

AN ABSTRACT OF THE THESIS OF

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Title: Sugar Uptake, Fruit Growth and Carbon Partitioning in the Strawberry

Abstract Approved: _____
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Sinks, plant organs which depend on the import of assimilates for growth, play a central role in determining assimilate distribution patterns within the plant. The strawberry fruit is a strong sink for dry matter and its effect on carbon partitioning and assimilation in the plant as well as the mechanism by which dry matter is assimilated by the fruit were studied.

All experiments were conducted with day-neutral strawberries (Fragaria X ananassa Duch. cv Brighton) grown in a greenhouse. Plants were deblossomed to determine the effect of fruiting on plant growth. Fruit was the dominant sink accumulating .16 g dry weight/plant and during the peak of fruit growth, fruit accumulated more dry matter than assimilated by the entire plant. Fruit limited the growth of all vegetative plant parts. During the peak of fruit growth net photosynthesis per unit area of single leaves was 60-80% higher in fruiting plants than in deblossomed plants.

To determine the chemical form in which dry matter arrived at the fruit, labeled photosynthate was collected from the phloem of

cut pedicels. Radioactive label in the phloem exudate was found to be primarily in sucrose (92%) with the remainder in glucose (2%), fructose (1%) and acidic and basic fractions (4%).

Growth of a strawberry fruit was dependent upon its ranking within an inflorescence. Primary fruit had the largest diameter, fresh weight, dry weight, expansion rate and relative growth rate from anthesis until fruit were ripe. All of these parameters decreased with lower ranking on the inflorescence. Rates of ^{14}C -sucrose uptake by fruit tissue discs cut from fruit having different growth rates were similar to rates of dry matter accumulation into the fruit in situ.

Uptake of ^{14}C -sucrose into fruit tissue discs was greatest at a pH of 5.0 and 40°C. High concentrations of metabolic inhibitors such as dinitrophenol stimulated sucrose uptake while reducing respiration and incorporation of label into insoluble components. Kinetic analysis of sucrose uptake revealed a linear and a saturable component. The uptake rates of fructose and glucose, which are the products of sucrose hydrolysis, were measured. The rates of fructose uptake were similar to that of sucrose, but glucose uptake was 2-3 fold greater and exhibited saturation kinetics.

SUGAR UPTAKE, FRUIT GROWTH AND CARBON
PARTITIONING IN THE STRAWBERRY

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SUGAR UPTAKE, FRUIT GROWTH AND CARBON PARTITIONING IN THE STRAWBERRY

Chapter 1

INTRODUCTION

Agricultural production is largely determined by the amount of carbon dioxide fixed by photosynthesis and how these photosynthates are distributed among economically important parts of the plant. It has been generally accepted that the sink regions, which depend on the import of assimilates for growth, play a central role in determining assimilate distribution patterns within the plant (120). Sink-mediated control of assimilate distribution may be achieved through the sink's ability to accumulate assimilates from the phloem (41).

The strawberry fruit is a strong sink for dry matter. Under favorable conditions a fruit may develop from anthesis through ripening in less than a month. In western Oregon the entire strawberry crop develops from bloom to harvest in about 3 months. At the end of this time over 40% of the plant's total dry matter is found in the fruit (91). During this period of fruit growth, fruit are able to out compete vegetative plant parts for dry matter (67, 76, 103).

Fruit size is an important yield component in strawberry, but often fruit do not appear to reach their full potential. Abbott et al. (1) have described shortfalls in yield of 50% or more

because of fruit failing to fully expand. The rate of assimilates imported into the fruit may be a process controlling fruit growth. The processes involved in importing assimilates into fruit tissue are not known. An understanding of these processes may lead to new ways of increasing growth and size of the strawberry fruit through genetic selection and/or chemical regulation.

The objectives of this study were to describe how fruit growth affects growth of the strawberry plant, to better understand the mechanism by which assimilates are transported into the strawberry fruit and to determine how this mechanism relates to fruit growth.

Chapter 2

REVIEW OF THE LITERATURE

Introduction

In horticulture the production of many fruits and vegetables depends on the translocation and partitioning of assimilates to these plant parts. In this review, the processes involved in assimilate translocation will be discussed. Many of these processes including translocation (46, 131), partitioning (49), phloem loading (46) and sugar uptake (73) have been recently reviewed. Therefore, a detailed description of all the work that has been done in these areas will not be given. Instead an attempt will be made to give an overview of our present understanding of these processes. The fruit's morphology, growth and composition as well as how fruiting affects plant growth will be discussed to understand how these processes operate in the strawberry.

Translocation and Partitioning of Assimilates

Importance to Crop Productivity

Plant growth rates are largely dependent on the plant's photosynthetic rates. Dry matter produced in the leaf through photosynthesis can remain in the leaf as structural or storage components or can be translocated to other parts of the plant. Agricultural

production is directly dependent on how much of this dry matter is translocated to the harvested portion of the plant, which is expressed as the harvest index. In the past, most increases in yield have not come from increased photosynthetic rates, but rather greater partitioning of the plant's dry matter into harvested organs (28). During domestication and improvement of most crop plants there has been no increase in the plants photosynthetic capabilities (49). In wheat the more primitive species have higher photosynthetic rates per unit leaf area than present cultivars (29). Improvement of wheat yields has come from increasing harvest indices, some reaching 50%. Austin et al. (7) suggests that further improvements may increase the harvest index as high as 62%.

In order to continue increasing the harvest index of cultivars, a better understanding of how assimilates are translocated is needed. Specifically, what controls the partitioning of these assimilates to harvested organs and how these processes are regulated may lead to greater productivity.

Phloem Translocation

Translocated Compounds

Many compounds are translocated in the phloem including sugars, sugar phosphates, organic acids, amino acids, nucleotide phosphates, hormones and mineral ions. Non-reducing sugars, however, are the largest fraction (16, 132). Zimmermann and Ziegler (133) have surveyed sugars and sugar alcohols found in phloem sieve-tubes of

over 500 species in about 100 dicotyledonous families. They found sucrose to be the major sugar translocated in the majority of these plants. Sizable amounts of sugars in the raffinose family of oligosaccharides, which include raffinose, stachyose and verbascose were found in some plants while in other plant families the sugar alcohols, mannitol, sorbitol, dulcitol and myo-inositol were also found in large quantities.

Mechanism of Translocation

Many mechanisms have been proposed to explain phloem translocation. Some of these include protoplasmic streaming (11), electroosmotic flow (107) and peristalsis of cell walls (34). Presently, however, pressure driven mass flow, which was first proposed by Münch, is the most widely supported mechanism for phloem translocation (47).

Through mass flow, sucrose and other phloem constituents are translocated by means of a passive bulk flow down a gradient of hydrostatic pressure (47, 86). The active loading of assimilates in the source and unloading in the sink produces a difference in osmotic potential in the sieve tubes in these regions resulting in the pressure gradient. The sieve elements along the path of translocation appear to have a passive role in translocation with the active processes being the loading and unloading of the phloem.

Phloem Loading

Phloem loading, recently reviewed by Giaquinta (46), is a process by which the major translocated sugars are selectively and actively transported into the phloem sieve element-companion cell complex prior to their export from the source region (38). The route in which assimilates are transported from the mesophyll to the sieve elements may be symplastic, through plasmodesmata, or apoplastic, through the free space (46). In sugar beet leaves evidence indicates an apoplastic loading of sucrose. Plasmodesmata connections between the mesophyll and companion cells or sieve elements are rare or absent (42). Geiger et al. (41) have shown that 20 mM sucrose fed to the apoplast of abraded sugar beet leaves was loaded and translocated at rates equal to those produced by leaves photosynthesizing $^{14}\text{CO}_2$. Fondy and Geiger (35) measured levels of ^{14}C -labeled sucrose in the free space of sugar beet leaves and found them to approach the activity of supplied $^{14}\text{CO}_2$. The use of the nonpermeable inhibitor p-chloromercuribenzenesulfonic acid (PCMB) inhibited phloem loading of exogenously supplied ^{14}C -sucrose as well as transport of ^{14}C -assimilates from leaves fed $^{14}\text{CO}_2$ suggesting an inhibition of a sucrose carrier on the plasmalemma (43). Finally, phloem has a substantially higher sugar concentration than surrounding mesophyll (40) indicating an active pumping mechanism which is difficult to envision as occurring via the plasmodesmata. In leaves of Cucurbita pepo, which translocates large amounts of stachyose and verbascose, Madore and Webb (81) did not find these sugars in the free space nor was exogenous stachyose loaded

into minor veins. They suggest that in C. pepo translocated sugars may be loaded symplastically.

