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CARBOHYDRATE CHANGES IN OLIVE DURING FRUIT RIPENING

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Abstract

Changes in carbohydrate content in olive during fruit ripening in on and off years showed that contents of fruit soluble sugars during fruit development increased up to 90 days after fruit set and then decreased during fruit ripening up to 120 days. A marked temporary increase was evident at the beginning of fruit color changes. The content of reducing sugars in fruit were considerably higher than that of non-reducing ones. In leaves the amount of non-reducing sugars was higher than that of reducing ones. During on-year, content of soluble sugars decreased after a primary increase whereas during off-year a different pattern was observed. Glucose, fructose and mannitol, main sugars of soluble fraction of fruits were in decreasing order. Changes of glucose and fructose content were contrary to each other up to 135 days after fruit set, thereafter concomitant to decrease of glucose and fructose, mannitol content increased. In leaves, mannitol, glucose and fructose are the major components of soluble sugars, with different pattern during on-and off-years where the content of mannitol in off-year was lower than that of on-year. Insoluble sugars of fruit and leaf in off-year were higher than that of on-year. The main constituents of this fraction after hydrolysis were glucose, rhamnose, arabinose, fructose, galactose, ribose, xylose and mannitol, in different amounts. Glucose was the major sugar of fruits in most cases.

Introduction

Problems of alternate (or biennial) bearing in fruit tree have been investigated (Singh, 1949; Davis, 1957; Jonkers, 1979). Alternate bearing is a very widely spread phenomenon, occuring in both deciduous and evergreen trees. This phenomenon has also been studied extensively in olive in the last decade. Flower induction in this tree, starts during late winter and requires winter chilling and the presence of leaves (Monselise & Goldschmidt, 1982). The degree of alternation is dependent on the species, the cultivar, environmental conditions and the fruiting history of each tree. Olive has a very marked alternate bearing pattern (Lavee, 1996). Changes in carbohydrate components of leaves during on (bearing) and off (non-bearing) year cycle are remarkable. Sugars and starch are much higher at the beginning of an on-than of an off-year (Fahmy, 1958). When we consider alternation of bearing, which is proverbially marked in olive tree, competition between vegetative and reproductive organs would cause reduced production of new branches during the on-year (competition with growing fruits during summer), producing a smaller number of flowers. The strong growth in the off-year again allows large amounts of flower to be initiated the next year (Monselise & Goldschmidt, 1982).

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Changes in the soluble carbohydrate composition of leaf and fruit tissues of olive have been previously investigated (Donaire *et al.*, 1975; Wodner *et al.*, 1988; Drossopoulos & Niavis, 1988; Romani *et al.*, 1988). The objective of the present study was to examine the changes of components of soluble and insoluble carbohydrates in the olive leaves and fruits during fruit development in on-and off-year in order to gain more information about the physiology of olive tree and determine the possible roles of carbohydrates in alternative bearing in this plant.

Materials and Methods

Leaves and fruit samples were collected from olive tree during fruit ripening at 2-week intervals from 75 to 165 days after fruit set, from Gilvan region in Gilan province. The samples were lyophilized for 48 hr, powdered in omnimixer and maintained at -2°C. For the determination of sugar content, 1g of powder was extracted using 10ml of ethanol-distilled water (8:2 v/v) and after centrifugation the supernatants were collected (Patumi *et al.*, 1990). The residue from ethanol extraction was subsequently used for polysaccharide extraction by boiling water (Ebrahimzadeh, 1969). Alcohol soluble fractions were passed through a column (2x20cm) of a strong acidic resin (Amberlite IRA-120, H^+ form, 20-25 mesh). The elute was then passed through a column (2x20cm) of a strong basic resin (Amberlite IRA 400, CO_3^{-2} form, 20-30 mesh). Free sugars were determined in the eluent (Donaire *et al.*, 1975).

