate Reserves

Temperature plays a dominant role in the productivity and survival of grasses.

Burt (12) studied the effect of temperature on carbohydrate utilization and plant growth. He confirmed the finding of Humphries (37), that growth and net assimilation rates may be controlled by the growth of the organs constituting the major carbohydrate storage areas and the size of these storage areas is determined by the temperature of the air surrounding them. At temperatures lower than the optimum for growth synthesized carbohydrates are stored, but at temperatures higher than the optimum synthesized carbohydrates are rapidly dissipated without serving as reserves since they do not contribute to the formation of new tissue.

With ryegrass, the maximum growth of new leaf tissue occurred between 60-70° F. and the maximum formation of carbohydrates also occurred within this range (19). In orchardgrass leaf expansion and cell division were found to increase with temperature from 5° to 30° C; the optimum temperature for cell division most likely being between 20° to 25° C. Dry matter accumulation and tillering were found to be higher at 14° C. while carbohydrates were highest at low temperatures. The above data suggested that photosynthesis has a lower optimum temperature than growth (17). Temperatures of 32.2° C. were found to stimulate top growth of <u>Agrostis</u> spp. as compared to 15.6° C.; this resulted in a sharp decrease in carbohydrate content in leaves and stolons (53). The net photosynthesis at these two temperatures was similar but lower than at 12.9° C. It was concluded that carbohydrate depletion caused by excessively high temperatures indicated that photosynthesis may be retarded more than growth in such environment.

Murata and Iyama (63,64), working with perennial ryegrass and orchardgrass, found that photosynthesis was faster around 10-15° C. and decreased slowly at lower temperatures and rapidly at temperatures above 35° C. On the other hand, respiration rate increased exponentially with increase in temperature throughout. Davidson and Milthorpe (17) suggest that photosynthetic capacity of the leaf surface of orchardgrass decreased at high temperatures (26° C.) as the plant aged. The effects of high rates of nitrogen on freshly defoliated cool season grasses during hot weather increase respiration and regrowth potential of the plants beyond their photosynthetic capacity (62).

Smith and Jewiss (88), working with timothy, found the amount of carbohydrate, tiller numbers and stubble weight were always greater in the lower temperature treatment which gave more total top growth at anthesis with each of the nitrogen regimes. The number of tillers and stubble weights were usually higher with added nitrogen and produced more new growth and higher crop growth rates after cutting at anthesis.

With ryegrass, defoliation was found to stimulate metabolic activities such as growth, nutrient absorption, translocation and respiration. The additional stimulus of high temperature at the same time may speed up the respiratory process and eventually lead to an exhaustion of reserves (63).

In controlled temperature studies with the bentgrasses, Kentucky bluegrass and many of the forages, investigators have reported deleterious effects of high temperature on root production (6,10,14,35,90,97,98).

Working with ryegrass, Sullivan and Sprague (90) reported that the roots were more seriously injured by high temperatures than was the stubble. In a period of 28 days, the roots changed color from white to brown and their sucrose and fructose content fell to near zero. Beard and Daniel (6), working with creeping bentgrass found that soil temperature at a 2" depth and light intensity were the predominant environmental factors that could be used with the most consistency in accounting for variations in root number and color. Soil moisture was the least significant factor in accounting for variations in root activity. Harrison (33,34) found that 60° F. was the optimum temperature for the growth of Kentucky bluegrass mowed regularly, and Beard (5) found that the yield of bentgrass was sharply decreased at temperatures above 80° F. Beard and Daniel (6) found that creeping bentgrass grown in a controlled environment at temperatures of 60°, 70°, 80° and 90° F., showed the total root production to be significantly reduced at 90° F. under both cut and uncut treatments, and that the total root production decreased as the temperature increased. Stuckey (97) reported that with unclipped grasses a cessation of root growth accompanied the advent of high soil temperature.

The microclimate of a plant may be altered by differences in cutting height. The soil temperatures under a close clipped population is higher than under a high clipped population. In a pasture grass study, Mitchell (58) recorded a difference of as much as 17° C. at a soil depth of 1/4 inch beneath a low cut pasture in comparison to a high cut pasture of 3 inch cover. Madison, et al. (54) have found that temperatures in the summer at Davis, California, reach greater extremes next to the turf than at 4.5 feet. In the winter at Davis, when growth is slow and temperatures favor storage, the turf does not appear to suffer from reduced mowing heights.

Respiration is not always associated with carbohydrates alone, it also affects the amino acids and the proteins leaving residues relatively rich in nitrogen such as amides and ammonium salts. The accumulation of one or more of these salts was found to be characteristic of plants at high temperatures (39).

Brown, et al. (11) found that in fescues total soluble carbohydrates increased in the fall of the year and that this increase was prompted by a drop in the mean temperature; there was a decrease in the growth rate. Laude (47) and others

(55), have demonstrated that temperatures and day length may be involved in the phenomena of growth retardation which many turf grasses undergo during the summer when climatic conditions are unfavorable. Burton (13) included shade, along with high nitrogen, as contributing factors in carbohydrate depletion. There is undoubtedly some relationship between carbohydrate accumulation and stability and the ability of a plant to withstand high temperatures.

Beard (6) has associated winter hardiness in grass to changes which include an increase in soluble carbohydrates, a reduction in level of water content in the plant tissue and alteration in the proteins. Julander (40) has shown that the resistance of plants to injury during unfavorable conditions has been correlated with carbohydrate reserves in the tissue.

Evidence is in agreement that both environment and maintenance practices regulate carbohydrate storage and therefore plant growth.

Effect of Mowing on Growth and Carbohydrate Reserves

In the mowing of grasses, pasture and turf results agree, when units are representative of either individual plants or the product.

A plant responds to a partial or complete removal of the top growth by transferring its reserves from the roots and other storage organs to the remaining shoot tissue in the production of new growth. This results in a loss of weight by the roots. Madison (53) found in creeping bentgrass "Seaside" and colonial bentgrass "Highland" that shorter mowing increased yield, and roots per unit of soil volume were affected little. But if verdure is considered rather than yield, verdure (quality of the green turf) was decreased as the turf was mowed more frequently or shorter. The root weight per plant was similarly decreased by the same treatment. Working with Kentucky bluegrass, Brown (9) found a reduction in roots and yield with frequent and severe mowing. The frequent removal of top growth was found to produce smaller, less fleshy, rhizomes producing leaf vegetative shoots in comparison to no removal. In cultures of Kentucky bluegrass adequately supplied with nitrogen and clipped at the same frequency, a one-inch cutting height produced fewer roots and rhizomes and a lower yield of clipping than a two-inch clipping height (10).

Clipping affects regrowth potential and tillering as demonstrated in perennial ryegrass clipped at a one-inch height, at intervals of 1, 2 and 3 weeks, resulted in a reduction in tiller development ratio of tillers to number of roots, weights of roots.

An increase in the frequency of clipping reduces the amount of roots produced and the total yield of tops. When grass plants are completely defoliated new top growth is initiated, largely at the expense of previously deposited root and stem reserves. Unless these reserves are sufficiently replenished during the period between successive cuttings a reduction in reserve content of the root occurs which progressively diminishes the amount of new top growth and root growth following each cutting to the point of extinction (35). Similar conclusions were obtained by other investigators (1,4,28,34,35,38,41,56,62,76,86).

Variations in the response of different species to various heights of cut were shown by Roberts and Sprague (80). For each specie there is a limit of tolerance to clipping which, when exceeded, causes a depletion in reserves and eventual death to the turf. Julander (40) has shown that low heights of cut, frequent mowing, and other cultural practices that lower the amount of food reserves have a detrimental effect on the plant under conditions of environmental stress.

The cutting of Kentucky bluegrass at intervals of one week for seven seasons resulted in the development of a surface soil higher in apparent specific gravity than the surface soil from areas which were uncut or cut at less frequent intervals (43).

Effect of Moisture on Growth and Carbohydrate Reserves

Irrigation practice as applied to both turf and pasture grass, is totally dependent upon the interaction of the biotic and physical properties of the plant and environment, in conjunction with a consideration of the maintenance practices, in the production of the end product "Verdure," the quality of the turf and a pasture yield of a nutritive and palatability optimum.

In general, irrigation treatments increase rooting, dry weight, verdure, chlorophyll and population of most turf and pasture grasses. Extreme changes in the irrigation schedule, amount, time and frequency, can produce detrimental results on the turf (5,14,30,53,54,59).

Madison and Hagan (54) reported that frequent irrigation of bluegrass turf produced a smaller and shallower root system. Bentgrasses irrigated 5 times per week showed an increase of population and chlorophyll, and a decreased verdure and rooting (53). Brown (9) reported that when drought was not limiting, irrigation reduced yields, roots, rhizomes, and carbohydrate storage due to the higher respiration rates at summer temperatures.

The growth of orchardgrass and Bermudagrass (<u>Cynodon dactylon</u>) under a favorable environment was reduced by restricting water gradually to the wilting point. Orchardgrass growth was reduced 57% by moisture stress and carbohydrate increased by 22% and 104% in the stubble and herbage respectively. Carbohydrates were found to be higher during the warm summer months for slow growing orchardgrass without irrigation as compared with more rapid growth with irrigation (8). Under field conditions, relatively high temperatures are sometimes accompanied by low soil moisture. Low soil moisture levels have been reported to have caused a decrease in the total available carbohydrate levels (107).

An examination of the interaction has revealed that growth and respiration at the expense of food storage can be stimulated only so much before growth is decreased by the depletion of food reserves.

Carbohydrate accumulation is favored by the influence of cool temperatures, low available soil moisture, low nitrogen (high potassium), and high light levels. In contrast, a reduction of carbohydrate content is favored by plentiful water and nutrients, high nitrogen, high temperature (inducing excess respiration), shade and defoliation. Other factors may also be involved, such as the stimulator effect on the turf through mowing practices. The frequency and severity of these practices may promote changes in the population such as frequent low mowing reported by Madison (53) as having lowered the ratio of mature to juvenile tissues.

The combination of daily mowing, irrigation and high nitrogen levels on Seaside bentgrass favored growth but, resulted in decreased yields compared to either weekly mowing or weekly irrigation. The combination of treatments appears to use up reserves to a point that yield is decreased. But, it was found that weekly irrigation and mowing also result in decreased yield. Therefore, the decrease in one case appears to result from exhaustion, and in the latter from conservation. A similar response was found with Highland bentgrass when daily irrigation, one-half inch mowing, and high nitrogen were combined (53,54).

Murata _et_ _al_. (65) compared the effect of soil moisture, from deficiency to excess on the photosynthetic and respiratory activities as well as leaf water content among the seed crops of eleven species which included representatives of grass, clover and alfalfa.

Photosynthesis, in most species, was little affected by soil moisture stress until the stress became considerably severe. As water stress was found to progress beyond a critical level, photosynthesis showed an abrupt decrease followed by the lowering of leaf water content. In contrast to this, respiration was found to decrease at a higher soil moisture level than in photosynthesis though the rate of decrease was smaller.

The species examined were found to differ in the critical soil moisture level at which the decrease in photosynthesis, respiration or leaf water content began. For example, alfalfa was found to be the most resistant, paddy rice the least resistant, and various cool season grasses such as ryegrass, and orchardgrass were found to be intermediate.

The overall effects of the different maintenance treatments cited in the literature review were designed to demonstrate the interaction of factors in producing what Madison (53,54) has referred to as "Verdure," the quality of the green turf.

Percent Nitrogen and Potassium in the Foliage

Macleod (51) found yield responses to additional potassium application for grasses increase up to a tissue content of 2 percent and level off between 2 and 3 percent. The percent protein, non-protein and nitrate nitrogen in the forage increased with nitrogen fertilization but decreased with potassium fertilization. Conversely, Laughlin *et al*. (46) reported a significant decrease in percent nitrogen in bromegrass as the potassium rate increased from 0 to 30 to 60 pounds of potassium per acre. Robinson *et al*. (81), working with orchardgrass, found a seasonal fluctuation of potassium content. The grass had a relatively high potassium content in the spring and the level declined as the season progressed. He suggested a summer application of potassium would overcome the tendency towards luxury consumption in the spring and starvation in the fall. An examination of orchardgrass by Walsh (105) revealed a potassium deficiency threshold at 0.4% potassium and a luxury threshold at 1.5% potassium. Mortensen (62) observed large reductions in percent potassium but small reduction in percent phosphorus when grasses were heavily fertilized with nitrogen. With increased increments of nitrogen fertilization, there is an increase of percent nitrogen in the plant. From one initial application of nitrogen the carry-over to the succeeding cutting diminished with each cutting (19,20).

The application of fertilizers to both pasture and turf grasses has been found to increase yield, population, verdure and color. A consideration of population and its influence upon carbohydrate level provides an interaction which must be considered as part of the total picture.

METHODS AND MATERIALS

Location

The field phase of the investigation was conducted from April to November in 1966 on the University of Massachusetts Plant and Soils Department Farm, Amherst, Massachusetts.

History

In April, 1966 the following three varieties of grass were sodded: Kentucky bluegrass (*Poa pratensis* L.) var. Merion, creeping bentgrass (*Agrostis palustris*) var. Penncross, and velvet bentgrass (*Agrostis canina*) var. Kingston. Colonial bent (*Agrostis tennuis*) var. Astoria was seeded. The plots were established on the northwest section of the Brooks Farm in a field previously seeded to orchardgrass. The textural class of the soil was a silt loam with a pH of 7. To insure adequate fertility in the establishment of the turf and to duplicate conditions as applied in golf course maintenance practices, the plots were raked, top dressed, and fertilized with an application of 9 lb. of 10-10-10. Throughout the entire paper, the above grasses will be referred to as follows: Merion, creeping, velvet and colonial.

Experimental Design

Plot Design

Table 1 shows the plot layout. The Roman numerals signify the replications and the numbers (plotted from a random number table) signify the treatments. Four varieties each in plot sizes of 360 sq. ft., were established as illustrated.

Grass Varieties

Four grass varieties were investigated in this study. A description of the growth habit and pertinent morphological features of each variety will be discussed.

Merion bluegrass. Merion bluegrass was chosen because of its popularity as a typical fairway grass. It is established in pure stands or in mixtures with grasses such as fescues and bents. It is characterized as a low growing, dense, medium textured variety that is more tolerant to close mowing in comparison to other Kentucky bluegrasses because the distance between the nodes is shorter. Merion requires heavier fertilization than other Kentucky bluegrass varieties because of its vigor, density and extensive root and rhizome development.

The following bentgrasses; creeping, velvet, and colonial were selected because of their popularity as typical grasses grown on putting greens.

Creeping bentgrass. As the name implies, creeping bentgrass spreads by creeping stems. It forms a very close-knit, dense sod which requires close cutting and frequent brushing

TABLE 1*

FIELD PLOT DESIGN

REPLICATIONS	Merion Blue	Creeping Bent	Velvet Bent	Colonial Bent
I.	2	3	4	1
	3	4	3	3
	1	2	2	4
	4	1	1	2
II.	1	4	1	1
	3	3	2	3
	4	1	4	2
	2	2	3	4
III.	4	1	1	1
	2	3	2	2
	1	4	3	3
	3	2	4	4

*Numbers 1,2,3 & 4 signify the fertilization treatments.

to avoid undesirable matting and graininess. Systematic watering and high fertilization practices are applied to ensure a good quality turf. Creeping bentgrass produces the best turf when the soil acidity is moderate to low and when soil aeration and soil water holding capacity are good.