Sucrose is loaded from the free space into the phloem via an active, carrier-mediated process (13, 44). It is believed plasmalemma bound ATPases pump H^+ from the sieve tube into the free space creating an electrochemical gradient. The 'downhill' movement of protons into the phloem is thought to be coupled with the transport of sucrose across the membrane (46). This active accumulation of sucrose in the sieve tube leads to osmotic uptake of water resulting in a hydrostatic pressure believed to drive mass flow in the phloem (49).

Phloem Unloading

The varying ability of sinks to remove sucrose from the phloem may determine how assimilates are partitioned within a plant (49). Although phloem unloading is important in determining economic yields in crops, the mechanism of this process is not well understood.

The path of unloading may be apoplastic or symplastic depending on the plant and the sink organ. In the developing seeds of corn (32), soybean (111) and wheat (69) no plasmodesmatal connections exist between the maternal tissue and the developing embryo and sucrose is unloaded into the apoplast. Apoplastic unloading has also been reported in the vegetative storage organs of sugar beet

(45) and sugar cane (51). In corn roots (48) and young sugar beet leaves (45) this process appears to be symplastic.

In attempts to study phloem unloading, sucrose efflux from the seed coats of soybean (112, 114), broad bean (127) and snap bean (94) was measured in the presence of various substances. Metabolic inhibitors and the sulfhydryl group modifier PCMBS inhibited efflux from seed coats. The rate of sucrose efflux from controls was similar to sucrose import rates into intact bean seeds, and efflux from the seed coat was believed to be a measure of phloem unloading. Wyse (131) has also shown that high concentrations of apoplastic sucrose and the inhibitor PCMBS both reduce sucrose efflux from stem segments of Vicia faba. This suggests that sucrose efflux is carrier mediated and energy dependent. Gifford and Evans (49) suggested that phloem unloading may occur by leakage wherever active uptake by the phloem ceases, or that unloading may actually be the loading system working in reverse.

The fact that apoplastic sucrose may inhibit apoplastic phloem unloading indicates that the rate of assimilate transport from the phloem in the sink may depend on the sink's ability to remove sucrose from the free space. Apoplastic sucrose concentrations can be maintained at low levels if sucrose is hydrolyzed to glucose and fructose by cell wall-bound invertase or sucrose is rapidly transported into sink cells (131). Cell wall-bound invertase is involved in phloem unloading in corn kernels (105) and in sugar cane internodes (51). In addition to lowering apoplastic sucrose concentrations, sucrose hydrolysis prevents reloading of the sucrose and

increases the efflux of water from the phloem by increasing the osmotic concentration in the apoplast (131). In other sinks such as sugar beet tap roots (45, 129) soybean cotyledons (111) and wheat ears (69) low apoplastic sucrose levels are maintained by rapid sucrose translocation into sink cells without hydrolysis.

Because phloem unloading often occurs down a concentration gradient of assimilates, this process may be a passive leakage. In a short review of loading and unloading, Ho and Baker (60) concluded that phloem unloading was not an energy-dependent process. They believe the rate limiting step for import into sink tissues is among the metabolic activities occurring beyond the initial unloading process and may not be the same in different sink organs. In the seed coats of soybeans, however, unloading of photosynthates was inhibited by metabolic inhibitors and cation chelators suggesting an active unloading system (114).

Sugar Uptake

Gifford and Evans (49) state that it is the ability of sinks to take up sugars from the sink free space which determines partitioning, not the relative adequacy of vascular connections or relative activity of various sources. Therefore to better understand partitioning, the mechanism of sugar uptake and what regulates this process must be understood.

In most tissues studied, sugar uptake is an active carrier mediated process. In sugar cane internodes, sucrose is hydrolyzed in the free space and glucose and fructose are actively taken

up (10, 51). Uptake of both of these sugars demonstrates saturation kinetics, which is sensitive to metabolic inhibitors, temperature and pH. Sucrose is taken up without hydrolysis in cotton hypocotyls (53), bean cotyledons (93) and sugar beet storage root (101, 130). Sucrose uptake in both cotton hypocotyls and bean cotyledons exhibits saturation kinetics and temperature dependence. Metabolic and penetrating sulfhydryl inhibitors decrease sucrose uptake in sugar beet. Sucrose uptake by the root tissue shows a biphasic dependence on sucrose concentration indicating the presence of two systems. The kinetics show both a saturable and a nonsaturable component similar to those seen in phloem loading (24) and sucrose uptake in soybean cotyledons (78). These kinetics may be explained by two forms of the putative sucrose carrier; one being a high affinity saturating type with the non-saturating form having a low affinity for sucrose (73).

The mechanism of sugar transport into sink cells appears to be a sugar proton co-transport system (73). Using sugarcane suspension cells, Komor et al. (74) demonstrated that hexose uptake was accompanied by proton uptake and K^+ efflux. A pH gradient across the plasmalemma was necessary for uptake and was abolished by uncouplers which also inhibited sugar uptake. An ATPase proton pump is believed to maintain an electrochemical gradient across the membrane (73). This sugar proton co-transport system has also been proposed to operate in maize scutellum (64), soybean cells (23) and Vinca protoplasts (109).

The concentration of sugars inside the sink cells affects the rate of sugar uptake (53). Various sinks are able to maintain low internal sugar levels by metabolism and/or compartmentalization. In sugar cane internodes, hexoses accumulated in the cytoplasm are rapidly converted to sucrose and stored in the vacuole (51). Dinar and Stevens (27) believe that sucrose levels in the tomato fruit regulate rates of sucrose uptake. The more rapidly sucrose was metabolized to starch or respired, the greater were the rates of uptake. In sugar beet roots, sucrose storage in the vacuole may limit sucrose import rates. Saftner and Wyse (101) have shown that sucrose uptake into the cytoplasm is passive with the active uptake occurring at the tonoplast. Because of the reverse electrochemical gradient present on the tonoplast compared with the plasmalemma (i.e., the H^+ concentration is lower in the vacuole than the cytoplasm), they proposed sucrose is transported into the vacuole with K^+ while a proton is released into the cytoplasm.

Although there have been many studies on sugar uptake into sink tissues, this process is still not well understood. If phloem unloading occurs through the apoplast, sugar uptake into sink cells may control the import of assimilates in sink tissue. With the present understanding of phloem unloading, it is not clear what the unloading pathway is. Furthermore, it appears that this system may differ in different plants, plant parts and even in the same plant part at different developmental stages. As a result, generalizations about the unloading mechanism cannot be made, and it appears

that phloem unloading must be studied in more detail and in different tissues before we can better understand this process.

Strawberry Fruit

Morphology

Inflorescence

The strawberry inflorescence (truss) is a dichasial cyme giving rise to a hierarchy of fruit (4, 20). The main axis terminates in a primary flower and 2 axes often branch from this giving rise to secondary flowers. Branching continues in this manner forming tertiary, quaternary and at times quinary flowers. A typical inflorescence would have 1 primary, 2 secondary, 4 tertiary and 8 quaternary flowers, however, inflorescences often vary from this norm. Darrow (19) described as many as 124 different types of inflorescences in a single strawberry cultivar.