Polysaccharides were estimated in the alcohol-insoluble fraction by the method of phenol-sulfuric (Dubois *et al.*, 1956), and reducing sugar in the alcohol soluble fraction by the method of Nelson (Bell, 1944). Nelson's method was also utilized for determination of total sugar content after hydrolysis of alcohol soluble fraction. Values for non-reducing sugars were obtained as the difference between the total and reducing sugars. Alcohol soluble fraction and polysaccharides were hydrolyzed by heating in a sealed tube with 4N H_2SO_4 at 100°C for 1 and 3 hr, respectively. These solutions were cooled, neutralized with $BaCO_3$ and centrifuged (@ 2700 rpm for 30 min at 25°C). The monosaccharides, formed by hydrolysis, were identified by TLC together with authentic monosaccharides on silicagel 60G impregnated with phosphate buffer (pH 5) using acetone-1-butanol-phosphate buffer (50-40-10) as developing solvent. The visualization was carried out by spraying with aniline-diphenylamine-phosphoric acid reagent (Jork *et al.*, 1990). The monosaccharides were also analyzed quantitatively and qualitatively by GLC of their TMS ether derivatives (Sweeley *et al.*, 1963). In this method up to 10 mg of standard sugars were treated with 1 ml of anhydrous pyridine, 0.2ml of hexamethyldisilazane (HMDS) and 0.1ml trimethyl chlorosilane (TMS). The reactions were carried out in plastic stoppered vials. The mixture was shaken vigorously for about 30 sec., and was then allowed to stand for 5 min., or longer at room temperature, prior to chromatography. A 2 μl sample was then injected into a gas chromatograph with flame ionization detector (Shimadzu GC 16-A) for analysis. A 5% SE-30 glass column was used under the following temperature program conditions: column 100-260°C (4°C per minute), detector and injector 280°C. Carrier gas was N_2 with a flow rate of 55 ml min^{-1}. Flow rate of hydrogen and air were 55 and 400 ml min^{-1}, respectively (Rinaldi *et al.*, 1994).

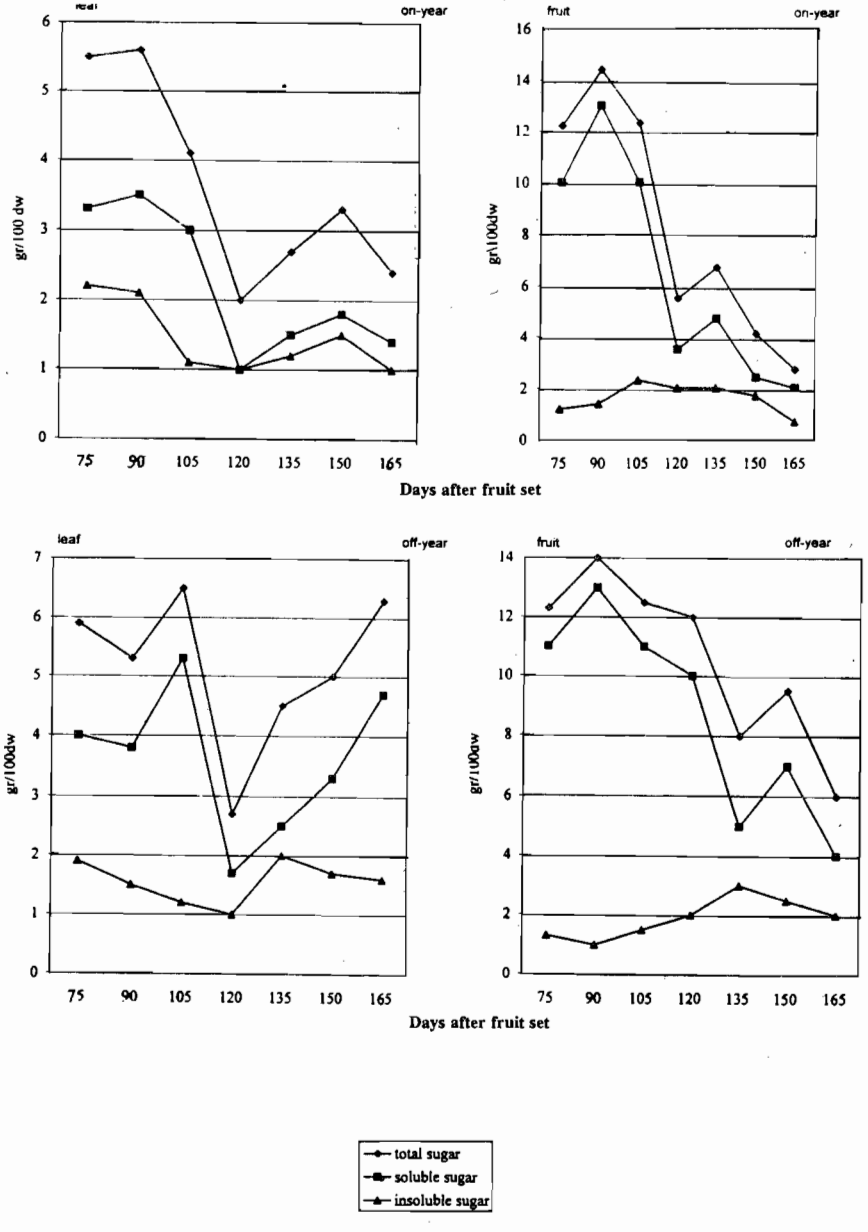

Fig. 1. Changes in soluble and insoluble sugars in leaves and fruits during fruit ripening in on-and off-year.