Velvet bentgrass. Velvet bentgrass spreads with a creeping stem although not as profusely as creeping bent and forms a close-knit sod. Under close clipping, it develops a fine textured dense turf; but also, like the creeping bentgrass, it has a tendency to mat. Velvet bentgrass will grow on a wide variety of soils, including wet and acid conditions and low fertility but it will also produce the best turf when fertility is maintained at a high level and acidity is corrected by the addition of lime.

Colonial bentgrass. Colonial bentgrass occasionally produces very short rootstocks and creeping stems. When heavily seeded it develops a fine texture dense turf under close clipping. Because of its rapid growth rate it requires a fertile soil and a program of liberal fertilization (67).

Fertilization Treatments

All the plots received a complete fertilizer application in early spring.

Four fertilization treatments were applied to the four varieties of grasses as reviewed in the following discussion and illustrated in Tables 1 and 2.

TABLE 2

FERTILIZER RATES*

TREATMENTS	Merion Blue	Creeping Bent	Velvet Bent	Colonial Bent
	N-K	N-K	N-K	N-K
1	6-6	8-8	4-4	4-4
2	6-0	8-0	4-0	4-0
3	0-6	0-8	0-4	0-4
4	0-0	0-0	0-0	0-0

*Data in actual pounds on nitrogen and potassium per 1000 sq. ft., per growing season.

TABLE 3

MONTHLY RAINFALL FROM APRIL THROUGH NOVEMBER 1966**

April	May	June	July	August	September	October	November
1.28	2.76	3.3	5.83	.68	5.14	4.52	3.48

** Data in inches of rainfall

Fertilization was applied at biweekly intervals and dispersed in a series of ten applications throughout the growing season. The high levels of both nitrogen and potassium were within the range required for good turfgrass development, and recommended for the particular varieties in question in golf course maintenance practices (101). The low levels of both nitrogen and potassium were designed to be below this desirable range and in the treatments were referred to as (0-K, N-0, 0-0) the absence of either one or both fertilizers.

Temperature

The soil temperature was recorded each day at a 2 inch depth for each variety at a random location in each replication and an average reading was obtained. A bare soil temperature was also recorded. The air temperature over the plots was recorded at a height of 4.5 feet, each day.

Mowing Practices

The bluegrass was cut twice weekly at a 1 1/2 inch cutting height. The bentgrasses were also cut twice weekly at a 1/4 - 1/2 inch cutting height. The clippings were always removed and discarded but at three periods during the season (spring, summer and fall), were collected for the analysis of potassium and nitrogen.

Watering Practices

The plots were maintained with a minimum of 1 inch of water each week. Rainfall was supplemented by one weekly irrigation application. The amount of rainfall and irrigation applied was recorded throughout the growing season.

Sampling Procedure

Plant samples included the remaining basal portion of the stem and leaf tissue collected after mowing. The carbohydrates in this part of the plant are considered to be a valid index of the reserve carbohydrate supply in the plants (16,21,68,86,100,101).

Harvesting Method and Sample Preparation

Samples were taken at approximately biweekly intervals, between 6:00 and 7:00 A.M., at the termination of those periods which were representative of the climatic conditions preceding the sampling date. This procedure was adopted to avoid the collecting of samples following short durations of unseasonal weather.

For the examination of the seasonal fluctuation of carbohydrates, plant samples were taken with a 4 inch sod plug sampler. A random selection of three plugs were collected per replication and per treatment.

The plugs were stored under refrigeration and cleaned on the same day. Representative plants were selected from each of the three plugs and the stems were cleaned from debris, dead plant tissue and the roots were removed with the aid of a wire brush. The samples were then dried in a force-draft oven at 90° C. for one hour and transferred to an alternate oven at a temperature of 70° C. for two days to complete the drying. The plant sample was then ground in a Micro Wiley Mill grinder to a 40 mesh size. One modification was introduced in that the tissue was vacuumed in a sieve prior to grinding. The vacuuming eliminated the fine soil particles which were not removed in the original cleaning of the sample. The ground sample was stored in 1/2 pint containers.

Samples were collected from established turf plots on the Montague Farm for the determination of the localization of carbohydrates at three periods (spring, summer and fall) in the growing season. The plants were separated into leaf, stem, rhizome and root tissue, cleaned and prepared in a similar manner.

The daily fluctuation of carbohydrates was examined in two varieties, Merion blue and Penncross, in August to take advantage of the large day and evening temperature variations. Samples were collected at four-hour intervals, separated into leaf and stem tissue, cleaned and prepared in a similar manner.

Analytical Methods

In specific color reactions from some groups of carbohydrates, for example, mono- and polysaccharides, the sensitivity of various classes of carbohydrates are found to be of the same order of magnitude and the absorption maxima are very nearly identical for more than one class of carbohydrates.

A reaction such as typified with the anthrone reagent, is particularly useful for the identification of substances as a carbohydrate and for the estimation of total amount of carbohydrate. Also included are reactions as typified with the total fructose analysis, which, in this case, the class of carbohydrates produces a different absorption spectra and which can be differentiated spectrophotometrically to determine the particular class of carbohydrates present (112). In the analysis for total extractable percent fructose and also total soluble carbohydrates, the digestate was extracted from 110 mg. of plant tissue in a single digestion procedure using .2N H_2SO_4 extracting solution.[1]

The dried sample was placed into a 110 ml. Kjeldahl flask, the .2N H_2SO_4 was added and the contents were heated over a steam bath for one hour at a temperature of 90° C. The necks of the flasks were rinsed two or three times during the digestion procedure. The flasks were then cooled, brought to 110 ml. volume, mixed and left undisturbed to allow the particles to settle.

In the analysis of total percent fructose, an appropriate aliquot of the digestate was transferred to a 90 mm. Evelyn Colorimeter cell graduated at 25 ml. Five ml. of alcoholic resorcinol (1 g. $C_6H_6O_2$/1L 90% E TOH) and 15 ml. of fresh 30% HCl were added to the sample and the contents was made to 15 ml. volume.

The standard solution was prepared with levulose dissolved in a saturated solution of benzoic acid with subsequent

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[1]During the course of this paper .2N H_2SO_4 total carbohydrates will be referred to as total soluble carbohydrates.

dilution to follow ranging from .05 to .35 mg. per ml. of sugar. Acid and resorcinol were also added as above and the color was developed. The unknown and standard samples were developed simultaneously in a hot water bath at 80° C. for a period of 20 minutes. After cooling the color intensity was determined on the Evelyn Colorimeter using a 540 mu filter.

The development of color depends, as Roe (83) has shown, on the quantity of alcohol, concentration of HCl, temperature of bath and the time tubes remain in the bath. Higher temperatures, or longer development periods are undesirable since a chemical reaction may occur between the HCl and other carbohydrates present. Color densities change after cooling at a negligible rate of about 1% per hour (57).

In the analysis of total soluble carbohydrates, an appropriate aliquot of the digestate was added to an Evelyn colorimetric cell made to 5 ml. volume and then 10 ml. of the anthrone reagent (1 g. anthrone to 500 ml. of 95% H_2SO_4) was added. The cells were mixed on a Vortex Genie mixer, allowed to cool and the color intensity was determined on the Evelyn cell using a 660 mu filter.

The standard solution was prepared with dextrose dissolved in a saturated solution of benzoic acid with subsequent dilution to follow ranging from .05 to .35 mg. per 1 m. of sugar. The anthrone reagent was also added as above and the color was developed along with the unknown and standard samples.

When mixtures of sugars were examined by the anthrone reagent method, the absorption curves were consistent with those expected from a summation of the individual sugars if the compound was first hydrolyzed and the sugars then determined (111,113). According to Beer's law, the sugar concentration is directly proportional to the product of the reaction; but, the law is applicable only in a narrow range of concentrations. Beer's law holds up between concentrations of 5 mg. to 30 mg. percent on the Evelyn Colorimeter. Above 30 mg. the depth of the color increases less than the concentration (48,57,95).

A standard Kjeldahl procedure was used for nitrogen determination in the plant tissue (61,97). Potassium was determined on the Perkin Elmer flame photometer after the samples were dry ashed and digested with the nitric acid method (28).

EXPERIMENTAL RESULTS

Diurnal Localization of Carbohydrate Reserves

The diurnal analysis conducted on both Merion bluegrass and on creeping bent revealed that the percent total fructose in both the leaf and stem tissue, fluctuated irregularly throughout the day. However, in the percent total soluble carbohydrate analysis, a diurnal pattern fluctuation was established. In the leaf tissue, the lowest percent carbohydrate was recorded in the early morning hours and the highest percent carbohydrate was recorded in late afternoon. In the stem tissue, a reversal in the trend was established with the high percent carbohydrate recorded in the early morning hours and the lowest percent recorded in the late afternoon. The percent total soluble carbohydrate in the roots was found to fluctuate irregularly.

The above observations point out the necessity of standardizing the time of day for sampling the basal stem tissue. Sampling in the early morning hours vindicates the procedures in sampling as pursued in this study.

Seasonal Localization of Carbohydrate Reserves

An examination of the localization of carbohydrate distribution in both percent total fructose and percent total soluble carbohydrates in Merion bluegrass (Figure 1 and 2), and in creeping bentgrass (Figure 3 and 4), showed that seasonal total of carbohydrates in all tissue areas sampled was the highest in the fall harvest and the lowest in the spring harvest period. However, the increase was relatively greater for Merion. In creeping bent, which typifies the carbohydrate distribution pattern in the bentgrasses, in comparison to Merion bluegrass, the total carbohydrate content was not substantially greater in the fall harvest period. Both Merion and creeping bentgrasses recorded the highest percent of both total fructose and total soluble carbohydrates in the stem tissue. In Merion bluegrass the highest percent carbohydrate was found localized in the rhizome tissue and in creeping bentgrass the highest percent carbohydrate was found localized in the main stem. Differences between Merion bluegrass and creeping bentgrass were recorded in both percent total fructose and percent total soluble carbohydrates in the leaf and root tissue. Merion bluegrass was found to contain a higher percent of carbohydrates localized in the root tissue, although in the fall harvest period, the percent carbohydrate in the leaf tissue surpassed the percent recorded in the root tissue. In creeping bentgrass, the leaf tissue was found to contain a higher percent carbohydrates in comparison to the root tissue at all harvest periods. In the fall, the percent carbohydrate localized in the leaf tissue surpassed all tissue areas analyzed. Colonial and velvet bentgrasses, which are not illustrated, resemble the distribution pattern in creeping bent but contain comparatively less total carbohydrates in all sample areas and exhibit less fluctuation in seasonal distribution. The rhizome tissue was not analyzed in both grasses since colonial bentgrass is not a rhizomatus grass, and in the case of velvet bentgrass, a sufficient amount of plant material could not be collected for the analysis.