Fruit

The strawberry is an aggregate fruit with achenes, small dry one seeded fruits, on the surface of a fleshy receptacle. The receptacle is composed of 5 zones: (1) epidermis, made up of polygonal cells; (2) hypodermis, consisting of meristematic cells; (3) a cortex composed of rounded parenchyma cells comprising most of the fleshy portion of the fruit; (4) bundle zone, comprised of a ring of vascular tissue with individual branches leading to each

achene; (5) pith, consisting of thin-walled cells which often separate during fruit growth leaving large cavities (125).

Fruit Growth and Composition

Fruit Development

In the strawberry fruit, cell division continues for about 7 days after petal fall after which fruit growth is primarily by cell expansion (71). Havis (56) has shown that the cortex grows most rapidly, whereas the pith grows at a slower rate, resulting in many larger fruit being hollow. During fruit development the cortex cells undergo considerable enlargement, reaching dimensions of 120 x 385 μm (110). When fruit are ripe, cells may separate from each other (90).

Increases in the fresh weight of strawberry fruit follow a sigmoidal curve with growth remaining logarithmic for the first 7 to 14 days after petal fall (71, 128). Crane and Baker (17) have observed that fruit diameter increases linearly throughout development.

During fruit development sugar accumulates logarithmically (71, 128). When fruit are ripe, they consist of 10% dry matter, half of which is free sugar (18, 104). Glucose and fructose account for most of this sugar with a lesser amount of sucrose being present. Selvaraj et al. (104) analyzed sugar content in 20 strawberry cultivars and found they averaged 3.1% glucose, 1.0% fructose and 0.6%

sucrose on a fresh weight basis. Ripe 'Benton' strawberries contain 1.8% glucose, 1.9% fructose and 0.5% sucrose (98).

Fruit Size

Strawberry fruit size is an important factor determining economic return of a strawberry crop. In addition to increasing tonnage, larger fruit bring a higher price and cost less to pick. Three factors involved in determining strawberry fruit size are the number of achenes on the fruit, the expansion of the receptacle relative to those achenes and the position of the fruit within an inflorescence.

The number of achenes or ovules a fruit has is determined at the time of flower initiation which occurs in the fall for 'June' bearing plants (1). The number of ovules that are fertilized and form viable achenes the following spring has been correlated with fruit size (68, 88). Abbott et al. (1) have reported that for a given achene spacing there is a linear relationship between achene number and fruit fresh weight. The number of achenes on a fruit decreases with inferior positions on the fruiting truss (37, 68, 88, 117, 124) but not at a consistent ratio between cultivars. Webb et al. (124) observed that quaternary fruit of the cultivar Grandee had over 60% the number of achenes counted on primary fruit. Whereas, quaternary fruit of other cultivars had only 30% of the primary fruit's achene number. Within a cultivar, Webb et al. (124) report the number of achenes on a fruit from a given inflorescence position is fairly constant.

In addition to the number of achenes per fruit, fruit size also depends on how these achenes are spaced. Abbott and Webb (2) proposed that 6 achenes/cm² is the maximum degree of expansion a strawberry can reach and any number greater than this represents a shortfall in size and yield. Receptacle expansion appears to be dependent on environmental conditions and management practices during the period from anthesis through ripening (124). Different cultivars experiencing the same environment also may differ greatly in the number of achenes/cm² on ripe fruit (91).

Fruit size decreases with inferior flower positions on the inflorescence (20, 37, 68, 88, 100, 106, 117). Part of this decline in fruit size can be explained by a corresponding decrease in the number of achenes, however, there also appears to be an apical dominance within the inflorescence. Fruit weight per achene decreased progressively in primary, secondary and tertiary fruit (88), causing a decrease in the size of lower order fruit. Janick and Eggert (68) increased the size of secondary fruit by removing primary fruit. Removal of secondary fruit, however, had no effect on the size of primary fruit. Similar results were obtained by Sachs and Izsak (100).

Effect of Fruiting on Plant Growth

Vegetative Growth

Fruiting decreases the growth of vegetative plant parts in the strawberry. The presence of developing fruit decreases root growth (76, 82), leaf area (14, 67), leaf number (67, 103) and runner

emergence (67, 103). Lenz and Bunemann (76) found a decrease in the growth of both roots and leaves with increasing numbers of fruit per plant. Plants with 16 fruit had roots and leaves weighing half as much as plants with no fruit.

Carbohydrate Content

Starch and sugar levels in strawberry roots and leaves decrease during fruiting. Mann (82) observed that starch stored in the roots was depleted during fruiting. In measuring starch and sugar levels in the plant throughout the season, Long and Murneek (80) also found the lowest levels occurred during fruiting. One day after fruit were removed, Hoffman and Lenz (61) reported 27% more leaf starch in plants without fruit than plants with fruit but no difference in the levels of soluble carbohydrate was found.

Carbon Assimilation

Fruiting has been shown to increase the rate of carbon assimilation in the strawberry plant. Unit leaf rates of fruiting plants were two fold greater than nonfruiting plants in the establishment year (91). Wolkowa et al. (126) found net photosynthesis rates were 30-40% higher in fruiting plants when compared to plants in which flower buds had not emerged. Net photosynthesis rates of fruiting 'Hecker' strawberry plants were also significantly greater than deblossomed plants (14). When fruit were removed 24 hr before measurements were made, Hoffmann and Lenz (61) found no difference in photosynthetic rates of plants with and without fruit.

Chapter 3

EFFECT OF FRUITING ON CARBON PARTITIONING
AND ASSIMILATION IN STRAWBERRY PLANTS

Abstract

Day-neutral strawberries (Fragaria X ananassa Duch. cv Brighton) were grown in a greenhouse and either continuously deblossomed or allowed to fruit over a 50 day period. Total plant dry weight was essentially the same in both treatments throughout this period. During a 25 day period the fruit was the predominant sink and for about 9 days fruit growth rate exceeded that of the whole plant and the growth rates of other plant parts were reduced to zero. At the end of the study, fruiting plants had accumulated 16 g dry weight in fruit and had 62%, 53% and 44% less dry matter in roots, crown and leaf blades than deblossomed plants. The starch content of roots, crown and leaf blades of deblossomed plants was about 18, 7 and 4 fold higher than plants with fruit. Fruiting reduced stolon, inflorescence and branched crown emergence by 80% or more. Leaf area was not significantly affected by treatments but at the end of the study was 15% higher in deblossomed plants. The specific leaf weight of these plants was as much as 44% higher than that of fruiting plants. Although fruiting had a small but non-significant effect on unit leaf rate, during the period of rapid fruit growth the net photosynthesis of leaves that had emerged simultaneously with the first inflorescence was 60-80% higher than

comparable leaves of deblossomed plants. Stomatal and mesophyll conductances of these leaves in fruiting plants were also about 40% larger than in deblossomed plants.

Introduction

The assimilation of dry matter through photosynthesis and its distribution within the plant are important processes determining crop productivity. The presence of plant organs with a net demand for dry matter (sinks) can strongly influence both of these processes (7). Sink activity and size largely determine the partitioning of assimilates within a plant and a large demand by sinks may also enhance photosynthetic activity of supply leaves (6).

The strawberry fruit is a strong sink for dry matter and has been shown to affect both its partitioning and assimilation. Fruit accumulate 40-50% of total plant dry matter (16, 22) resulting in a decrease in growth of vegetative plant parts (16). The rate of net assimilation was higher during the fruiting season than during plant establishment the previous summer (2). Similarly, net photosynthetic rates of fruiting plants were higher than those of deblossomed or nonfruiting plants (2, 27). The objective of this study was to determine the effect fruiting has on dry matter partitioning and carbon assimilation in the strawberry plant throughout fruit development.