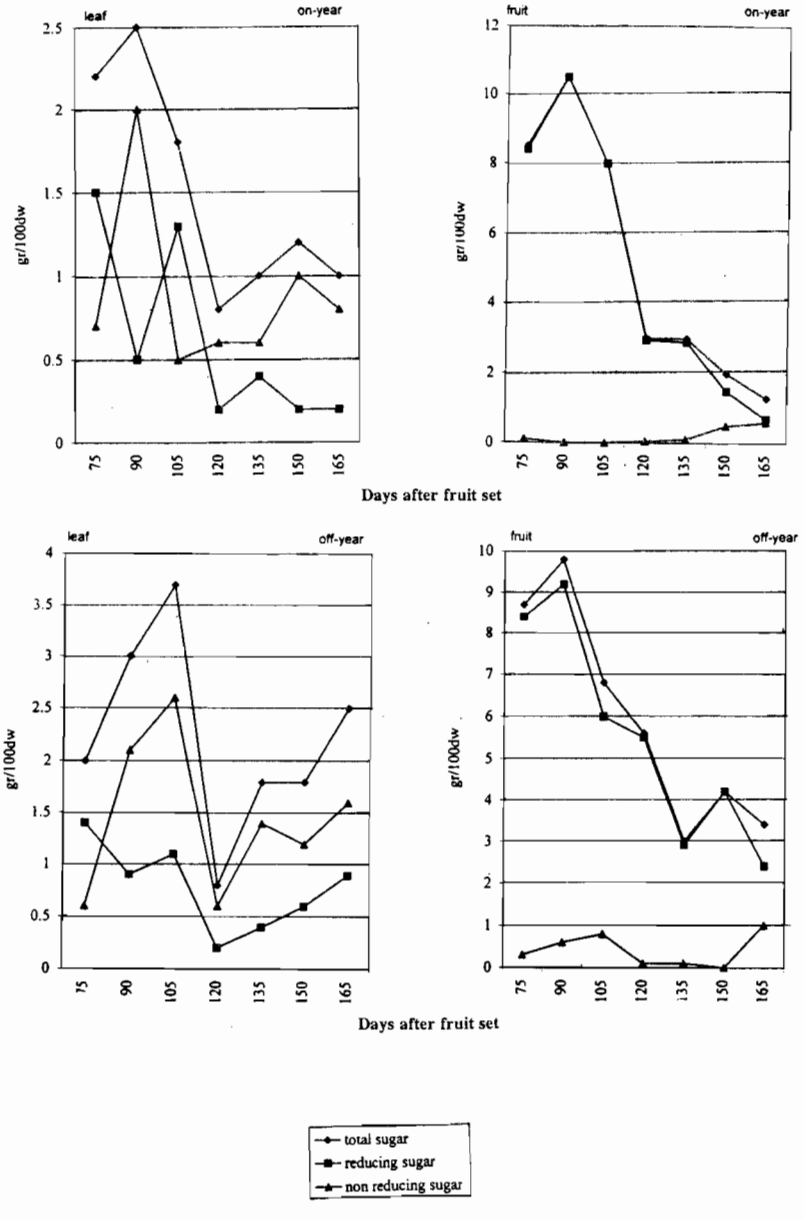

Fig. 2. Changes in reducing and non-reducing sugars during fruits ripening in on-and off-year.

Results and Discussion

Changes in the content of soluble and insoluble sugars in leaves and fruits after fruit set during on-and off-years are given in Fig.1. Content of fruit soluble sugars during fruit development increased up to 90 days after fruit set and afterward decreased during fruit ripening up to 120 days. A marked temporary increase was noticed at the beginning of fruit color change. The content of fruit insoluble sugars is almost constant during fruit development. It was higher during off-year. In contrast to the fruits, the content of sugar showed similar pattern in on-and off-years, the changes of sugars in leaves did not show the same pattern during fruit development. The changes of sugar content showed fluctuation in leaves during off-year with augmentation of total and soluble sugars at the end. The content of total sugars being higher than that of on-year.