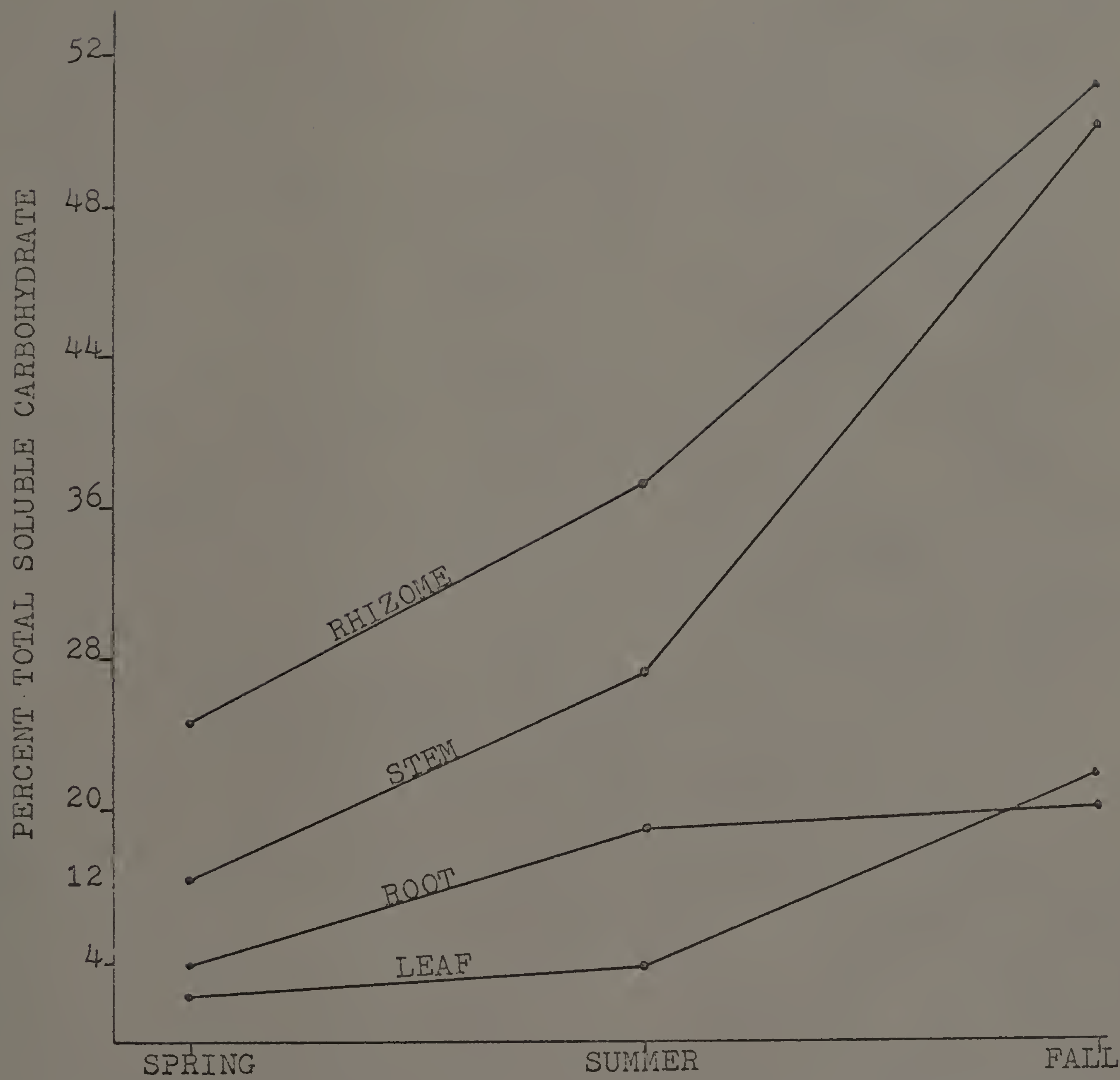

FIG. 2. SEASONAL CHANGE OF TOTAL SOLUBLE CARBOHYDRATE DISTRIBUTION IN MERION BLUEGRASS

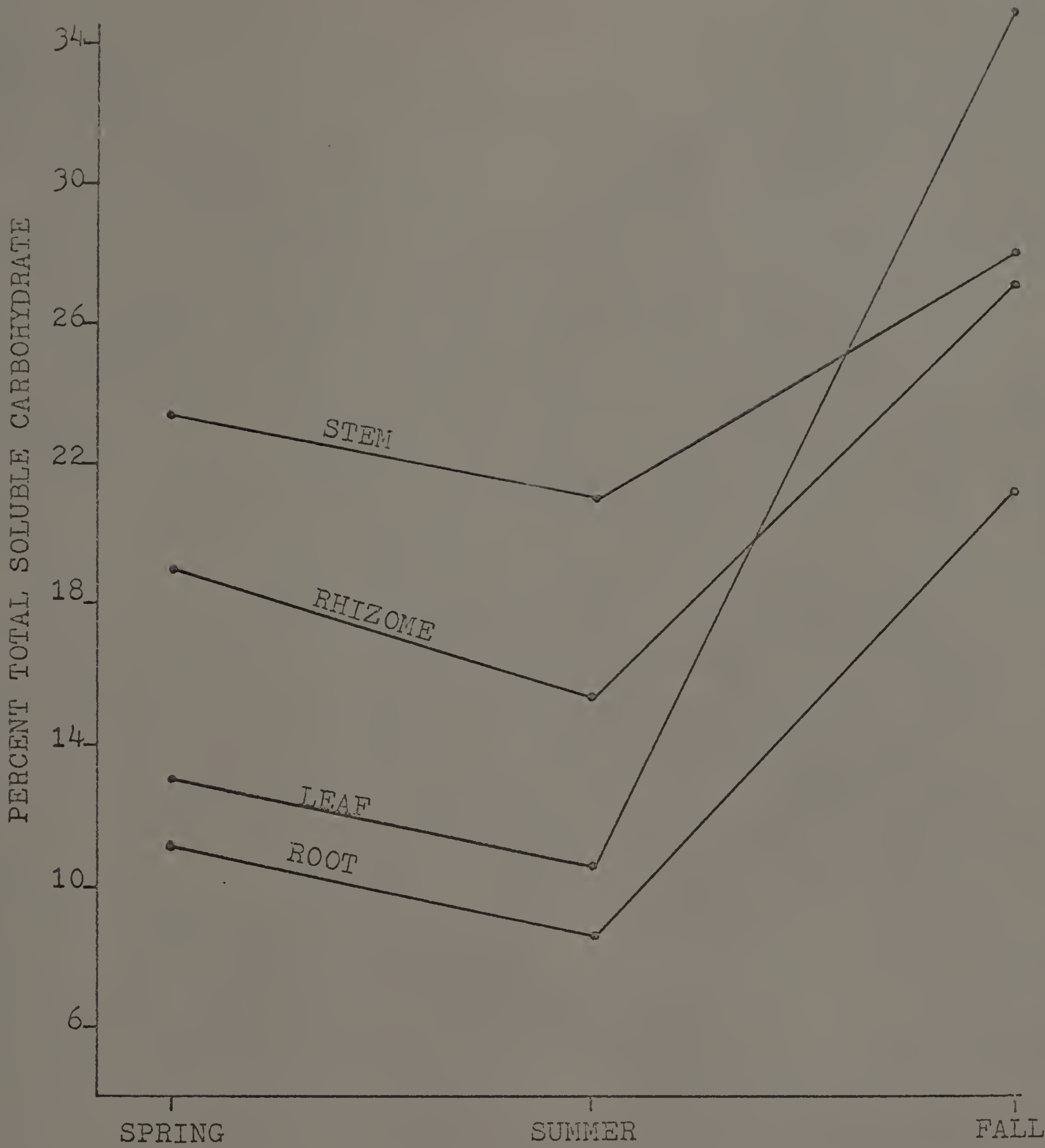

FIG. 4. SEASONAL CHANGE OF TOTAL SOLUBLE CARBOHYDRATE DISTRIBUTION IN CREEPING BENTGRASS

Seasonal Fluctuation of Carbohydrate Reserves

Figure 5 shows the percent total soluble carbohydrates in the (Harvest-Varieties) interaction. The percent total soluble carbohydrates was found to be proportionally higher than the percent total fructose for all varieties and at all harvest periods. The bentgrasses follow similar patterns in the seasonal fluctuations of carbohydrates. Colonial and velvet bentgrasses reacted similarly throughout the growing season, by displaying minor fluctuations in carbohydrate level in the summer sampling periods. Creeping bentgrass contained the highest percent of both total fructose and total soluble carbohydrate among the bentgrasses and displayed more erratic seasonal fluctuations in carbohydrate levels throughout the growing season. Merion bluegrass contained the highest percent of both total fructose and total soluble carbohydrates at all harvest periods and also displayed proportionally greater peaks and depressions in carbohydrate levels in response to the harvest periods.

Figure 6 shows the percent total fructose plotted in the (Harvest-Fertilization) interaction. Treatments 0-0 and 0-K and treatments 0-N and N-K resemble each other in both percent total fructose and percent total soluble carbohydrates at all harvest periods throughout the growing season. Treatment 0-K was found to reduce the total fructose level to a greater extent than treatment 0-0. Similarly, treatment N-0 reduced the percent total fructose to a greater extent than treatments N-K. The greatest increase in the percent total fructose was in the fall of the year for all treatments. A comparison of Figure 6 with Figure 5 (Harvest-Varieties) reveals that the total carbohydrate levels recorded in relation to the fertilization treatment are highly influenced by the carbohydrate levels recorded in Merion bluegrass. This is especially true with respect to the high peaks recorded in the summer and the fall.

Figure 7 shows that both percent total fructose and percent total soluble carbohydrates plotted in the (Fertilization-Variety) interaction. Fertilizer treatments O-K and O-O displayed a prominent increase in carbohydrate level over treatments N-O and N-K. As pointed out previously, but displayed more diagrammatically in Figure 7, the percent total soluble carbohydrates exhibited a proportionally higher carbohydrate level under all four treatments. Under all four treatments, colonial and velvet bentgrasses contained the lowest total percent carbohydrates analyzed and followed a similar pattern in carbohydrate distribution under both percent total fructose and percent total soluble carbohydrates. Both grasses showed a slightly higher increase in carbohydrate level in treatment O-K in comparison to treatment O-O. Merion bluegrass contained the highest percent of both total fructose and total carbohydrates under all fertilization treatments and creeping bent occupied an intermediate position. The greatest variation in carbohydrate level in all the grasses occurred under the treatments 0-0 and 0-K and the least variation occurred under treatments N-K and N-0.

An examination of the interaction among (Harvests, Varieties and Fertilizations) in Figures 8 through 15 revealed that under all varieties treatments N-K and N-0 and treatments 0-K and 0-0 resembled each other in the seasonal fluctuation and in the magnitude of the percent total fructose and percent total soluble carbohydrate analyzed from sampling periods throughout the growing season. With the exception of a number of harvest periods, treatments 0-K and 0-0 were found to contain the highest percent carbohydrates and treatments N-K and N-0 were found to contain the lowest percent carbohydrates in both percent total fructose and percent total soluble carbohydrates.

In Merion bluegrass and creeping bentgrass, treatment 0-0 was found to contain the highest percentages of both total fructose and total carbohydrates. In comparison to velvet and colonial bentgrasses, treatment 0-K was found to contain the highest percentages of both total fructose and total carbohydrates.

All grasses were similar in that they displayed a prominent depression in the carbohydrate level from late fall to early spring. A comparison among the four varieties revealed that the bentgrasses resembled each other in the fluctuation of both percent total fructose and percent total