Material and Methods

Plant material. On May 29, 1982 strawberry crowns, weighing 5-8g, of the day-neutral cultivar Brighton were planted in 3 liter pots containing a mix of soil: coarse sand: peat (1:1:1, by volume). Plants were grown in a greenhouse with 4 hr of supplemental lighting from high pressure sodium lamps ($175 \pm 25 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ 400-700 nm) in the morning and evening to maintain a 16 hr photoperiod. Temperatures were maintained at $30 \pm 5^\circ \text{C}$ during day and $18 \pm 2^\circ$ at night.

During the first 30 days after planting flower buds were continuously removed from all plants to permit establishment. Thereafter half of the plants were allowed to flower and fruit (fruiting plants) whereas flower bud removal continued in the other half (deblossomed plants). To ensure good fruit set, flowers were pollinated daily using a camel hair brush. Emerging stolons were removed throughout the experiment.

At 3 day intervals, from 30 to 80 days after planting, 4 plants were randomly chosen from each treatment and divided into roots, leaf blades, petioles, crowns (stems) and fruit. Fruit also included the pedicels and peduncles of the fruiting truss in addition to fruit and flowers. Most plant parts were weighed after drying at 80°C for 48 hr except ripening fruit which were frozen and freeze-dried. Total projected leaf area, which included bracts, was determined with a Li-Cor Model LI3100 Area Meter.

During the summer of 1983 the effect of deblossoming on starch accumulation and net photosynthesis was studied. Plants were grown under the conditions previously described. Eight plants each from the fruiting and deblossomed treatments were harvested at 50, 65 and 81 days after planting and the starch content of leaf blades determined. Starch content of root, crown and petiole was assayed at the 81 day harvest. Net photosynthesis was monitored on the middle leaflet of a single leaf of 8 plants from each treatment. The leaves selected for monitoring emerged simultaneously with the first inflorescences of fruiting plants at 35 ± 2 days after planting. Such leaves might be expected to have a strong source-sink relationship with that inflorescence.

Growth analysis. The patterns of dry weight change of whole plants and plant parts grown during 1982 were fitted to polynomial functions using regression analysis. Natural log transformations of total plant dry weight, organ dry weight and leaf area were fitted to polynomials of increasing degree up to a cubic function (14). Terms of higher order than cubic did not add significantly to any of the models. Relative growth rate ($\text{g dry weight} \cdot \text{g}^{-1} \text{ plant dry weight} \cdot \text{day}^{-1}$), unit leaf rate ($\text{g dry weight} \cdot \text{m}^{-2} \text{ leaf area} \cdot \text{day}^{-1}$) and absolute growth rate ($\text{g dry weight} \cdot \text{day}^{-1}$) were derived from the fitted functions for dry weight and area (14).

Starch analysis. Dried plant parts were ground to pass through a 40 mesh screen. Tissue samples weighing about 20 mg were extracted in 10 ml of boiling 80% (v/v) ethanol. Samples were cooled,

centrifuged at 2800 x g for 10 min and the supernatant decanted and discarded. After repeating the extraction twice, the pellet was dried at 50°C, suspended in 10 ml of 100 mM acetate buffer (pH 4.8) and boiled for 10 min. Starch was digested by adding 20 mg of amylogucosidase (Sigma) to each sample and incubating at 55° for 90 min (1). The supernatant was analysed for glucose using glucose oxidase, peroxidase and o-dianisidine (Sigma Technical Bulletin 510). Corn starch was used as a standard.

Photosynthesis measurements. Net photosynthesis was measured in the greenhouse using a CO₂ depletion technique similar to that described by Ehleringer and Cook (4). A 0.53 liter Plexiglas chamber, fitted with closed-cell foam gaskets, was placed around the leaflet. A small fan in the chamber mixed the air and reduced the leaf boundary layer. After the chamber was in place for 5 sec, a 10 ml sample of air was removed using a plastic syringe and a second sample was taken 30 sec later. Air samples were analyzed for CO₂ using a flow through infrared gas analyzer system (3). Net photosynthesis was calculated from the CO₂ depletion over 30 sec and from leaf area determined from tracings. Stomatal conductance was determined using a Li-Cor Model LI-1600 steady state porometer on the same leaflet immediately following the photosynthetic measurement. Mesophyll conductance was calculated according to Gaastra (5). Measurements were generally taken between 1000 and 1200 hr under natural light. On several cloudy days supplemental light (500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 400-700 nm) was used.

Results

Fruiting had a strong effect on dry matter partitioning in the strawberry plant. The fruit trusses accumulated an average of 16 g dry matter per plant which was 40% of the total plant dry weight by 80 days after planting (Fig. 3.1A). Dry matter accumulation in the fruit increased logarithmically from the time of first bloom (40 days after planting) until the first fruit began to ripen (55 days after planting), then the rate decreased. Between 70 to 80 days after planting there was little increase in fruit dry weight. During this time many primary and some secondary fruit were overripe and a large number of tertiary and quaternary fruit failed to develop.

Fruiting did not affect total plant dry weight over the 50 days (Fig. 3.1B), however it significantly decreased dry matter accumulation in roots, crowns, leaf blades and petioles (Fig. 3.1C, D, E and F). Dry matter in these plant parts increased very little between 55 and 80 days after planting in fruiting plants. At the final harvest, roots, crowns, leaf blades and petioles of fruiting plants weighed 62%, 53%, 44% and 44% less, respectively, than comparable plant parts of the deblossomed plants.

Curves representing the absolute growth rates of these plant parts were derived from the curves in Figure 3.1 (Fig. 3.2). From 50 to 75 days after planting the fruit was the predominant sink for dry matter (Fig. 3.2A). At the peak of fruit growth, between 60 and 70 days after planting, fruit growth equaled or exceeded total