The amount of reducing sugars in fruit during ripening was considerably higher than that of non-reducing ones (Fig. 2). This result is in agreement with the reports of Lavee, (1988). Besides, changes in pattern of reducing sugars and non-reducing sugars are against to each other. In contrast to fruit, non-reducing sugar content was higher than that of reducing ones in leaves. In the present study the content of sugars was determined by utilizing two different methods where by using Nelson's method, the sugars were partially destroyed. Glucose, fructose and mannitol are the main constituents of alcohol soluble sugars of fruits during ripening both in on- and off-years (Fig. 3) as also observed by Lavee (1988).

Besides, the content of glucose was higher than that of fructose and mannitol which has also been reported previously (Fernandez, 1971). Glucose and fructose varied to each other in fruits during ripening in on-year. This pattern of changes can be seen up to 135 days after fruit set, then the contents of both sugars drop. Furthermore during the decrease of glucose and fructose, the amount of mannitol considerably increased. Same variations of glucose and fructose amount are seen in off-year in fruit, though with less fluctuations, and the mannitol without intense augmentation in the last period of ripening.

In leaves, the amount of fructose was very low and the two other sugars, glucose and mannitol which are the main monosaccharides of alcohol soluble fraction, varied in opposite directions both in on-and off-years, the mannitol being the most abundant, especially in on-year.

In general, the fruit is fed by translocation of metabolites produced in the leaves. The accumulated reducing sugars which were found in the fruits (glucose and fructose) are not translocated in the phloem of higher plants. It has been shown, however, that in some plants the polyols serve as translocatable carbohydrates, in addition to sucrose and other non-reducing oligosaccharides (Wodner *et al.,* 1988). Thus the mannitol in the olive fruit as well as mannitol and other polyols in the fruits of many other plants might be of specific importance in the metabolic transformation and synthesis of specific first storage material. It was observed that in the first period of fruit development (from 75 to 135 days of fruit set), the amount of sugars is similar among the fruits in on-and off-years, but in leaves, glucose and fructose are lower and mannitol is higher in on-year. In the second period (from 135 to 165 days of fruit set), the amount of mannitol increases in fruits following the augmentation of this sugar in the leaves. By analysis of