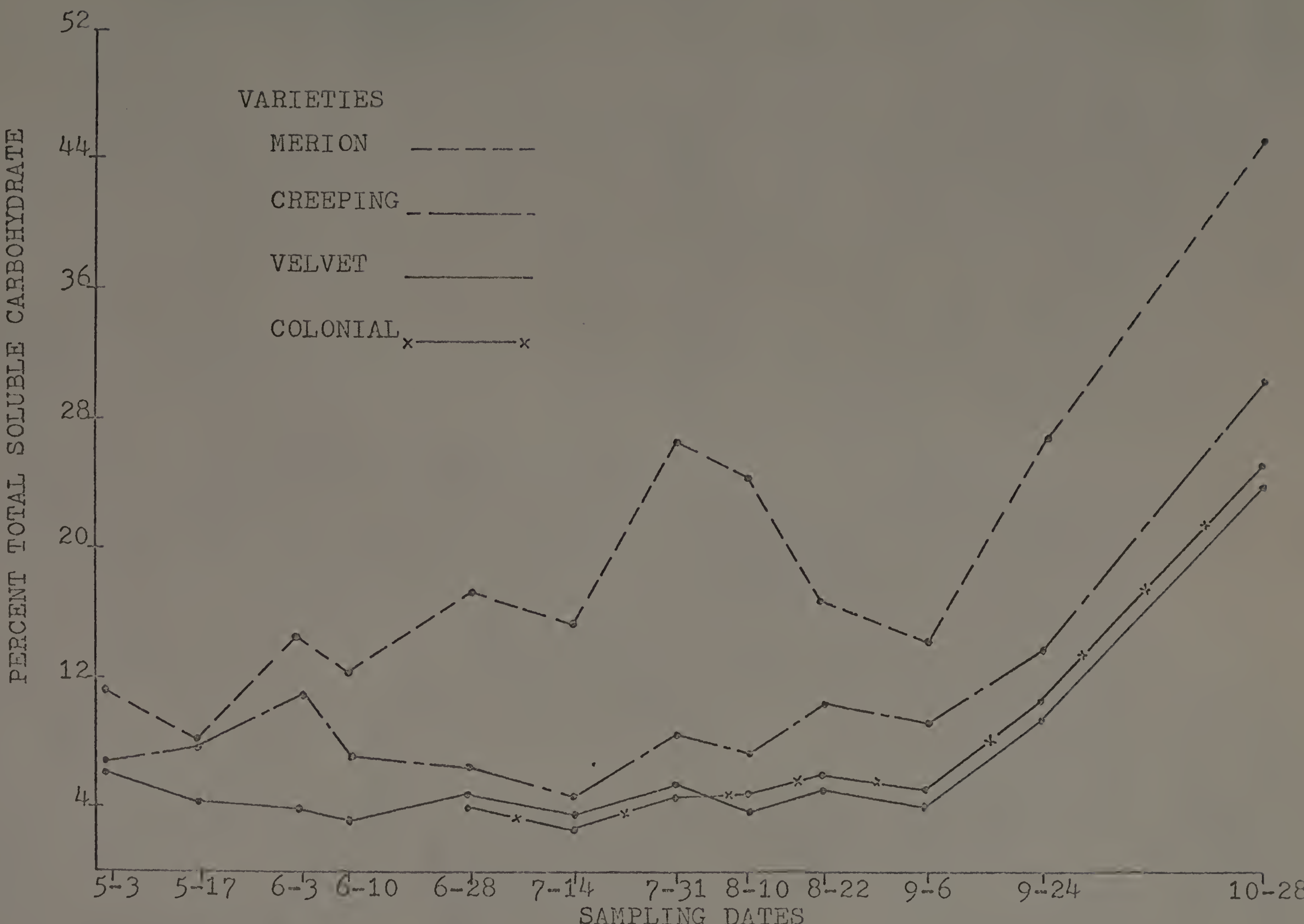

FIG. 5. SEASONAL EFFECT OF TOTAL SOLUBLE CARBOHYDRATE$ FLUCTUATION WITHIN THE VARIETIES

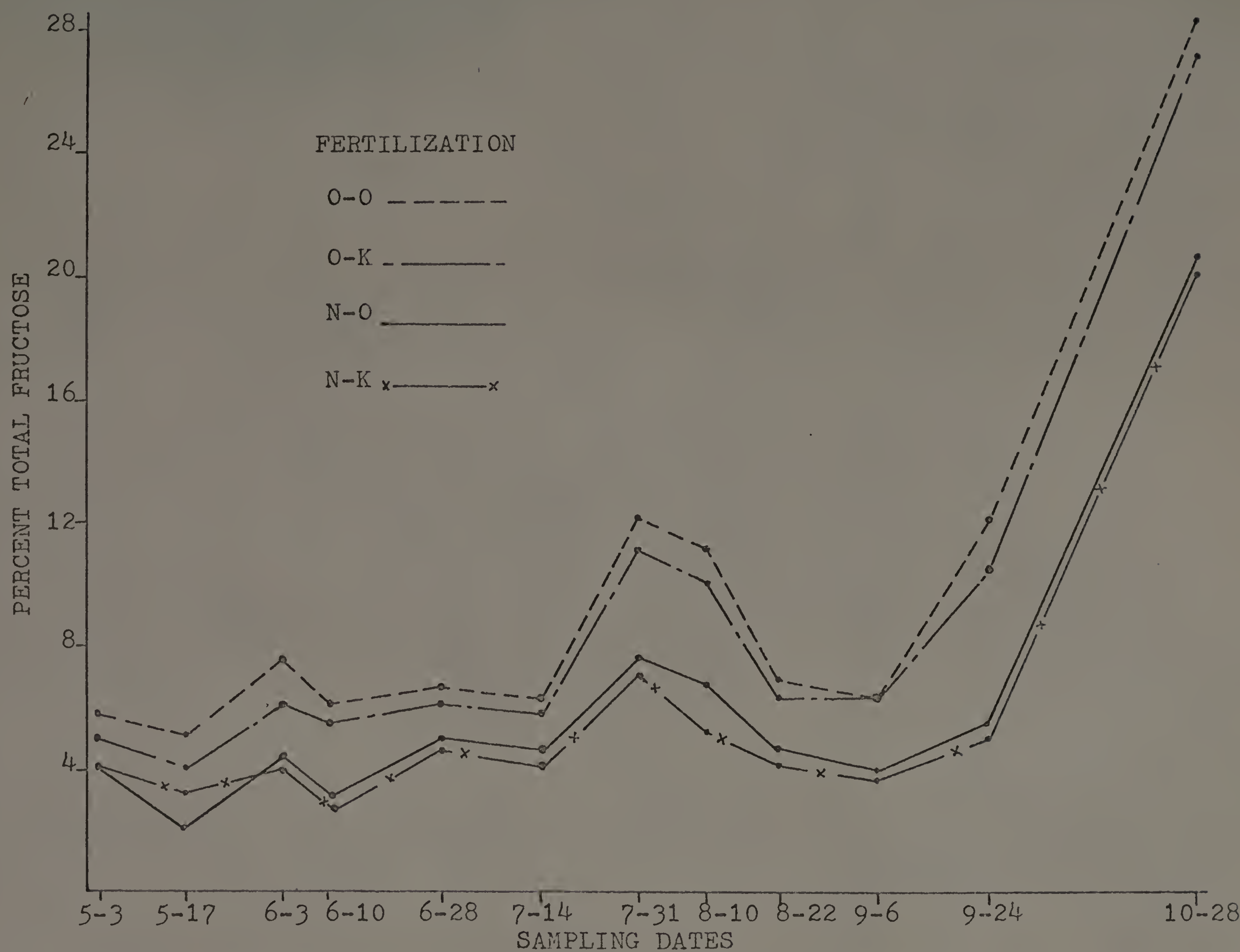

FIG. 6. SEASONAL EFFECT OF TOTAL FRUCTOSE FLUCTUATION WITHIN THE FERTILIZATION TREATMENTS

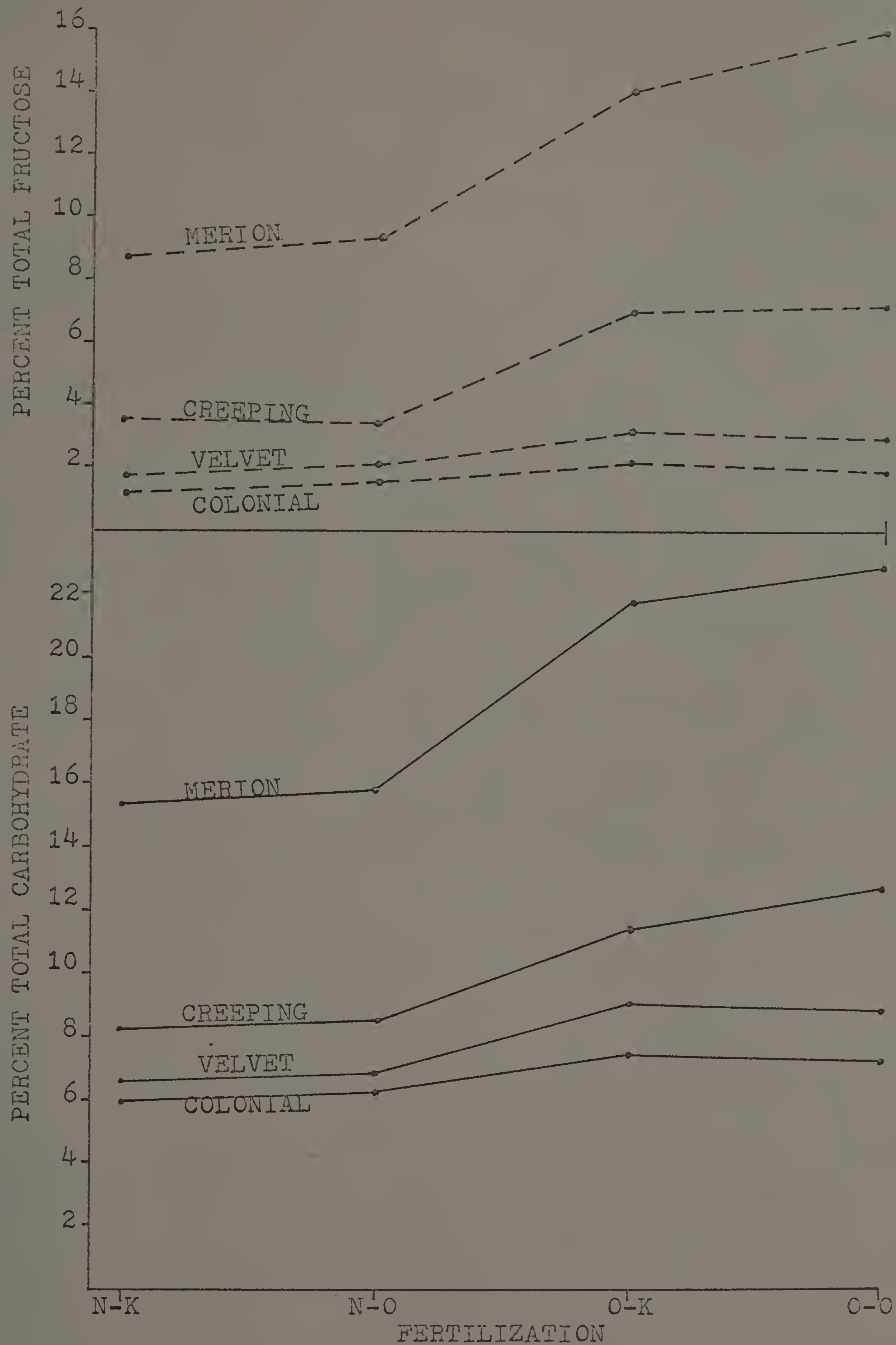

FIG. 7. EFFECT OF FERTILIZATION ON TOTAL SOLUBLE CARBOHYDRATES AND TOTAL FRUCTOSE WITHIN THE VARIETIES

soluble carbohydrates. Creeping bentgrass, in comparison to colonial and velvet bentgrass, contained the highest percent carbohydrates analyzed under all treatments exhibiting a prominent increase in carbohydrate level in the spring and early summer followed by a sharp decline in percent carbohydrates in mid-summer. The lowest percent of carbohydrates recorded for the bentgrasses was on the sixth (July 14) harvest period. Velvet bentgrass recorded the lowest percent of both total fructose and total carbohydrates in early summer through early fall but recorded prominent peaks in the spring and fall harvest periods. Similar results were recorded for colonial bent with the exception of the early spring through early summer harvest periods which were not illustrated because the grass was too immature and representative samples could not be obtained for analysis at this point in the growing season.

Bluegrass, velvet and creeping bentgrass displayed differences in the magnitude of the spring depletion of carbohydrate reserves and in the period in the season at which the spring depletion of carbohydrate reserves was terminated. The termination of the spring depletion of reserves in creeping bent was on the first (May 3) sampling period; in bluegrass, the spring reserve was terminated on the second (May 17) sampling period; and in velvet bentgrass, the spring depletion of reserves was on the fourth (June 10) sampling period.

Merion bluegrass displayed a prominent rise in both percent total fructose and total soluble carbohydrates in the spring and summer with the maximum increase recorded on the seventh (July 14) harvest period followed by a sharp decrease in late summer and subsequent rise in carbohydrate level in the fall. Aside from the increase in carbohydrate level recorded in the fall, Merion bluegrass did not resemble the bentgrasses either in the magnitude of percent carbohydrates analyzed or in the seasonal fluctuations from harvest periods analyzed throughout the growing season.

In all varieties examined, and under all treatments, the percent total carbohydrates was found to be proportionally higher than the percent total fructose at all harvest periods throughout the growing season.