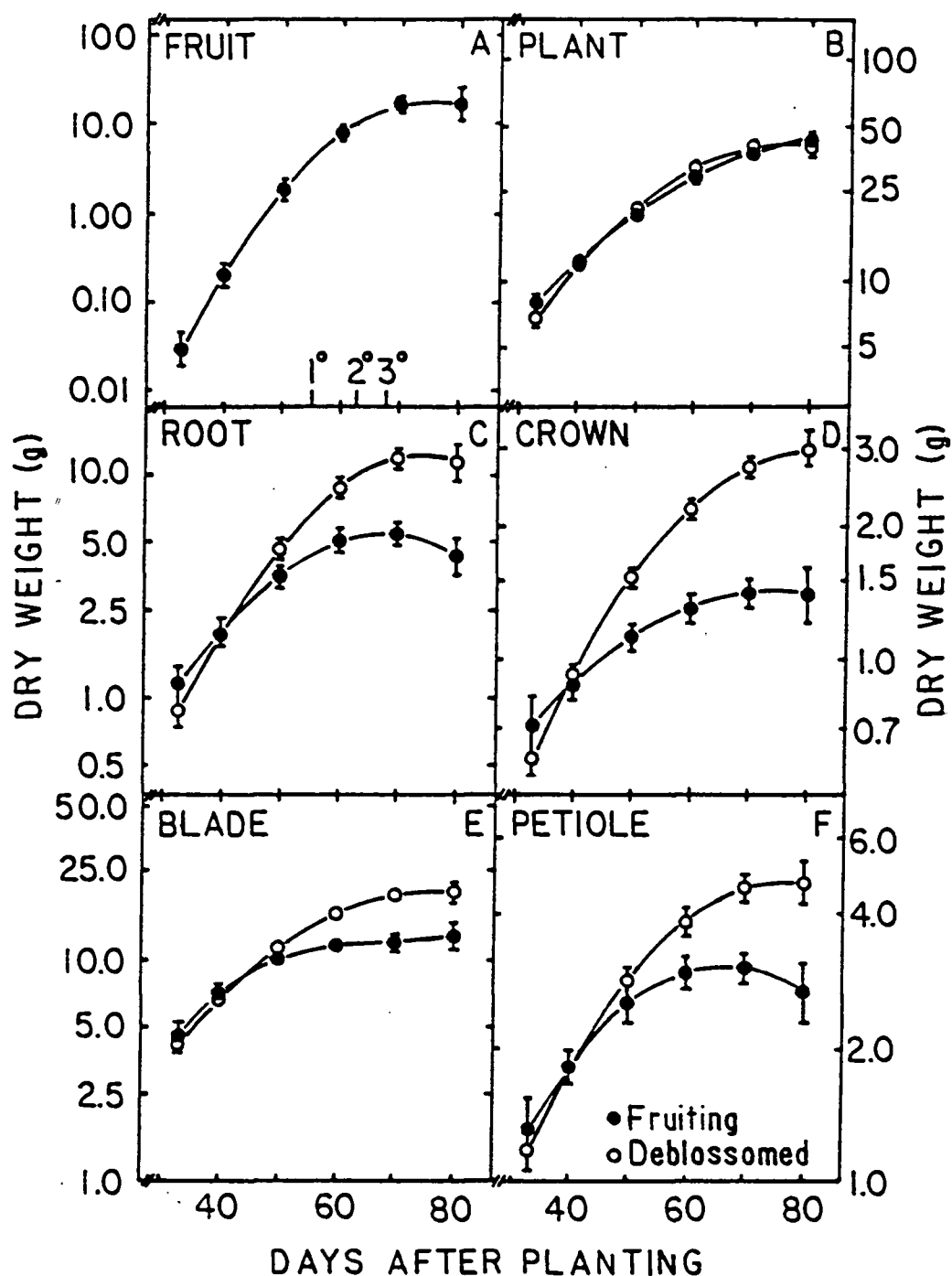


Fig. 3.1. Dry matter accumulation in fruit (A), whole plant (B), root (C), crown (D), leaf blade (E) and petiole (F) in fruiting and deblossomed strawberry plants. (Note Log Scales) Points are fitted values on the regression lines and error bars represent 95% confidence intervals. The time of first color change in primary (1), secondary (2) and tertiary (3) fruit is indicated in A.

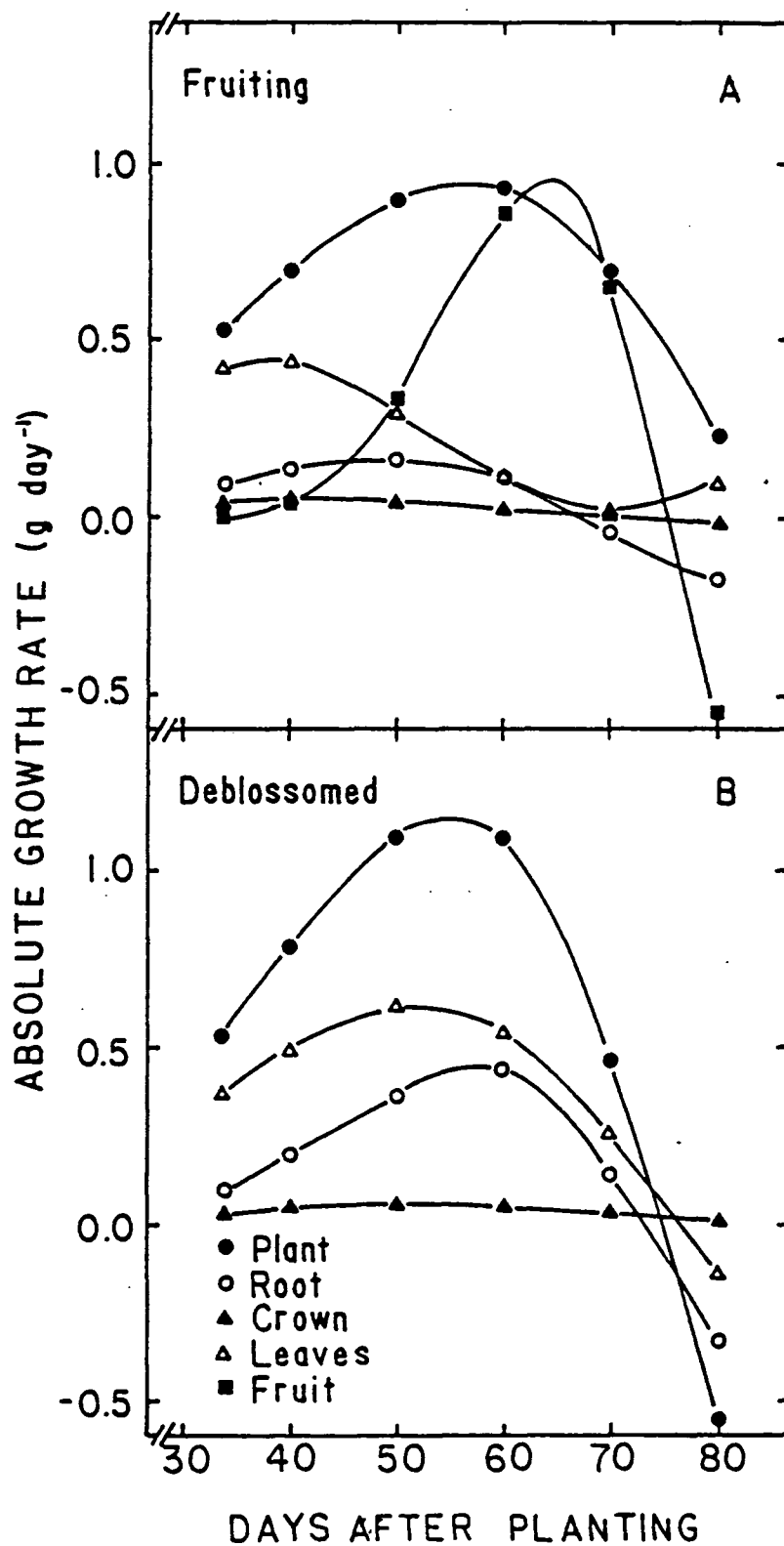


Fig.3.2. Absolute growth rates of whole plant, root, crown, leaves (leafblade and petiole) and fruit of fruiting (A) and deblossomed (B) strawberry plants.

plant growth. Growth rates of other plant parts in the fruiting plants were reduced to near zero during this time. When fruit growth was highest, growth rates of roots, leaves and crowns were 86%, 89% and 64% less in fruiting than deblossomed plants.

Relative growth rates (RGR) were derived from the curves of total dry matter accumulation (Fig. 3.3A). RGR of both fruiting and deblossomed plants decreased throughout the experiment, with that of deblossomed plants declining at a slightly faster rate.

The treatments did not significantly affect total leaf area per plant, although deblossomed plants tended to have greater leaf area near the end of the experiment (Fig. 3.4A). The number of leaves per plant increased at the rate of 0.15 leaves per day throughout the experiment in deblossomed plants. In fruiting plants this rate slowed and reached zero about 65 days after planting. At the end of the experiment deblossomed plants had an average of 8 more leaves than fruiting plants (34 vs 26). Specific leaf weight increased steadily in the deblossomed plants, but during rapid fruit growth in the fruiting plants there was no significant increase (Figure 3.4B).

Unit leaf rate, also known as net assimilation rate, was calculated from the functions of total plant dry weight and leaf area (Figure 3.3B). Because there was no significant difference between the 2 treatments in either plant dry weight or leaf area, the unit leaf rate curves cannot be considered significantly different. There was, however, a trend that the unit leaf rate of deblossomed plants decreased more rapidly than the fruiting plants.