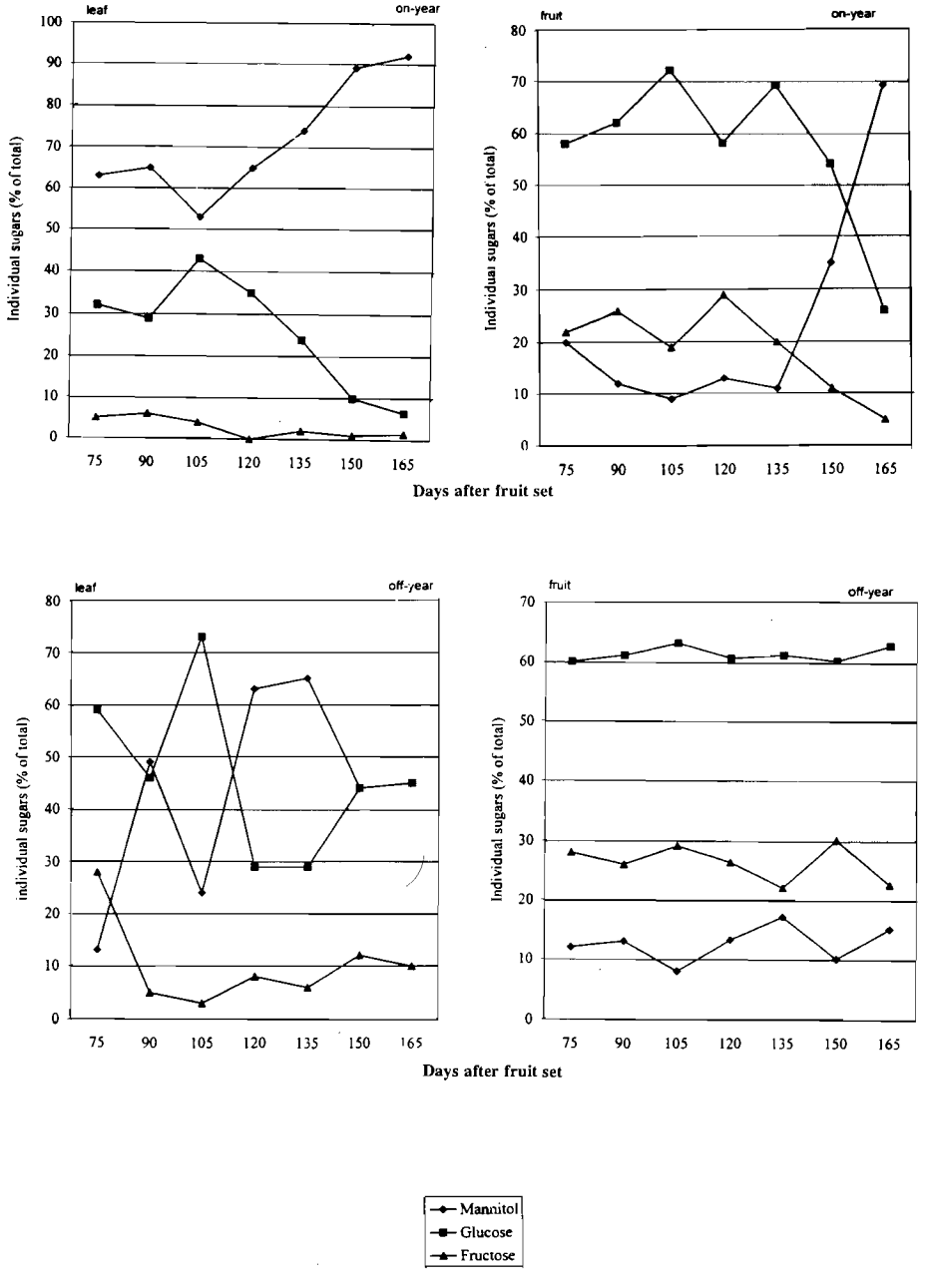

Fig.3. Changes in the concentrations of monosaccharides derived from hydrolysis of soluble sugars of leaves and fruit during fruit ripening in on-and off-years.