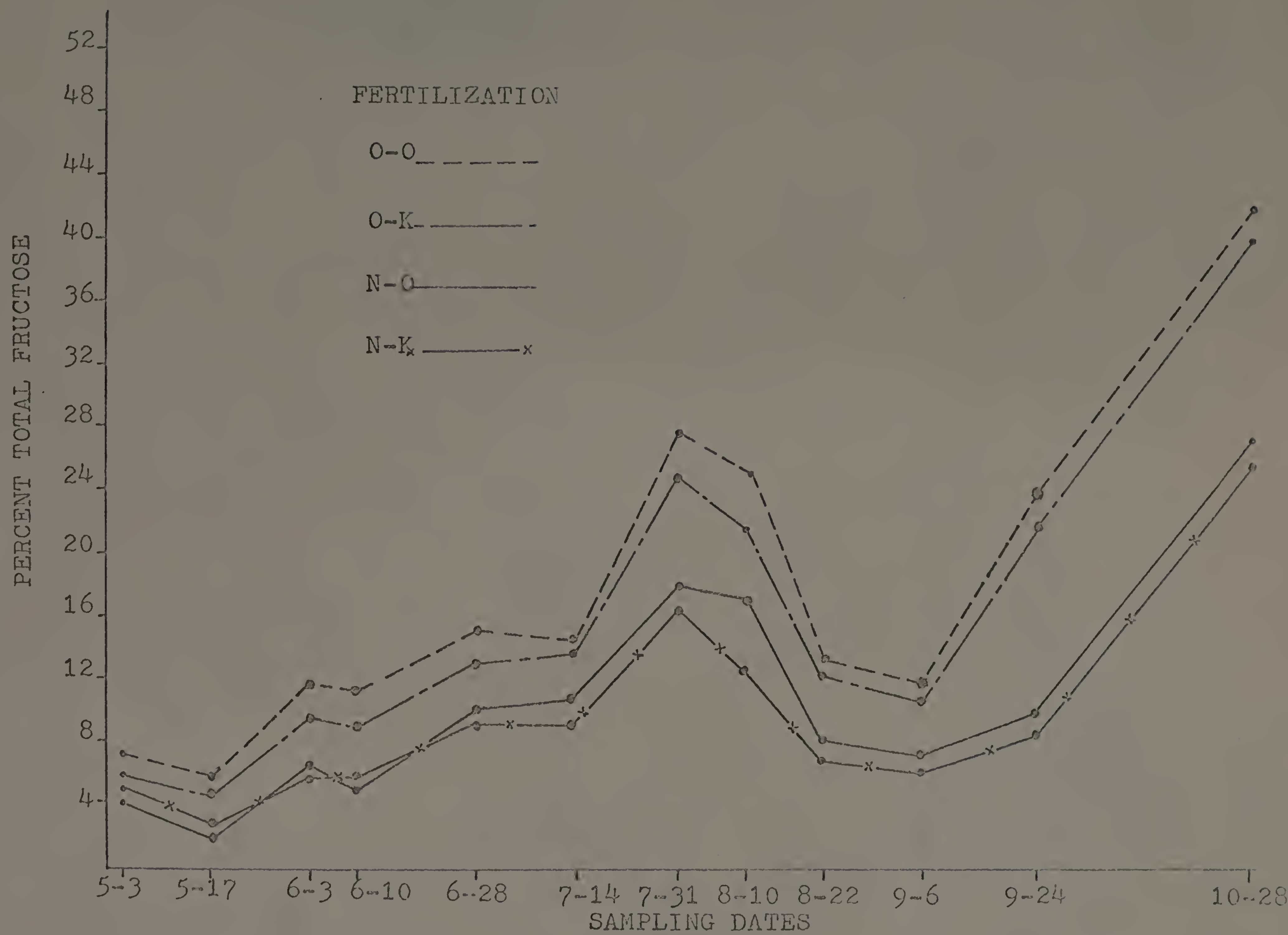

FIG. 8. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL FRUCTOSE CONTENT IN MERION BLUEGRASS

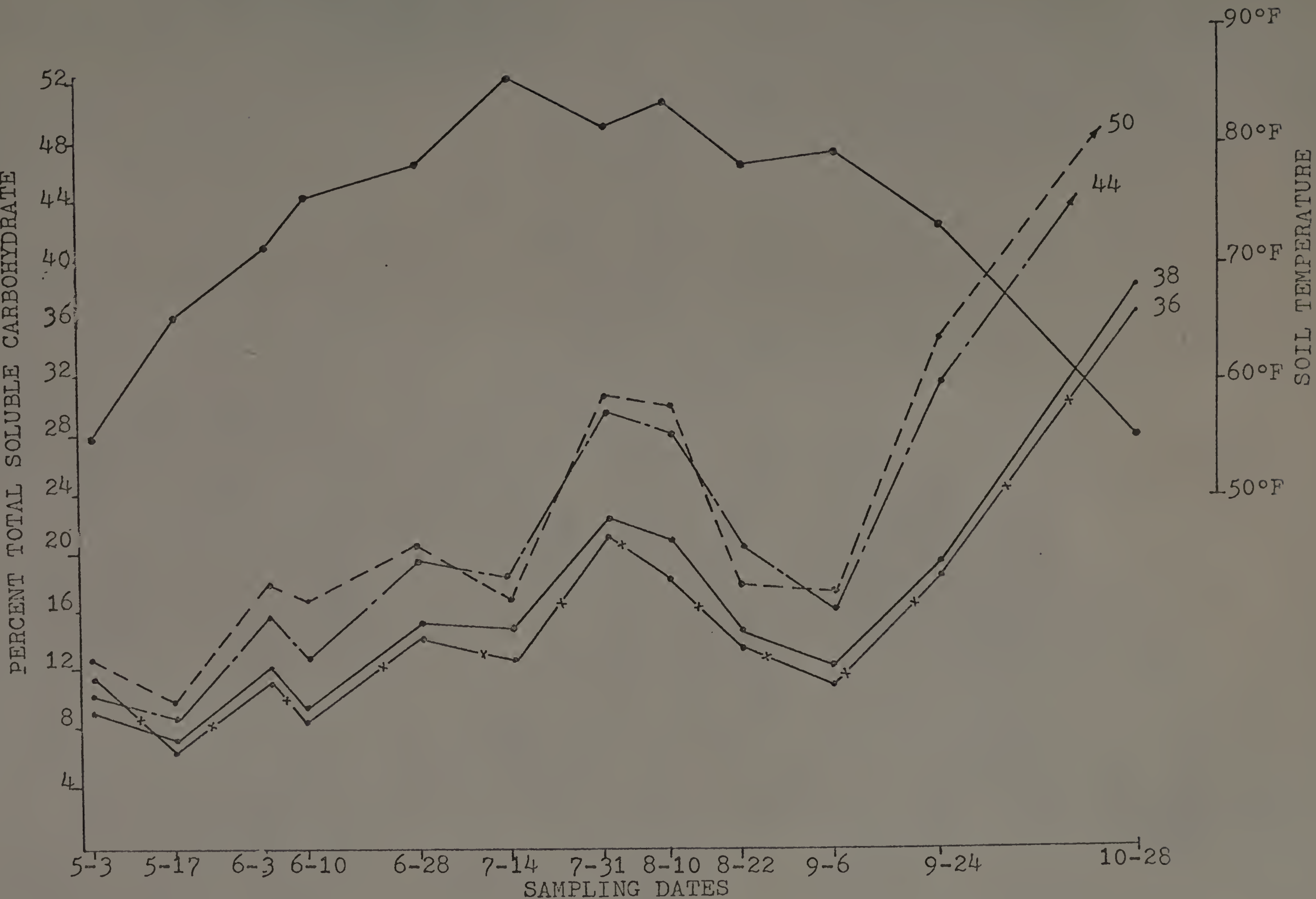

FIG. 9. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL SOLUBLE CARBOHYDRATE CONTENT IN MERION BLUEGRASS

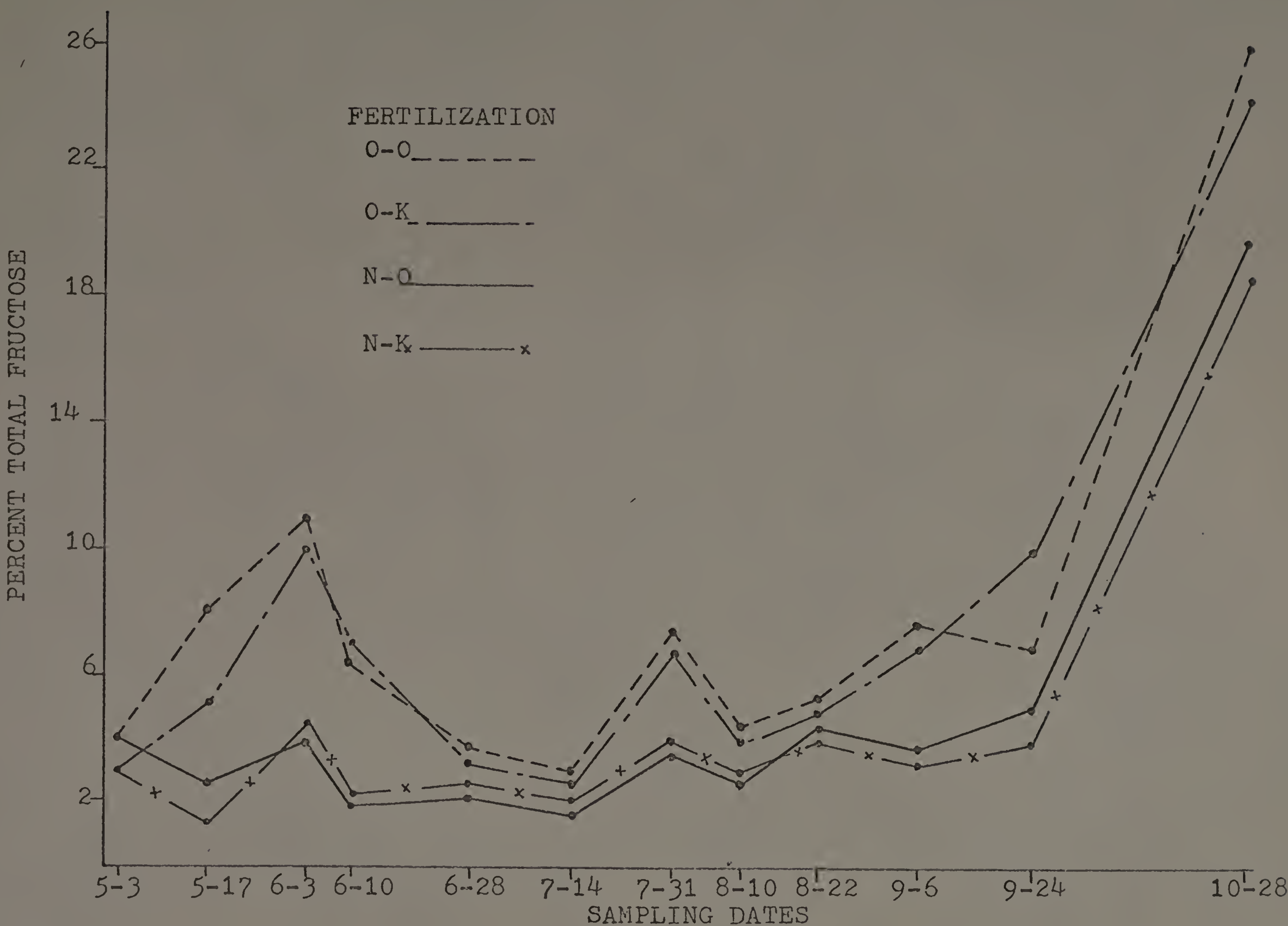

FIG. 10. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL FRUCTOSE CONTENT IN CREEPING BENTGRASS

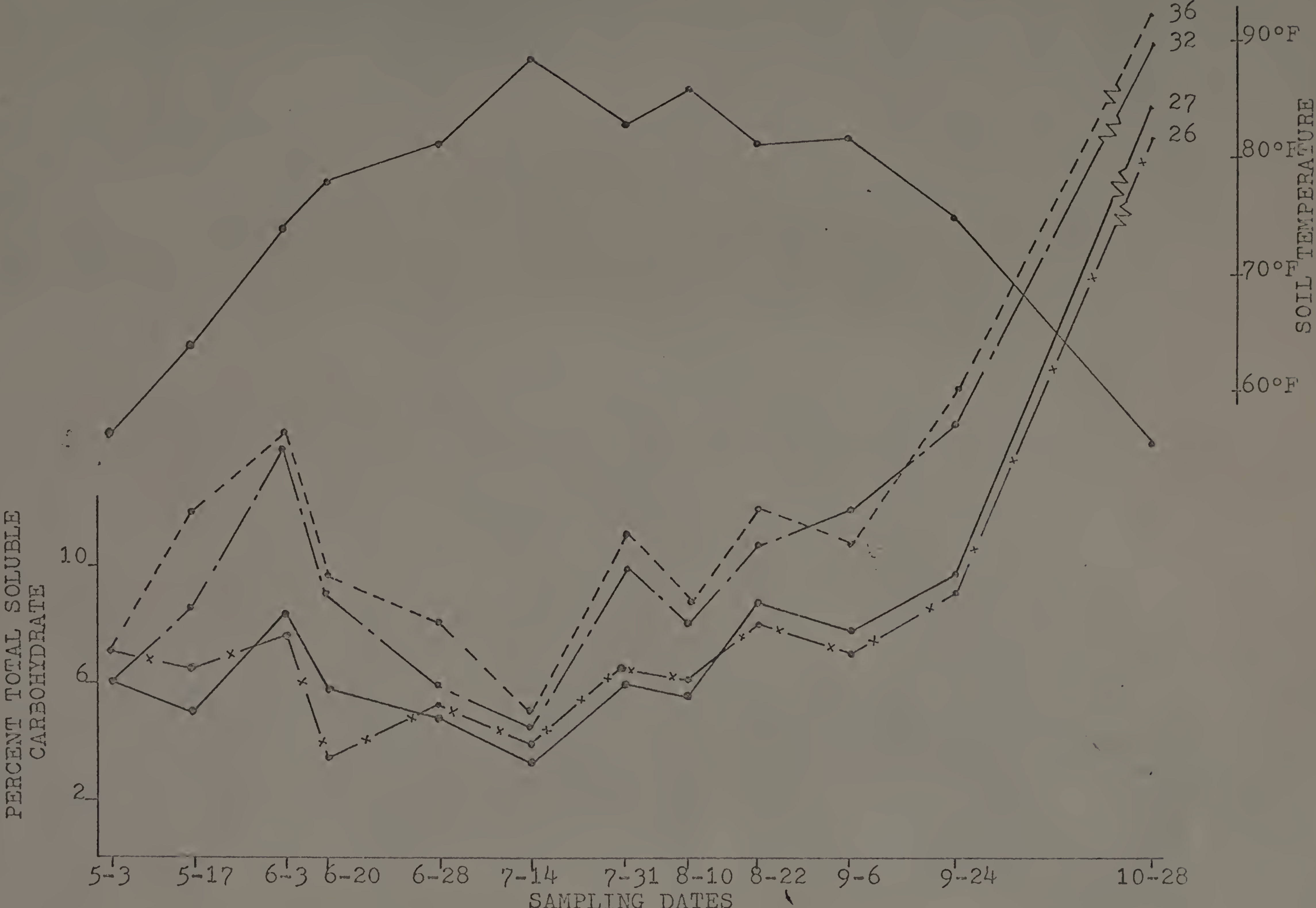

FIG. 11. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL CARBOHYDRATE CONTENT IN CREEPING BENTGRASS

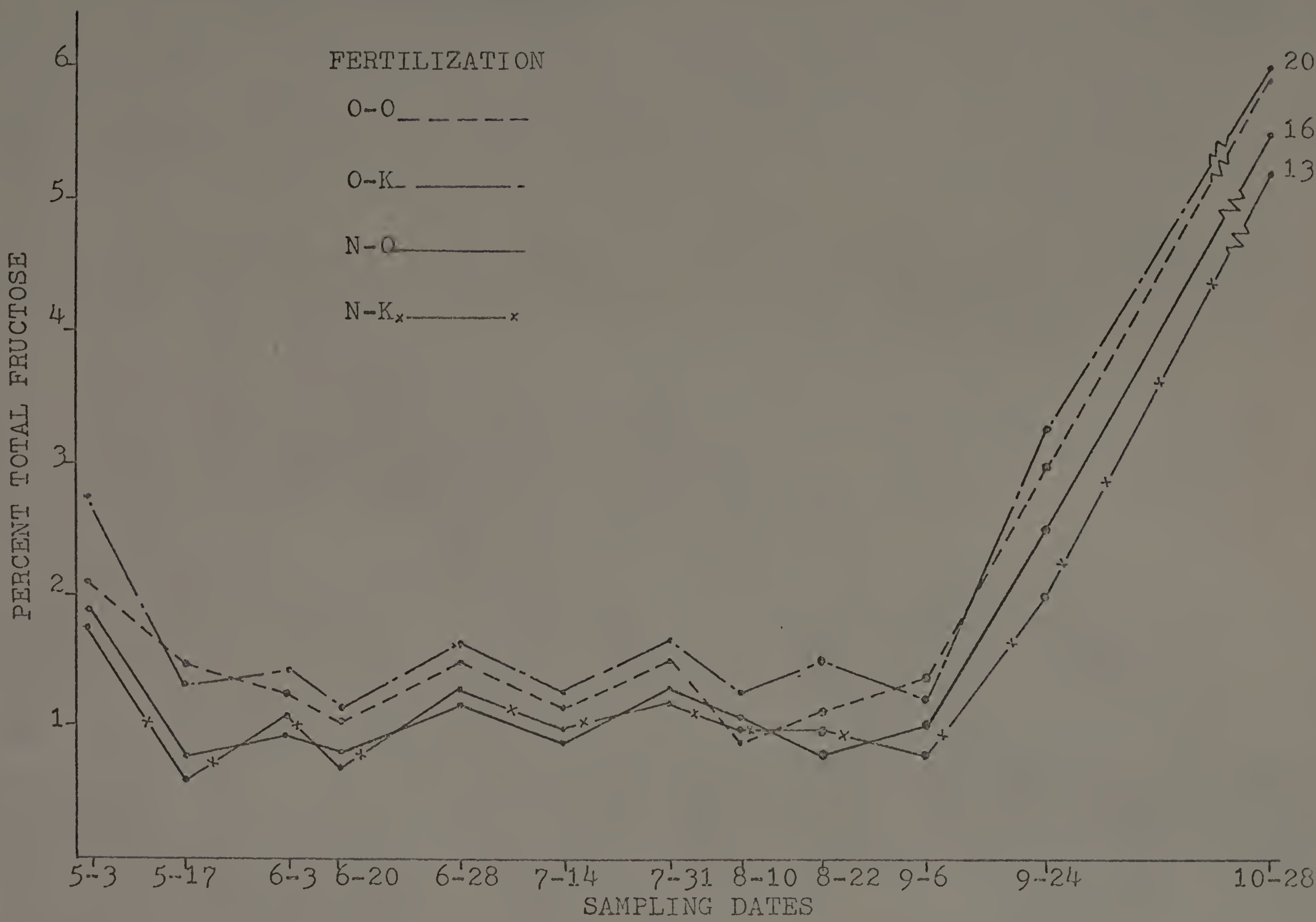

FIG. 12. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL FRUCTOSE CONTENT IN VELVET BENTGRASS

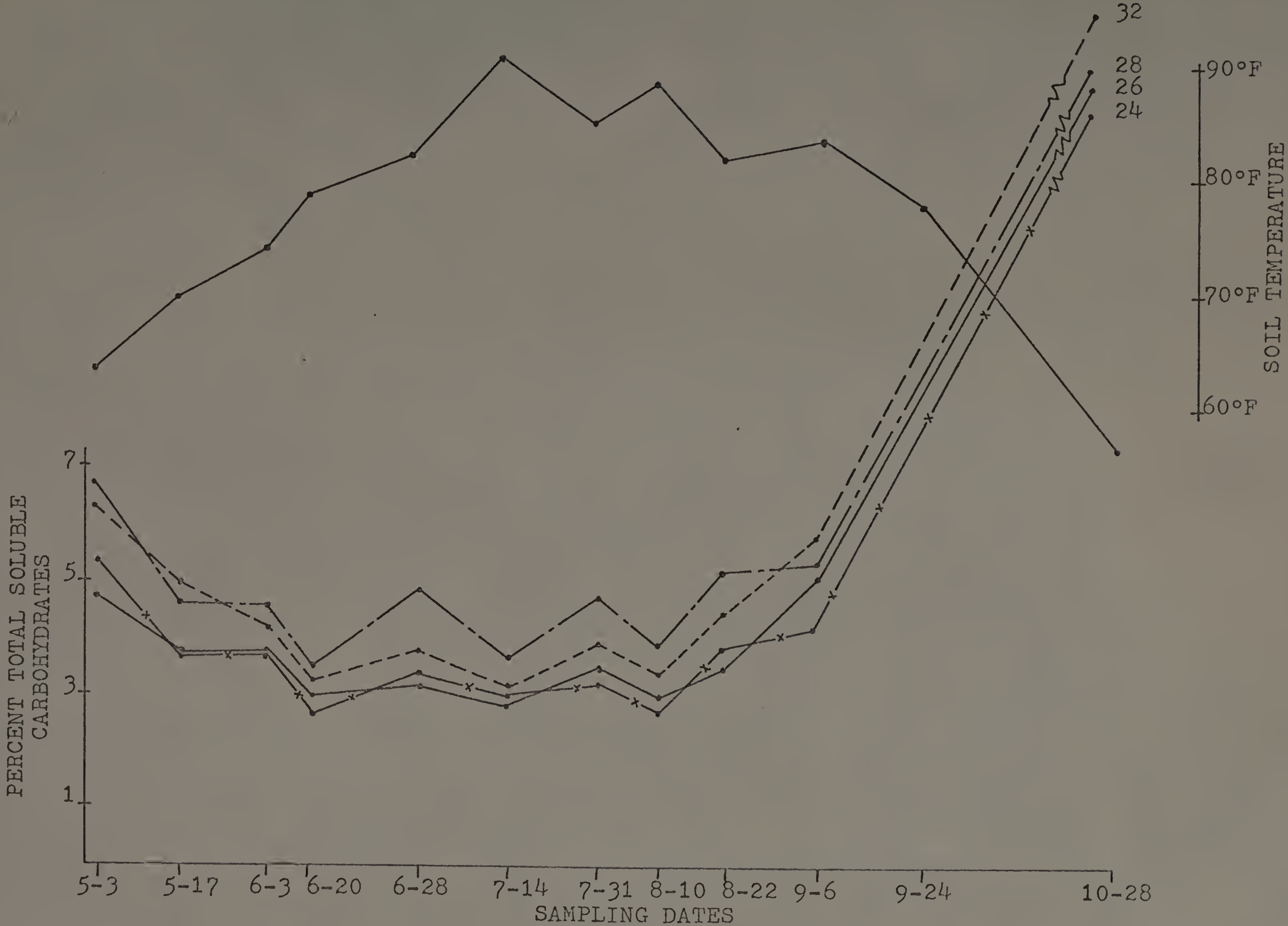

FIG. 13. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL CARBOHYDRATE CONTENT IN VELVET BENTGRASS

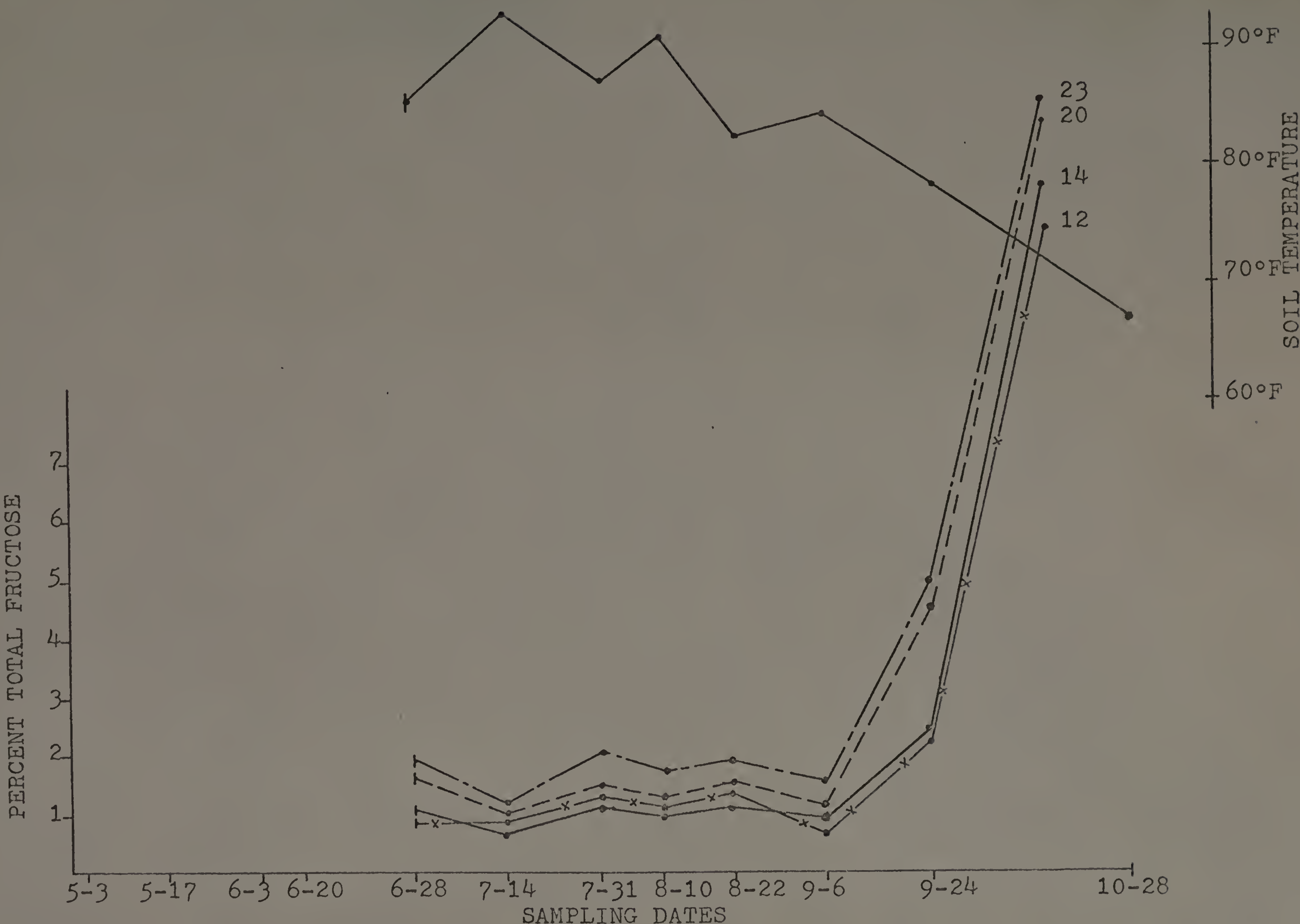

FIG. 14. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL FRUCTOSE CONTENT IN COLONIAL BENTGRASS

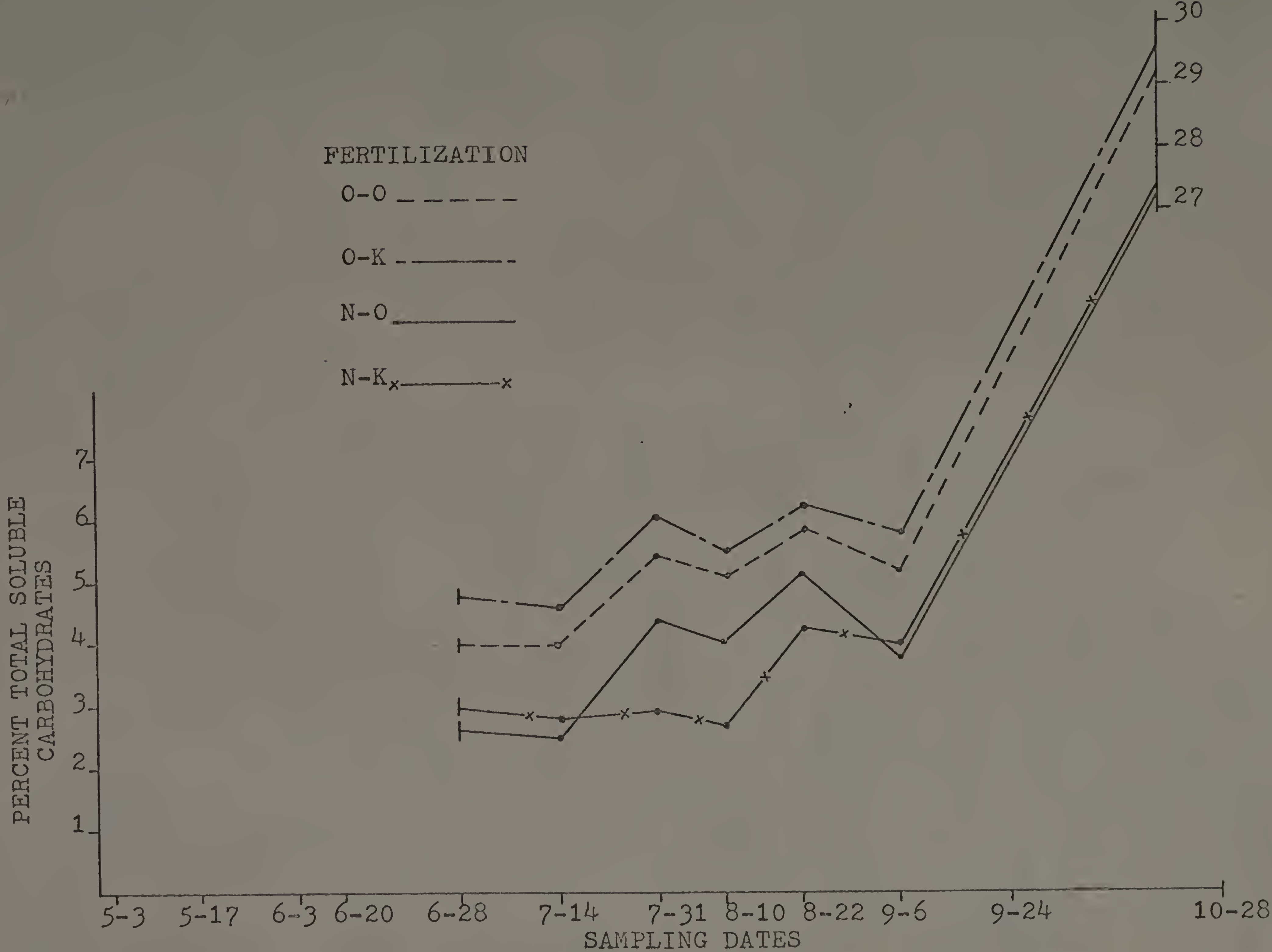

FIG. 15. EFFECT OF HARVEST TIME AND FERTILIZATION ON TOTAL SOLUBLE CARBOHYDRATE IN COLONIAL BENTGRASS

Seasonal Fluctuation of Soil Temperature

Figure 16 indicates differences in soil temperature recorded under the four turf species at two-inch soil depth, Merion bluegrass recorded the lowest average seasonal soil temperature and colonial bentgrass recorded the highest average seasonal soil temperature. Creeping and velvet bentgrass were found to be intermediate.

The bare soil temperatures were higher than the soil temperatures recorded under the turf in spring and summer, but in the fall, a reversal of this trend occurred.