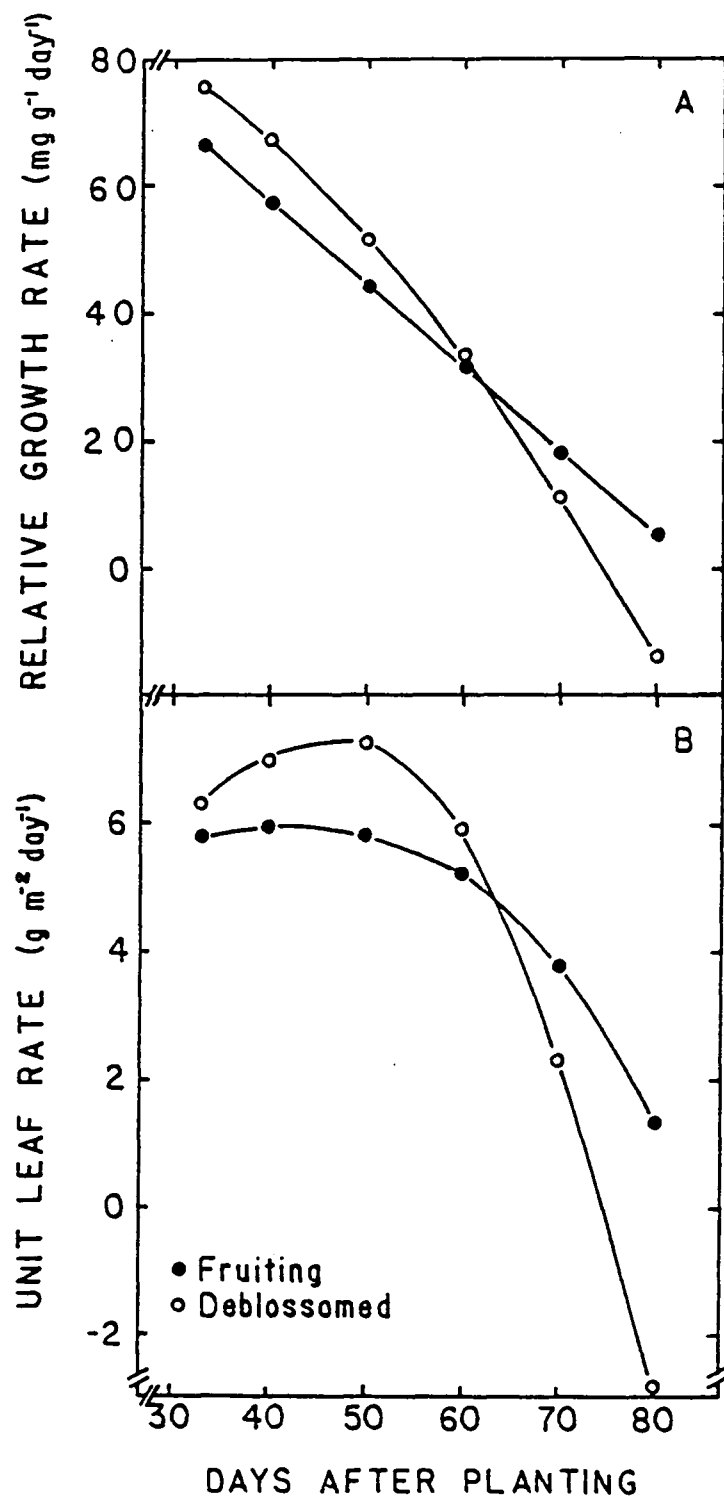


Fig. 3.3. Relative growth rate (A) and unit leaf rate (B) of fruiting and deblossomed strawberry plants.

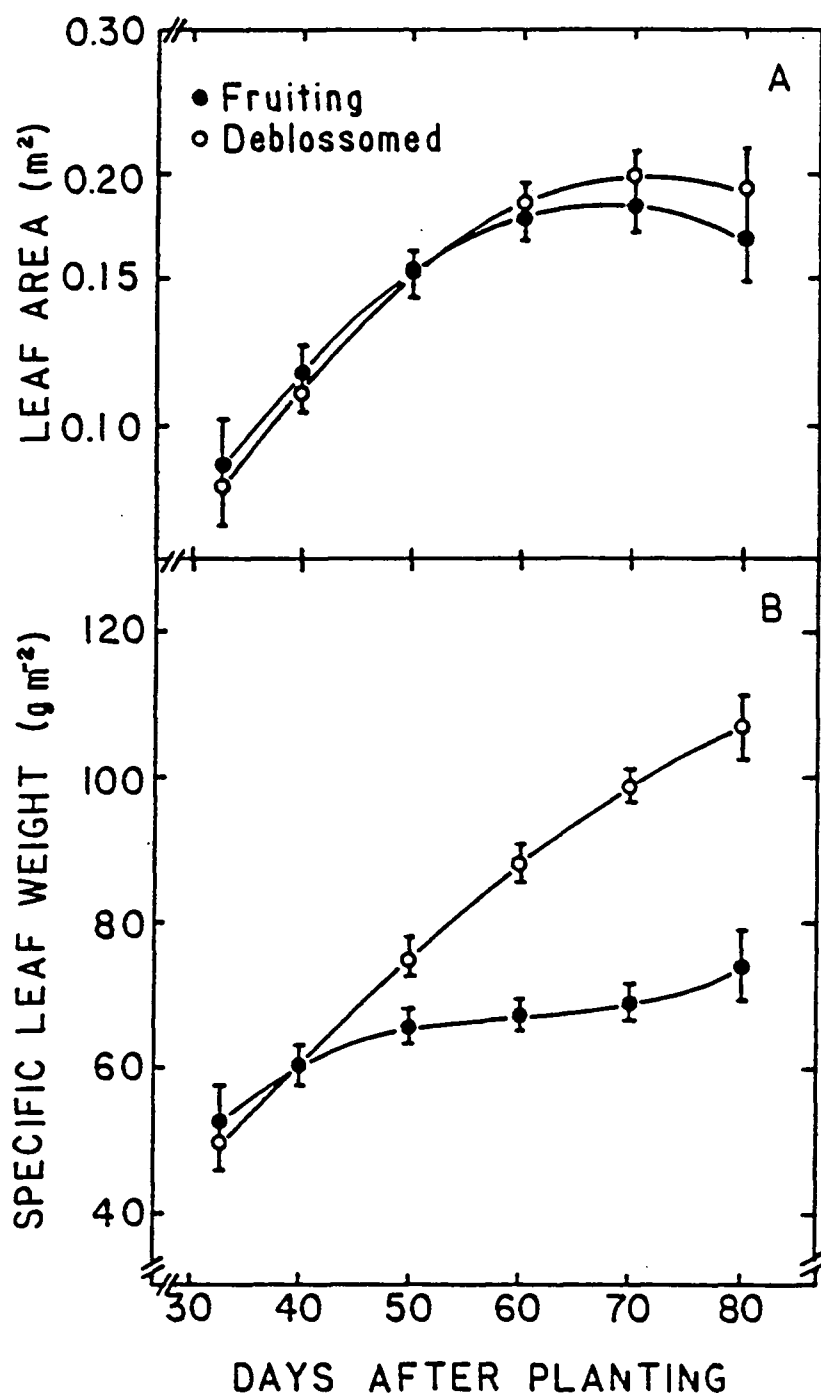


Fig. 3.4. Leaf area (A) and specific leaf weight (B) of fruiting and deblossomed strawberry plants. Points are fitted values on the regression lines and error bars represent 95% confidence intervals.

Fruiting affected stolon emergence, branch crown formation and flowering. Deblossomed plants produced stolons at a constant rate of 0.19 per day. Stolon formation nearly stopped in fruiting plants by 55 days after planting and few stolons emerged during fruit development. Branch crowns were initiated at a rate of 0.06 per day in deblossomed plants while there was no significant increase in the number of branch crowns in the fruiting plants. Fruiting also decreased the number of inflorescences formed. At the end of the experiment fruiting plants were producing only 0.04 inflorescences per day whereas deblossomed plants were continuing to form 0.3 per day, the rate maintained throughout the experiment.

Starch content in the various plant parts was measured in the 1983 experiment. Starch per unit leaf area accumulated in the leaf blades of deblossomed plants to values over 4 fold higher than those of blades from fruiting plants (Table 3.1). Specific leaf weight followed a similar but less dramatic pattern. Starch accounted for about 10% of the difference in specific leaf weight. At the end of the experiment starch levels in roots, crowns and petioles of deblossomed plants were 18-, 7- and 5- fold higher than in fruiting plants (Table 3.2), but the higher levels accounted for less than 8% of the difference in dry weight.