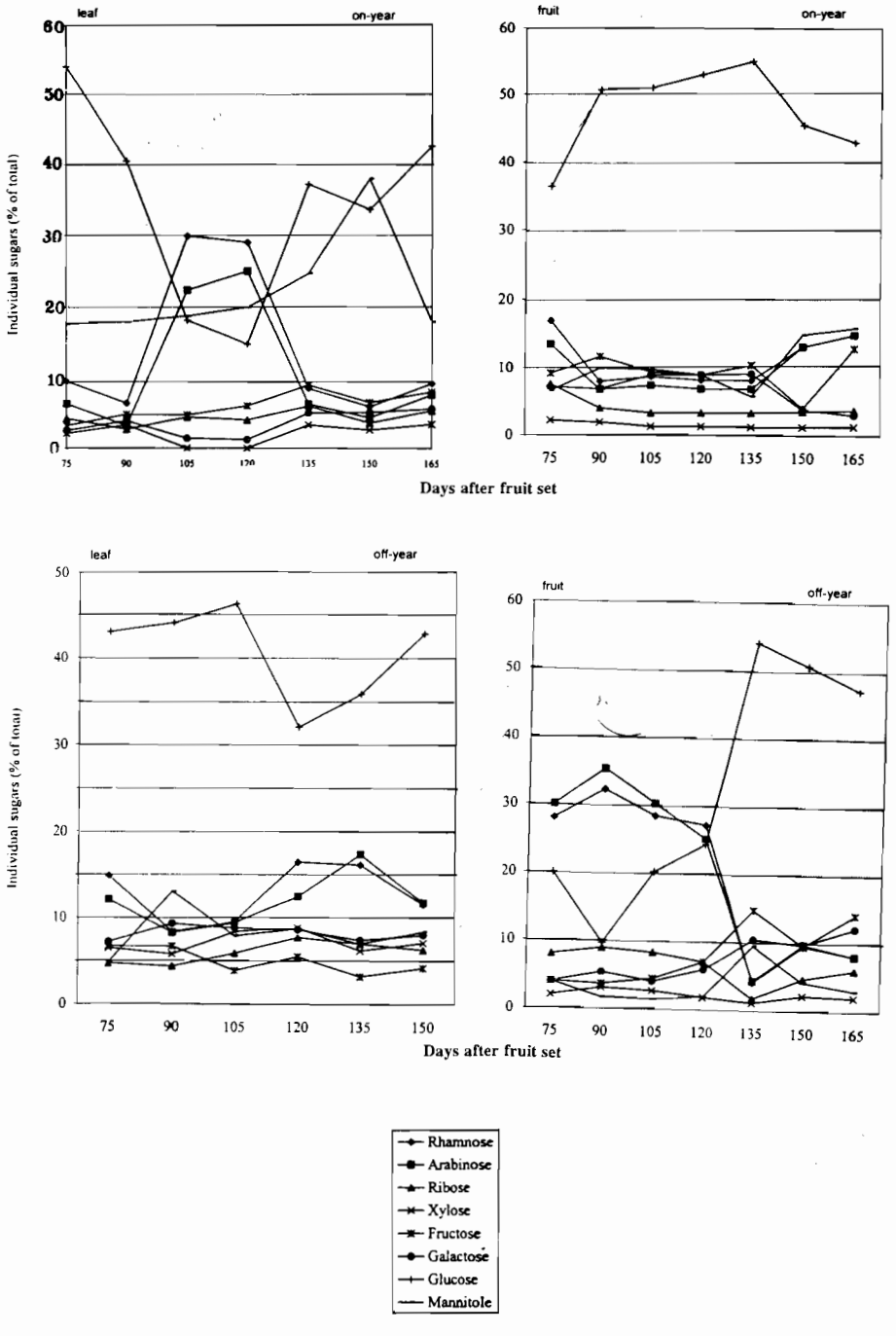

Fig.4. Changes in the content of monosaccharides derived from insoluble sugars of leaves and fruits during fruit ripening in on-and off-years.

water soluble sugars of leaves and fruits, seven monosaccharides and mannitol (a sugar alcohol) were determined by GLC and TLC. Of these constituents, glucose was the major component, followed by rhamnose, arabinose, fructose, mannitol galactose, ribose and xylose, respectively. It is possible that by using boiling water for extraction of storage polysaccharides, some pectins compounds would have been extracted. According to the results based on the monosaccharide composition, this compound could be an arabinorhamnan that alongwith uronic acids are the main contituents of cell wall pectic compounds (Goodwin & Mercer, 1992). The content of glucose in fruits, during on-year was higher than that of the off-year except in the last stages. But in leaves the amount of glucose in off-year was high, while during on-year the amounts of rhamnose and arabinose were higher than that of the off-year except in the last stages.

The contents of rhamnose and arabinose in fruits during off-year were higher than that of the on-year and concomitant to decreasing of rhamnose and arabinose, the content of glucose increased (Fig.4). The levels of soluble and insoluble carbohydrates in photosynthesizing leaves are principally the result of the balance between the rate of carbon assimilation and the rate of carbon exported to a short or long distance, under the influence of the various sinks. The balance between the carbon assimilated in leaves and the carbon for export is regulated by two different mechanisms. In addition, polyols have an important physiological role, representing the main carbohydrate reservoirs in many woody plants (Drossopoulos & Niavis, 1988). Our results showed that with regard to possible role of carbohydrates in alternative bearing, the content of these compounds in leaves was high in off-year. Thus, we deduce that the rate of carbohydrate transport in off-year is low. As the carbohydrate content of the fruits is similar both in on-and off-years, we can conclude that the new shoots lack the capacity for carbohydrate mobilization. Such similar observations have previously been reported for apple (Monselise & Goldschmidt, 1982). Since the level of certain elements in off-year is lower than that of the on-year (Villemur, 1984) thus the decrease in mannitol transport could be attributed to the low level of these elements. The biosynthetic pathway of mannitol in *Apium graveolens* (Umbelliferae) has already been established. Triose phosphate, exported from the chloroplasts and converted to fructose-6-phosphate is used for mannitol and sucrose synthesis in equal amounts. Fructose-6-p is converted to mannose-6-p, mannitol-1-p and free mannitol by involvement of three enzymes: mannose-6-phosphate isomerase, NADPH-dependent mannose-6-phosphate reductase and mannitol-1-phosphate phosphatase, respectively (Smirnoff, 1995). Based on the results obtained in the study it would suggest that the same pathway is used during the on-year and mannitol is accumulated but during off-year the amount of glucose is more than that of mannitol, this pathway is not followed. The possible pathway could be the conversion of fructose-6-p to glucose-6-p and free glucose.

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