In the discussion, reference will be made to the insulating effect of the vegetative growth on soil temperature. The average dry weights of the vegetation including roots from core samples taken from established plots on the Montague Farm revealed values of 12.8 g. for Merion bluegrass, 9.2 g. for creeping bentgrass, 6.2 grams for velvet bentgrass and 8.2 grams for colonial bentgrass.

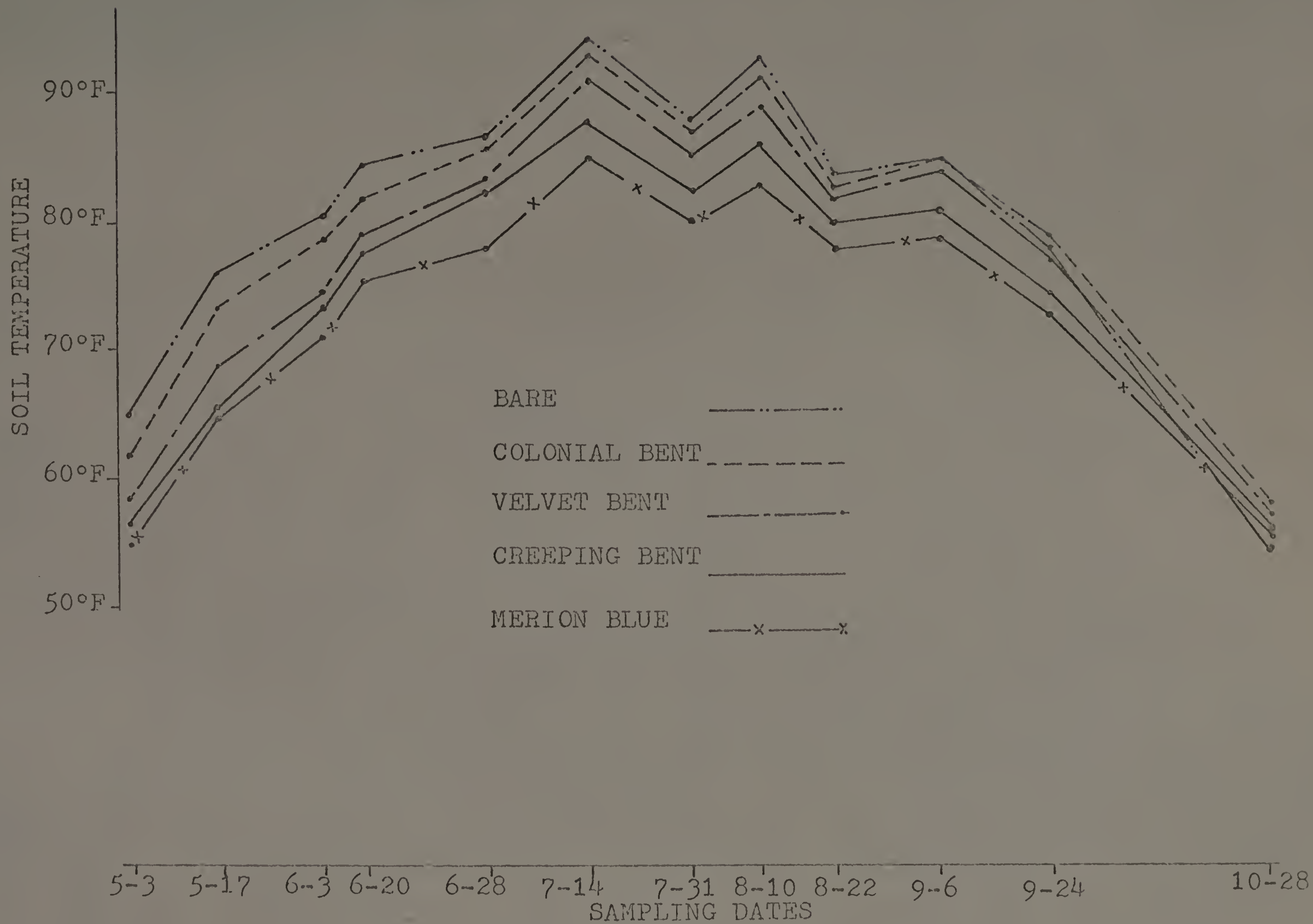

FIG. 16. SEASONAL FLUCTUATION OF SOIL TEMPERATURE

Percent Nitrogen and Potassium

The interaction between varieties and fertilization (Figure 17), shows that the percent nitrogen in Merion bluegrass and creeping bentgrass increased similarly under all fertilization treatments. The percent nitrogen in creeping bentgrass was considerably higher than in Merion bluegrass under all fertilization treatments. Velvet bentgrass comparatively exhibited a more gradual linear increase in percent nitrogen and recorded the lowest percent nitrogen in the N-0 and K-N treatments.

Creeping and velvet bentgrasses are similar in percent potassium under all fertilization treatments, with creeping bentgrass continuing a proportionally higher level of potassium than velvet bentgrass. Merion bluegrass occupies an intermediate position in potassium content with the percent potassium increasing for treatments N-0, 0-K and N-K respectively.

In all four grasses (Figure 18), the percent nitrogen for each fertilization treatment was the highest in the spring, intermediate in the summer and lowest in the fall. The highest percent nitrogen was found in treatments N-K and N-0, and the lowest percent nitrogen was found in treatments 0-K and 0-0.

The potassium level (Figure 19) was generally found to be the highest in the spring harvest and the lowest in the fall harvest. In the bentgrasses, treatments N-0 and N-K were similar and treatments 0-0 and 0-K were similar in percent potassium. Also, in the bent grasses, a sharp decrease in the percent potassium was observed in treatment O-K in the fall.