Net photosynthetic rates of leaves did not differ between treatments until 67 days after planting (Fig. 3.5). Thereafter net photosynthesis in fruiting plants remained 60-80% greater than deblossomed plants. Stomatal conductance in fruiting plants 79 days after planting was also significantly greater than in

Table 3.1. Starch content of strawberry leaf blades 50, 65 and 81 days after planting

Treatment	Starch ($\text{g}\cdot\text{m}^{-2}$)		
	Days after planting		
	50	65	81
Fruiting	0.198	0.275	0.878
Deblossomed	0.244	1.145*	3.30*

*Significant difference from fruiting treatment at 5% level.

Table 3.2. Starch content of strawberry plant parts 81 days after planting.

Treatment	Starch (mg.g ⁻¹)		
	Root	Crown	Petiole
Fruiting	1.2	1.8	2.9
Deblossomed	21.8*	12.1*	14.4*

*Significant difference from fruiting treatment at 5% level.

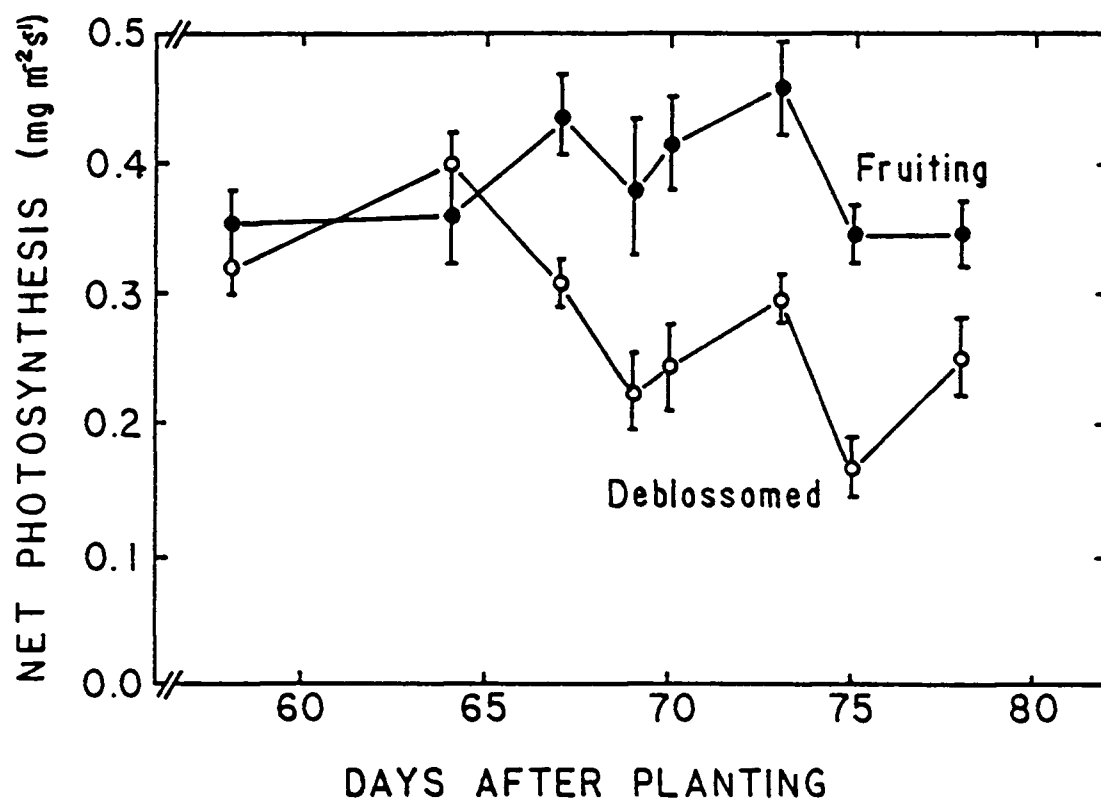


Fig. 3.5. Net photosynthesis of a single leaflet on fruiting and deblossomed strawberry plants. Error bars represent \pm standard error of the mean of 8 replicates.

deblossomed plants, 0.014 and 0.0096 $\text{m}\cdot\text{s}^{-1}$, respectively. Mesophyll conductance was calculated to be greater in fruiting than deblossomed plants (5.91×10^{-4} vs 4.31×10^{-4} $\text{m}\cdot\text{s}^{-1}$). Specific leaf weight and starch levels of the leaflet in which net photosynthesis was measured were 182% and 30% higher in deblossomed plants.

Discussion

During most of its development, the strawberry fruit was the dominant sink for dry matter in the plant. Other fruit such as apple (9), citrus (18) and pepper (8) have been shown to accumulate most of the plants' dry matter. During a 9 day period, 61-70 days after planting, the strawberry fruit accumulated more dry matter than the entire plant assimilated (Fig. 3.2A). This was probably accomplished by translocation of reserve carbohydrates from other plant parts as seen in potato (20) and wheat (26). During this period growth rates of the leaf blades and crowns approached zero, while roots and petioles actually lost dry matter. This decline in vegetative growth of strawberry plants due to fruiting was also reported by Lenz and Bunemann (17).

When strawberry fruit were absent because of deblossoming, no other plant part was able to dominate dry matter accumulation to the extent that fruit did. It has been suggested the ability of a sink to remove assimilates from the phloem determines how well it can compete with other sinks for dry matter (7). It appears that the mechanism of phloem unloading differs in different plant parts and the mechanism in the strawberry fruit may allow the fruit to be more competitive than other organs.

The strawberry root was a poor competitor for dry matter. In soybean, Huber (13) found that during the night root dry weight decreased while available assimilates derived from starch were accumulated in the stem.

The lower leaf growth rate observed in fruiting plants is attributed to reduced specific leaf weight since the difference in leaf area was not significant. Because of the demand for assimilates by the fruit, more dry matter was exported from the leaf blades. Ho (11) has shown that a large sink demand caused dry matter export to exceed assimilation in a single leaf in tomato. In the present study, specific leaf weight of fruiting plants did not increase significantly during rapid fruit growth indicating that most of the assimilated dry matter was exported. Starch can account for only about 10% of the increase in specific leaf weight in deblossomed plants. The other additional dry matter may have accumulated in cell walls, sugars, proteins or other leaf constituents.

Starch accumulation in deblossomed plants may indicate that vegetative plant growth was not adequate to utilize available assimilates. Mann (19) has shown that starch accumulated in strawberry plants as the dormant season approached and was depleted during fruiting. Thorne and Koller (24) found that increasing sink demand can reduce the starch content of soybean leaf by 10-fold in 8 days. Because roots of deblossomed plants filled pots, the size of the pots, even though they were 3 liters, may have limited plant growth and thus promoted starch accumulation. Pot-bound tobacco plants were shown to accumulate starch (10) and when sunflower root activity was reduced by flooding, starch accumulated in the leaves (25).

The observed stimulating effect of deblossoming on emergence of leaves, inflorescences, stolons and branched crowns agrees with previous reports of deblossoming increasing runnering and leaf number (15, 23). Because most previous work was done with 'June bearers' no effect has been reported on flower emergence.

The slightly lower unit leaf rate of deblossomed plants during rapid fruit growth was caused by a 15% greater leaf area in the nonfruiting plants. This trend corresponds with the greatly enhanced net photosynthetic rates of fruiting plants' leaves which emerged simultaneously with the first inflorescence and appear to have a strong source-sink relationship with the inflorescence. Other leaves on the plant may not have been as strongly affected by fruiting, therefore limiting the effect of fruiting on unit leaf rate. Photosynthesis was measured at midday when rates would be expected to be highest. At other times of the day differences may have been less, again being reflected in only slight differences in unit leaf rates.