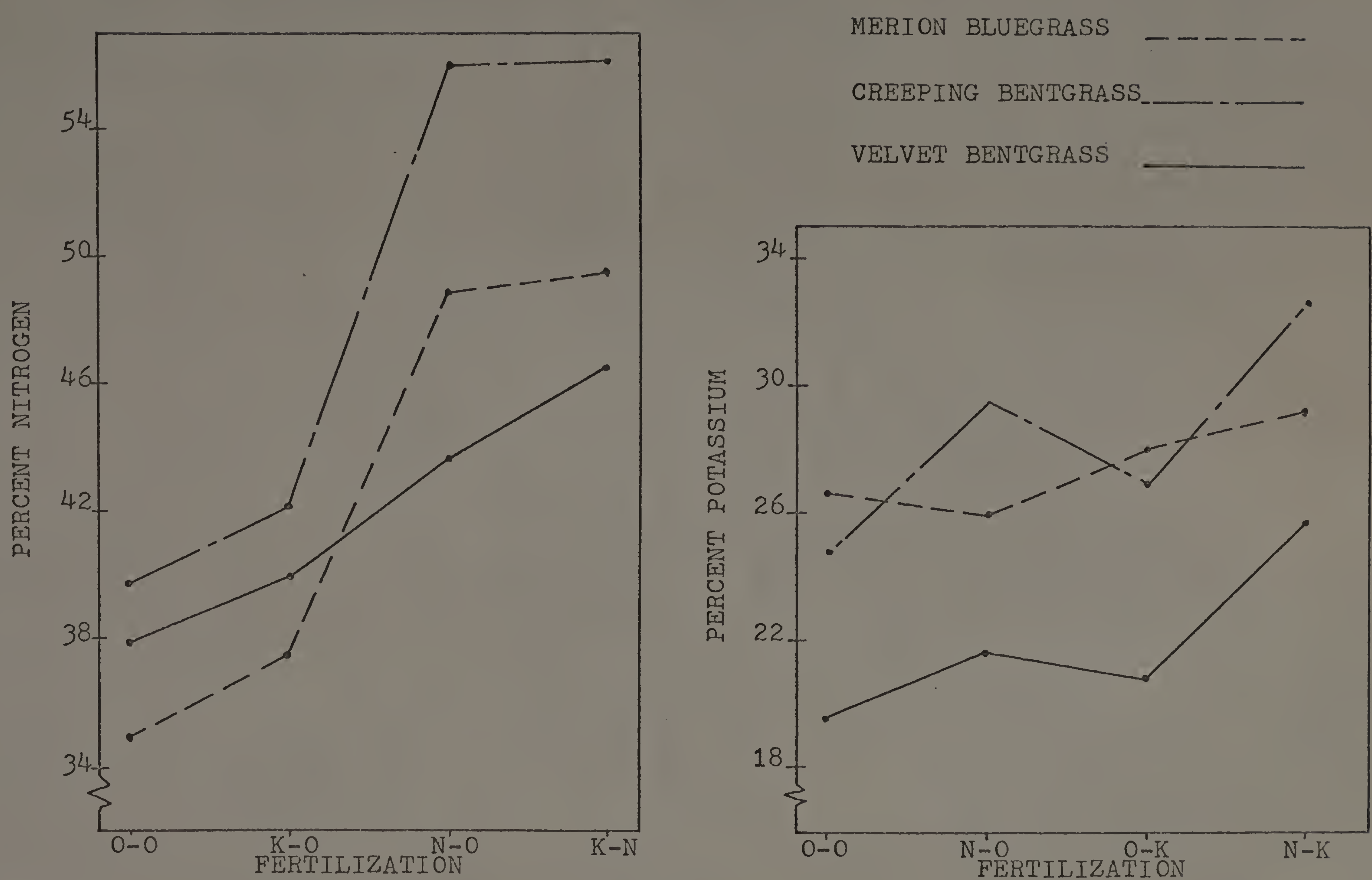

FIG. 17. EFFECT OF FERTILIZATION ON THE PERCENT NITROGEN AND PERCENT POTASSIUM

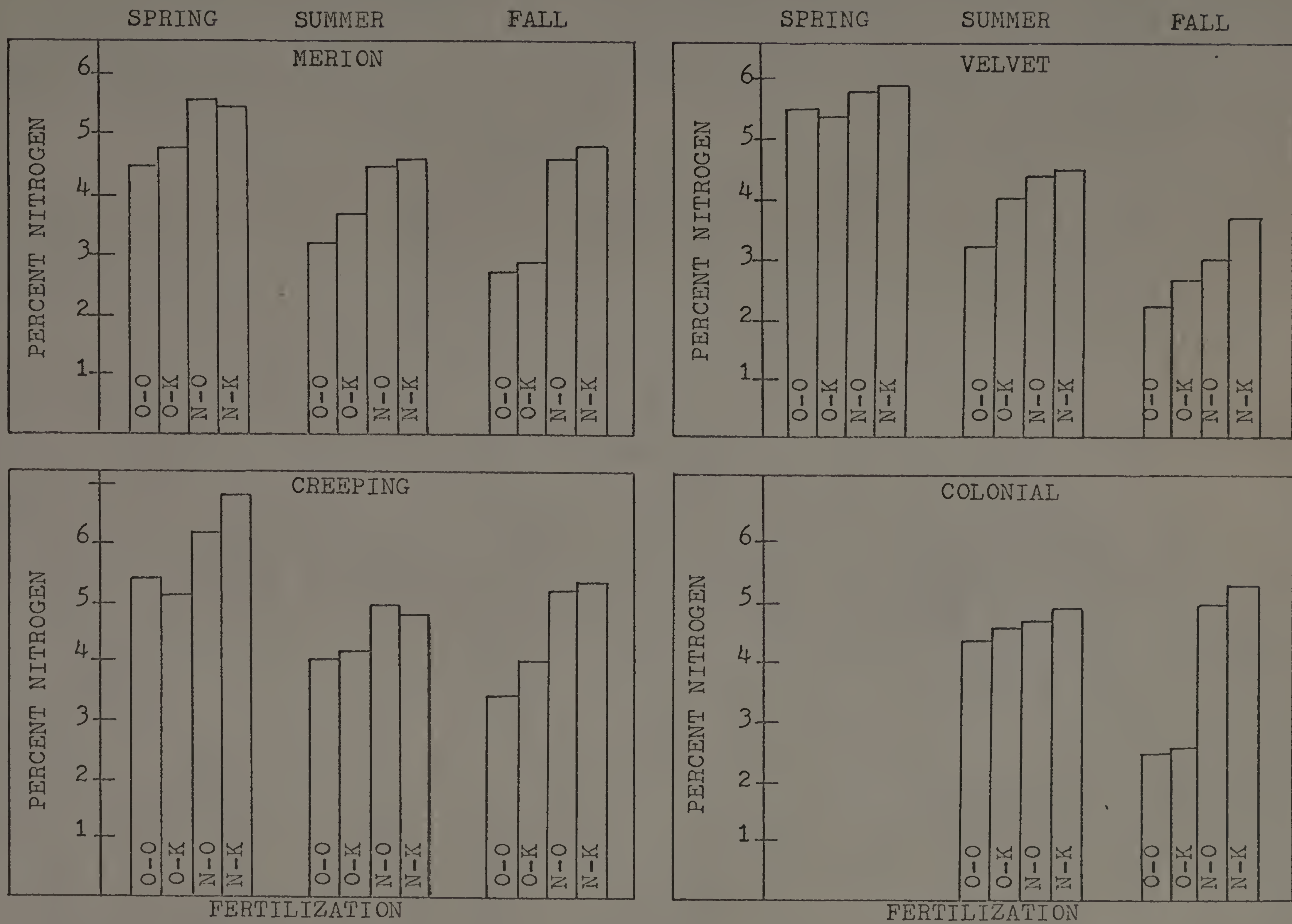

FIG. 18. EFFECT OF HARVEST TIME AND FERTILIZATION ON PERCENT NITROGEN

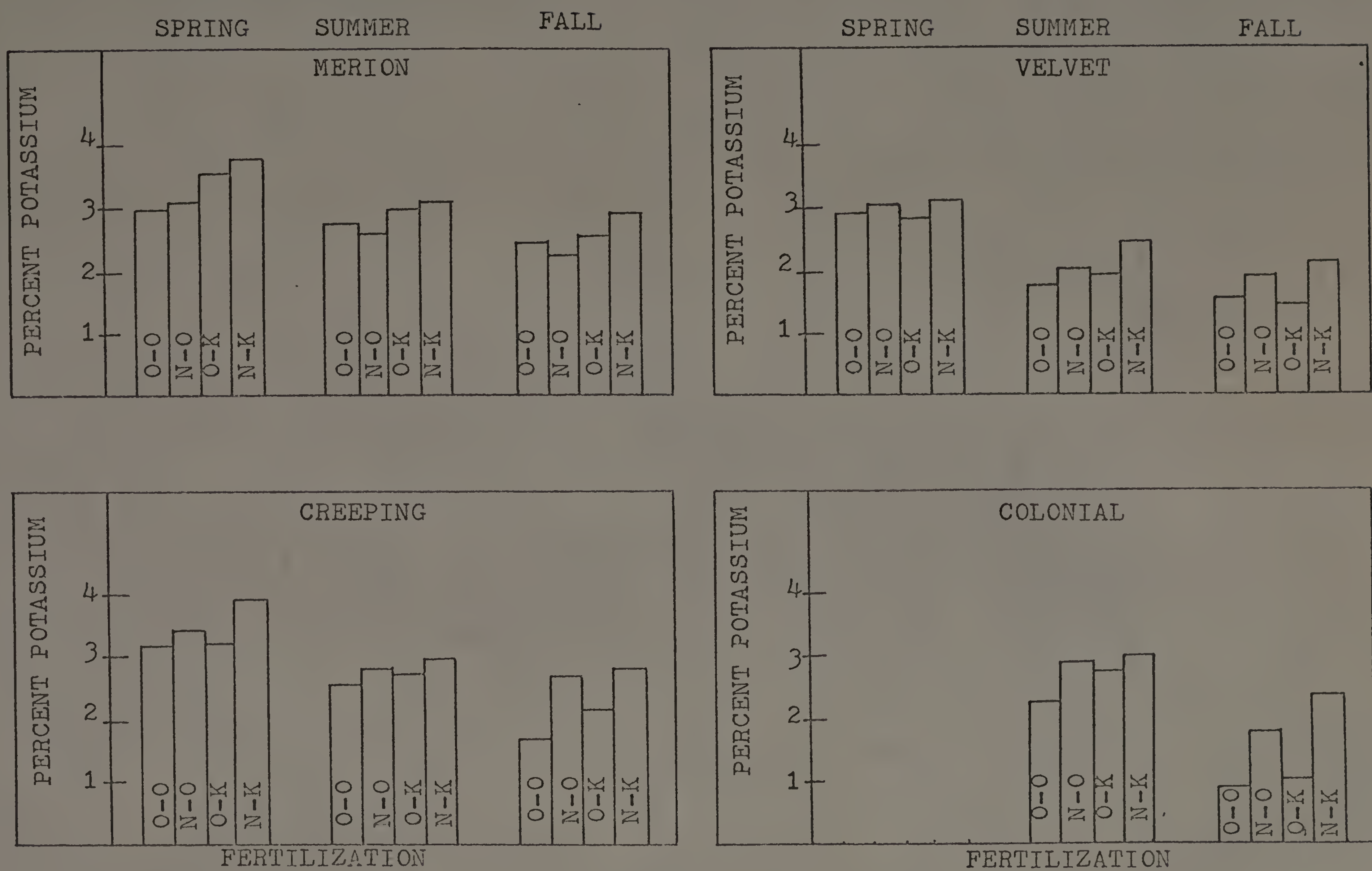

FIG. 19. EFFECT OF HARVEST TIME AND FERTILIZATION ON PERCENT POTASSIUM

DISCUSSION

General Introduction

The references discussed in the literature review represent a selection of work pertaining to cool season grasses as related to carbohydrate content. The majority of investigators have approached this study on the basis of the forage and silage potential of the grass. No literature which considered the esthetic value was found of Merion bluegrass or the bentgrasses in reference to the seasonal fluctuations of carbohydrates.

Upon reviewing literature in this field it is my opinion that although forage and turfgrasses vary in respect to their gross morphological and physiological characteristics, they appear to react similarly to the influence of cultural and environmental factors. The degree of the influencing factors and magnitude of the response is related to the morphological characteristics and genetic adaptation of the different grasses.

During the course of the discussion, frequent reference will be made to a number of terms which for the sake of clarity should be defined.

1. Vegetative characteristics - denotes the leaf, stem, and root structures inherent in the genetic endowment of the plant.

2. Growth habit - refers to the gross morphological form which the plant adopts as a result of its vegetative characteristics and as a result of the maintenance practices applied.

3. "Verdure" as adopted by Madison (53) refers to the quality of the green turf.

4. "Verdure durare" refers to the maintenance of an adequate carbohydrate level in the turf during periods of environmental stress.[1]

Seasonal Localization of Carbohydrate Reserves

A proportionally higher concentration of total carbohydrates in the plant was found localized in the stem tissue areas (rhizome and main stem) in bluegrass, in comparison to the same stem tissue areas sampled in the bentgrasses. Also, in both bluegrass and bentgrasses, the leaf and root tissue accumulated the lowest percent carbohydrates in comparison to the stem tissue sample areas. The bentgrasses displayed a more even distribution of carbohydrates among the four tissue areas sampled containing proportionally a greater amount of carbohydrates in the leaf and root tissue areas in comparison to Merion bluegrass.

These results reflect a response related to the differences observed in the vegetative characteristics among the grasses. Total stem tissue production in cool season grasses appears to be an important factor in determining and establishing the main carbohydrate reserves. Merion bluegrass develops more stem tissue in comparison to the bentgrasses

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[1]"Verdure durare" is a term adopted by the author.

since the total distance between nodes is greater and since it produces more abundant underground stem tissue. Noticeable differences were observed among the bentgrasses in underground stem production, with creeping bent producing the most abundant underground stem tissue and colonial bent producing the least.

Therefore, on the basis of the magnitude of the seasonal fluctuation of carbohydrates, the growth processes reflect to a greater extent, the stem tissue reserves in Merion bluegrass in comparison to the bentgrasses which have a lower carbohydrate concentration in the stem tissue areas. Since there is a more even distribution of carbohydrates in the four tissue areas sampled in the bentgrasses, the growth processes are not as dependent upon any one main carbohydrate reservoir.

Carbohydrate localization studies on both turf and forage cool season grasses appears to be consistent in that the main reservoir of reserve carbohydrate is localized in the stem tissue areas. Therefore if the vegetative characteristic of a cool season grass discloses a higher proportion of stem tissue, by virtue of its stem tissue, the grass has a high potential for the storage of reserves, particularly under favorable cutting management.

This concept of a main carbohydrate "reservoir" has been shown in the following turfgrass study and further substantiated in forage grass studies. The stubble was found to exhibit the largest differences in the carbohydrate content as influenced by cutting and other maintenance practices. Moreover, the region of principal photosynthetic activity, the leaf, contains considerably less reserve carbohydrates than the stem tissue. It can be concluded (from the following study) that the roots in turfgrasses in comparison to the stem tissue contribute considerably less carbohydrate in proportion to the magnitude of carbohydrate found in the stem tissue. As substantiated in literature, root activity is considerably influenced by the fluctuation of the carbohydrate content in the stem tissue.

Seasonal Fluctuation of Carbohydrates as Influenced by Nitrogen and Soil Temperatures

The influence of nitrogen in the fertilization treatment is demonstrated diagrammatically in Figures 6 and 7. In all varieties examined and under all sampling periods, the presence of nitrogen in the fertilization treatments was found to reduce the carbohydrate level and in the absence of nitrogen in the fertilization treatment was found to result in a substantially higher carbohydrate level.

The nitrogen effect in influencing the carbohydrate level was found to be expressed to the greatest extent in Merion bluegrass and to the least extent in colonial bentgrass. Again, this appears to be consistent with what has been discussed previously in reference to the reduction in carbohydrate level as related to the potential reserve capacity of the turfgrasses. For example, on the July 31 sampling period Merion bluegrass was found to exhibit a 7-8% decrease in carbohydrate level under the influence of high nitrogen. On the same sampling period colonial bentgrass was found to exhibit an approximate 1% decrease in carbohydrate level under the influence of high nitrogen in the fertilization treatment.

As was shown with the nitrogen effect, the carbohydrate level was influenced by the vegetative characteristics and growth habits of the grasses. The results from the seasonal fluctuation of carbohydrates in bentgrasses suggest that the level of potassium fertilization may also be a factor in establishing the ultimate carbohydrate reserves in turfgrasses.

In the velvet and colonial bentgrasses, potassium fertilization produced a higher seasonal carbohydrate level in comparison to low potassium. Although the difference in percent carbohydrate between the present and absence of potassium in the fertilization treatment in the percent carbohydrate recorded was not significantly different, the results do suggest the importance in establishing a proper nitrogen-potassium balance in turfgrasses.

The results showed that the seasonal fluctuation of carbohydrate appears to be directly related to soil temperature at a 2" depth and that the magnitude of the carbohydrate level was highly influenced by the presence or absence of high nitrogen in the fertilization treatment. This nitrogen-soil temperature interaction follows the most consistent pattern in the bentgrasses particularly in creeping bent, Differences in the carbohydrate levels recorded under the presence of high nitrogen in the fertilization treatment, and low nitrogen in the fertilization treatment can be seen in the graphic representations of the seasonal distribution of carbohydrate in respect to the fertilization treatment. This effect is most noticeable in creeping bentgrass (Figure 11) in a few isolated examples as shown on the third (June 3), seventh (July 31), and eleventh (September 24), sampling periods. The large differences are consistent with lower average soil temperatures recorded at the time of the sampling. It is concluded from the peak and depressions observed in the seasonal distribution of carbohydrate, that the nitrogen effect in the fertilization treatment in influencing the carbohydrate level is more greatly influenced during periods at which lower average soil temperatures were recorded.

During periods of higher recorded soil temperatures the influence of high nitrogen fertilization treatments did not influence the magnitude of the carbohydrate level in the same degree as was shown with the interactioh of high nitrogen and a low soil temperature. All turfgrasses examined resembled each other in the effect of nitrogen in reducing the carbohydrate level at sampling periods throughout the growing season.

The air temperature, which was also recorded over the plots, does not correlate with the seasonal fluctuation of carbohydrate to the same degree of consistency as observed with soil temperature at a two-inch depth. The reason for this lack of continuity between soil and air temperature as related to the fluctuation of carbohydrate is that the air temperature is subject to violent change by changing climatic conditions. The soil zone itself, with its specific heat capacity in addition to the buffering influence of the vegetative cover, aids in maintaining a more consistent soil environmental temperature for plant growth.

The insulating effect of vegetative cover upon the soil temperature recorded at a two-inch soil depth can be readily observed when comparing the lower soil temperatures recorded under the Merion bluegrass in comparison to the higher soil temperatures recorded under the bentgrasses. Refer to Figure 16. Differences in soil temperatures are also observed among the bentgrasses with creeping bentgrass recording the lowest soil temperature and colonial bent, the highest. From sod core samples taken from established plots, Merion bluegrass was found to contain the highest average dry matter by weight in comparison to velvet and colonial bents which recorded the lowest average dry matter by weight. Again an examination of the vegetative and growth characteristics of the grasses is an important consideration in explaining these differences.

Soil temperature effect as influenced by the vegetative cover is further substantiated by the bare ground temperature readings. Higher bare ground temperatures were recorded in the summer and during the warmer periods in the growing season in comparison to the lower temperatures recorded under the turf. In the fall a reversal of this trend occurred with lower bare soil temperatures recorded in comparison to the higher soil temperatures recorded under the turf. Colonial bentgrass which was characterized by developing the most erect growth habit among the bentgrasses was found to record soil temperatures which follow the closest similarity to the bare ground temperature readings. Since soil temperature at a two-inch depth was found to be the factor best correlated in respect to the seasonal carbohydrate fluctuation one can further infer that soil texture and structure, soil moisture, and soil color, could also be contributing factors which would ultimately affect the soil temperature at a two-inch depth and which would eventually establish the soil temperature environment of the plant and thus affect growth.

Aside from the prominent increase in carbohydrate level in the spring of the year in creeping bentgrass, all the bentgrasses resemble each other in the seasonal fluctuation of carbohydrate throughout the entire growing season. To explain this trend in creeping bentgrass it is important to re-examine the vegetative characteristics and growth habits of the grasses. Creeping bentgrass, in comparison to velvet and colonial bentgrass, produces the largest quantity of underground storage tissue and in addition, its growth habit is such that the stem tissue does not grow erect and therefore there is more stem tissue remaining after mowing in comparison to either velvet or colonial bentgrasses. Because of its vegetative characteristics and growth habit, the more abundant stem tissue represents a potential reservoir for the establishment of a carbohydrate reserve. Among the bentgrasses, creeping bentgrass displayed the largest carbohydrate level change at all sampling periods throughout the growing season, whereas colonial bentgrass recorded the least. In colonial bentgrass one would expect the lower carbohydrate value because with its erect growth habit and proportionately less total stem tissue volume it has a lower potential for the accumulation of reserve carbohydrates.

Merion bluegrass showed a gradual increase in carbohydrate reserves from spring to mid-summer followed by a prominent depression in carbohydrate level in late summer and a subsequent rise in the fall. This late summer depression in carbohydrate content appears to be consistent to what Musser (67) and other investigators have referred to as a "summer dormancy period." In forage studies Kentucky bluegrass has been found to exhibit this depression in growth as shown by a decrease in clipping yield. The low clipping percent was found to parallel the low carbohydrate period observed in the turfgrasses examined.

In all the bentgrasses examined, with few exceptions, the entire late spring to late summer period in the growing season displayed a seasonal depression in carbohydrate level that followed an extended U-shaped curve. This depression was especially prominent in both velvet and colonial bentgrasses which obtained the lowest carbohydrate values recorded among the four grasses and also displayed the least variation in the degree of carbohydrate fluctuation. The prominence of the extended U-shaped curve, appears to be a response to the high soil temperatures recorded in the summer in comparison to the low soil temperatures recorded in the spring and fall. Again, as discussed previously, the difference in the magnitude of the carbohydrate level recorded among the grasses could be related to the vegetative characteristics and growth habit of the grasses.

Forage studies reveal a decrease in herbage yield from mid-summer to fall. Yields were found to decrease at an early date in Rhode Island bentgrass (9). This period coincides with the low recorded carbohydrate level at the onset of the August 20 sampling date. Refer to Figures 10, 12 and 15 for comparison. In Kentucky bluegrass (9) an abrupt decrease in herbage yield was found to occur. This period was found to coincide with the low carbohydrate level recorded at the onset of the August 22 sampling date. Refer to Figure 8 for comparison. The abrupt decrease in forage yield observed in Kentucky bluegrass may be explained in part by the sharp depression in carbohydrate level recorded in late summer in Merion bluegrass.

This summer depletion of carbohydrate reserves represents a critical period for the growth and maintenance of "verdure" in turfgrasses. This period coincides with the summer lag period in grazing yield characteristic of the forage grasses. As will be discussed in further detail in the following section, the intensive routine maintenance practices applied in golf course turf maintenance further stimulates the depletion of this reserve capacity and establishes a situation in which the turf is on the threshold between success and failure. The response during this critical growth period will be dependent upon the environmental condition existing and the maintenance practices.

One can conclude that soil temperature at a two-inch depth, as influenced by soil moisture, was found to be the most predominant environmental factor accounting for the seasonal fluctuation of carbohydrates. The nitrogen effect in the fertilization treatments was the most important factor accounting for the greatest magnitude of increase or decrease in carbohydrate reserves. The combined effect of low nitrogen and low soil temperatures were the factors most conducive for carbohydrate accumulation, whereas the combined effect of high nitrogen and high soil temperatures were the factors most conducive for carbohydrate reserve depletion.

The carbohydrate reserve in grasses represents an accumulation or surplus of materials in excess of the immediate needs of the plant for growth and maintenance. These reserves have been referred to by Blaser et al. (8) as a "dynamic system of energy balance." Synthesized carbohydrates are dissipated when the demands for growth (respiration) exceed photosynthesis and in turn, synthesized carbohydrates are stored when growth demands are low. Madison (53) has interpreted this energy balance by suggesting that "good verdure - a good product - is obtained only when carbohydrate accumulation occurs." Although the results in the following turfgrass study are consistent to what Madison has suggested in his original hypothesis, in lieu of the interpretation of these results, there is need of further re-defining this hypothesis.

The bentgrasses, in particular velvet and colonial, recorded the lowest carbohydrate levels during the hot summer period in the growing season and yet a good quality green turf was maintained. The low carbohydrate values recorded represent a critical period for the growth of turfgrasses. The carbohydrate reserves are depleted to low levels and the further lowering of these reserves places the turf in a highly variable condition in establishing "verdure" SUCCESS OR FAILURE with the maintenance practices applied.

With the objective of obtaining good "verdure" the routine maintenance practices adopted in golf course maintenance of turf are continued even through this critical growth period, while the potential effect of any one or more of these maintenance practices are most conducive to the further lowering of the reserves. The combined effect of these maintenance practices in conjunction with climatic conditions which are not conducive from the standpoint of carbohydrate accumulation, the reserves are depleted to the point that the plant may be weakened and unable to recover. In this carbohydrate depleted state, the major problems in turf culture are intensified. The turf may lack the durability which the game of golf demands and in a weakened state it may be more susceptible to disease conditions.

Since the turfgrasses have been found to vary considerably in respect to seasonal fluctuation of carbohydrates, this concept of the maintenance of an adequate carbohydrate level especially during periods of environmental stress may be further applied in the selection of turfgrasses which have a higher potential capacity for the storage of carbohydrate reserves. This may explain why varieties of (Agrostis palustris L.) creeping bentgrass have been highly accepted in the northeast and in particular, in cool regions and in areas more susceptible to climatic extremes. A similar analogy could be applied with varieties of (Poa pratensis L.) Kentucky bluegrass, although a similar comparison could not be made among the bluegrasses since Merion was the only representative variety examined.

Since it is reported that the establishment of an adequate carbohydrate reserve is essential for producing good "verdure" during this critical growth period, the maintenance practices should be adjusted to favor the accumulation of a reserve or regulated, so as to prevent the further depletion of the reserves.

In the study of cool season grasses, Pellett and Roberts (72) concluded that with food reserves available, differentiation of the protoplast can take place that increases the resistance of plant tissue to high temperatures and other unfavorable conditions.

In the study of warm season grasses Burton (13) concluded that "there is a relationship between carbohydrate accumulation and stability and the ability of a plant to withstand high temperatures." This relationship is most applicable to cool season grasses since these grasses are not genetically adapted for perpetuation especially during extended periods of hot unfavorable weather.

From the results of this study, an interesting hypothesis which could be proposed is that "Verdure durare" is established when carbohydrate accumulation occurs, and that good "verdure" is not promoted during periods of environmental stress unless an adequate carbohydrate level is maintained. In selecting cultural practices in the maintenance of turfgrasses one must consider an examination of the vegetative characteristics and growth habits of the turfgrasses in promoting resistance to verdure deterioration.

Cutting and Moisture Factors in Influencing the Fluctuation of Carbohydrate Reserves

It would be impossible to treat the factors such as moisture, cutting and nutrient level separately in discussing the effect of any one particular factor or in determining the level of the carbohydrate reserve since they are all interrelated and therefore, cannot be treated separately.

Conclusions reported from pasture and turf studies as discussed in the review of literature reveal in general that the clipping of turfgrasses results in a transfer of reserves, affecting the yield of both top and root growth, reducing the re-growth potential of the grass plant, and decreasing the carbohydrate reserves of the plant. These conditions thus reduce the resistance of the plant to drought and winter hardiness.

The moisture level has been found to influence the carbohydrate level when below optimum conditions are established, such as insufficient supply of water, or when an excess amount of water is supplied. This aspect of moisture relation, as related to the growth process, was investigated by Murata _et al_. (66). In most species examined, photosynthesis was little affected until the stress became considerably severe. As water stress was found to progress beyond a critical level, photosynthesis showed an abrupt decrease. In contrast to this, the decrease in respiration started at a higher soil moisture level than with photosynthesis; although the rate of decrease in comparison to photosynthesis was smaller.

The moisture factor as well as the cutting factor are contributing factors as determined by the climatic conditions and the maintenance practices applied. This interaction has been investigated by Madison (52,53,54). Madison found that when maintenance practices which stimulated growth (respiration) were combined (high nitrogen, frequent irrigation, and short or frequent mowing) growth was decreased and "verdure" was decreased. The combination of an alternate series of maintenance practices (weekly irrigation, high weekly mowing, and less than maximum nitrogen) produced low yield but increased the "verdure." Upon an interpretation of the literature, Madison (53) suggested that yield is decreased in one instance by exhaustion of carbohydrates, and in another by accumulation of carbohydrates.

In this turfgrass study, both the cutting and moisture factors were maintained as accurately as field conditions allowed, and it can be concluded that both influence the carbohydrate supply in relation to the climatic conditions and maintenance practices applied. Since the experiment was set up to duplicate conditions as established in golf course maintenance practices the influence of cutting and moisture is not considered in the interpretation of the results.

The influence of the maintenance practices such as fertilization, temperature, cutting and moisture as discussed in the literature review has been shown to be instrumental in altering the carbohydrate reserve capacity in turf. A decrease in the intensity of these maintenance practices as frequently as the sport or environment allows is desirable in maintaining an adequate carbohydrate reserve during this period of environmental stress.

Percent Nitrogen and Potassium

With maximum metabolic activity occurring in the foliage and with the high demand for nitrogen in the growth process, the high percent nitrogen over the percent potassium is consistent with what is expected. Also, the influence of nitrogen increased the uptake of potassium. These effects can be observed graphically in Figures 17 and 18. Therefore, we can conclude that when environmental conditions are favorable for growth, nitrogen stimulates growth amd thus an increase in metabolic activity will in turn further stimulate the uptake of nitrogen and potassium.

Differences appeared among the grasses in the percent foliage nitrogen and potassium in the spring, summer and fall harvest periods. The highest percents of nitrogen and potassium were recorded in the spring and lowest percents were recorded in the fall. Again, this may reflect the higher metabolic activity occurring in the spring of the year. Also, this period coincided with the low carbohydrate levels recorded in the spring which were continuously being depressed as a result of the stimulation of new growth.

Low nitrogen levels limit growth so that available carbohydrates are not used up as completely in foliar production. According to Pellett _et al_. (72) with food reserves available, differentiation of the protoplast and other differentiation processes can take place that increase resistance of plant tissue to high temperatures and other unfavorable conditions. The high nitrogen treatments decreased carbohydrates and food reserves and also produced a turf that had a low resistance to high temperatures whereas the high levels of potassium increased the resistance of turf produced on high nitrogen.

In forage studies, nitrogen fertilization has been found to raise the total yield considerably with the effect being greater in the spring than in the summer or autumn. The crude protein content has been also found to increase with nitrogen fertilization, with this increase dependent upon the fertilization level and the length of the growing period. Deinum (19) reported that shortly after fertilization this rise is considerable (as the growing period is longer the increase will be smaller). After long growing periods the content in the fertilized and non-fertilized treatments may never be the same.

SUMMARY

Four turfgrass species maintained under four fertilization treatments were investigated.

The percent total soluble carbohydrates were found to be proportionally higher than the percent total fructose for all species and at all sampling periods.

The main carbohydrate reserves were localized in the stem tissue and these reserves responded with the greatest seasonal fluctuation.

Soil temperature was found to be the most predominant factor which accounted for the greatest seasonal fluctuation of carbohydrates wherein an increase in the soil temperature caused a decrease in soluble carbohydrates and vice versa. Similarly an increase in nitrogen fertilization decreased carbohydrate reserves.

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FACTORS WHICH INFLUENCE THE LEVELS OF SOLUBLE

CARBOHYDRATE RESERVES OF COOL SEASON TURFGRASSES

A Dissertation

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