The large demand for assimilates by fruit may have caused photosynthetic rates of the single leaf to be higher in fruiting than in deblossomed plants. Wolkowa et al. (27) found a greater net photosynthetic rate of fruiting strawberry plants when compared with plants in which flower buds had not emerged. Fruit may enhance photosynthetic rates by keeping stomates open as seen by larger stomatal conductance. Lenz (16) also observed higher stomatal conductance in fruiting eggplants and citrus when compared with plants in which fruit had been removed. Increased leaf starch in

deblossomed plants may have contributed to lower mesophyll conductance as reported by Nafziger and Koller (21) in soybean. Declines in net photosynthesis of deblossomed plant may in part be due to limitation of root growth by pots as was observed in tobacco (10). Differences in net photosynthetic rates between treatments may therefore be a result of both fruiting and limited root growth.

Choma et al. (2) reported that fruiting 'Hecker' strawberry plants had higher rates of net photosynthesis than deblossomed plants from full bloom through fruit maturation. The relatively small demand for assimilates caused by strawberry flowers would not appear to be large enough to cause enhanced photosynthetic rates. In the present study with 'Brighton' strawberry plants, differences in net photosynthesis were not seen until fruit maturation. It appears that changes in photosynthetic rates as a response to high assimilate demand by fruit is not immediate as seen by Hoffman and Lenz (12), but may take several days.

Choma et al. (2) reported significantly greater leaf area in deblossomed plants during the first week of the experiment. In the present study no significant difference in leaf area due to deblossoming was observed, and it was not until 40 days after treatments began that deblossomed plants showed a trend toward greater leaf area. Choma et al. (2) indicate that fruiting plants had gone through a fruiting cycle before the experiment, it is unclear whether subsequently deblossomed plants were allowed to fruit at this time. Differential treatment of plants before the experiment

may explain the greater photosynthetic rates and smaller leaf area of fruiting plants during flowering.

In conclusion, large assimilate demand from strawberry fruit growth strongly inhibited vegetative plant growth, both in terms of dry matter accumulation and emergence of new plant organs. Fruit were able to out compete other plant parts for assimilates. Understanding how this was accomplished may indicate ways to regulate partitioning for more efficient fruit production. Fruiting also enhanced net photosynthetic rates, but only during a period of maximum assimilate demand by the fruit. Limitations on root growth imposed by pots may have adversely affected net photosynthetic rates. Measurements of the effect of fruiting on photosynthesis under conditions where root volume is not limited, are needed to actually determine if fruiting affects photosynthesis in the field.

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Chapter 4

COLLECTION AND CHARACTERIZATION OF
PHLOEM EXUDATE FROM STRAWBERRY PEDICELS

Abstract

Fruit was removed from greenhouse grown strawberry plants and ethylenediaminetetraacetate (EDTA) used to collect phloem sap from cut pedicels attached to the plant. The rate of sugar exudation increased with the size of the fruit, but was lower than the rate of dry matter accumulation of the fruit at its removal. When plants were fed $^{14}\text{CO}_2$, radioactivity appeared in the phloem sap after 3 hr and the rate of exudation of labeled assimilate was maintained at a fairly constant rate for 6 hr. About 92% of the ^{14}C -activity in the exudate was in sucrose, with the remaining in glucose (2%), fructose (1%), and acidic and basic fractions (4%). The results indicate that sucrose is the major assimilate translocated to the fruit.

Introduction

An understanding of the chemical nature of organic compounds transported in the phloem may give some indications of the inter-relationship and nutrition of various plant parts. Sucrose forms the bulk of the translocated compounds in most plants although sugar alcohols and oligosaccharides of the raffinose type are also found in varying amounts (18). Determining the composition of the phloem sap arriving at the strawberry fruit would be helpful in understanding the growth and nutrition of the fruit.

When the phloem is cut, most plants fail to exude sap, in part by the blockage of sieve tubes by callose. King and Zeevaart (8) have demonstrated that chelating compounds, such as ethylenediamine-tetraacetate (EDTA), prevent callose formation and stimulate exudation of labeled assimilates from the phloem of detached leaves. This method has been used and modified by other workers to sample phloem sap from various plants and plant organs (1, 5, 15, 16). By using this technique, phloem exudate of photosynthetically derived translocate was collected from strawberry pedicels. This exudate was analyzed to determine the composition of assimilates arriving at the strawberry fruit.

Material and Methods

To determine the effect of EDTA on maintaining exudation in strawberry phloem, fruit at various developmental stages were removed from greenhouse grown 'Brighton' strawberry plants (Fragaria X ananassa Duch.) and pedicels were cut under 0, 5 or 10 mM EDTA solutions (pH 7.0). Pedicels were placed in 10 ml of the corresponding solution and phloem sap was collected for 6 hr. The solutions and sucrose standards were analyzed with anthrone (12). Fruit growth rate was determined by regression of the dry weights of 95 primary fruit of various ages.

In order to analyze phloem sap from current photosynthate, plants were reduced to one crown consisting of 3 fully expanded leaves and one fruiting truss. The following day plants were sealed in polyethylene bags and exposed to $^{14}\text{CO}_2$ (40 μCi) for 20 min. The fruiting truss was covered with aluminum foil to prevent fixation of label in fruit or pedicel tissues. Following exposure, primary and secondary fruit were removed and pedicels were cut under 5 mM EDTA (pH 7.0) and placed in 10 ml of EDTA solution. Every hour EDTA solutions were changed and their activity was determined by liquid scintillation counting. Activity in leaf blades was monitored every hour using a Geiger-Müller detector.

Phloem exudate was fractionated and analyzed according to Atkins and Canvin (3). Exudates were passed through coupled Dowex 50-X8 (H^+) and Dowex 1-X8 (formate) cation and anion exchange columns, resulting in a neutral fraction. Basic and acidic

fractions were eluted from the cation and anion resins, respectively, with 2N HCl. All fractions were taken to dryness under reduced pressure, the residue dissolved in a known volume of water, and radioactivity determined.

The neutral fraction was analyzed for individual sugars by descending paper chromatography. Known sugars and sugar alcohols were co-chromatographed with samples on Whatman No.1 filter paper and developed for 72 hr in either 2-butanone: acetic acid: boric acid saturated water (9:1:1) (11) or n-butanol: acetic acid: H₂O (12:3:5) (9). Radioactivity of individual sugars was measured by cutting 18 mm squares from the chromatograms and counting activity according to Wang and Jones (17).

Results and Discussion

Sugar from cut pedicels accumulated in both 5 and 10 mM EDTA solutions while only trace amounts were found in water (Fig. 4.1). The rate of exudation of sucrose equivalents from cut pedicels was also related to the rate of dry matter translocated into the fruit (growth rate) at the time it was removed. Pedicels cut from more rapidly growing fruit ($4.4 \text{ mg}\cdot\text{hr}^{-1}$) exuded 8 fold more sugar than those from slow growing fruit ($0.8 \text{ mg}\cdot\text{hr}^{-1}$). The maximum rate of exudation observed was only 25% of the removed fruit's growth rate, and most exudation rates were 10% or less. Dickson (4) found that whereas EDTA promoted exudation from bean leaves at rates similar to translocation in an intact plant, cottonwood leaves exuded only 6% of normal translocate. In the present study, as with cottonwood leaves, EDTA may not entirely prevent phloem blockage.

When leaves were fed $^{14}\text{CO}_2$, cut pedicels exuded labeled phloem sap continuously for the entire 10.5 hr of the experiment (Fig. 4.2). Appreciable amounts of labeled assimilates did not appear in the exudate until about 3 hr after $^{14}\text{CO}_2$ -feeding. The rate of radioactive exudation peaked an hour later and remained fairly constant thereafter. Radioactivity was exported from the leaves at a steady rate during the experiment (Fig. 4.2). Activity in leaves decreased 36% over the 12 hour time period, which was similar to that observed by Starck (14) and Antoszewski and Dzieciol (2) in fully expanded strawberry leaves.

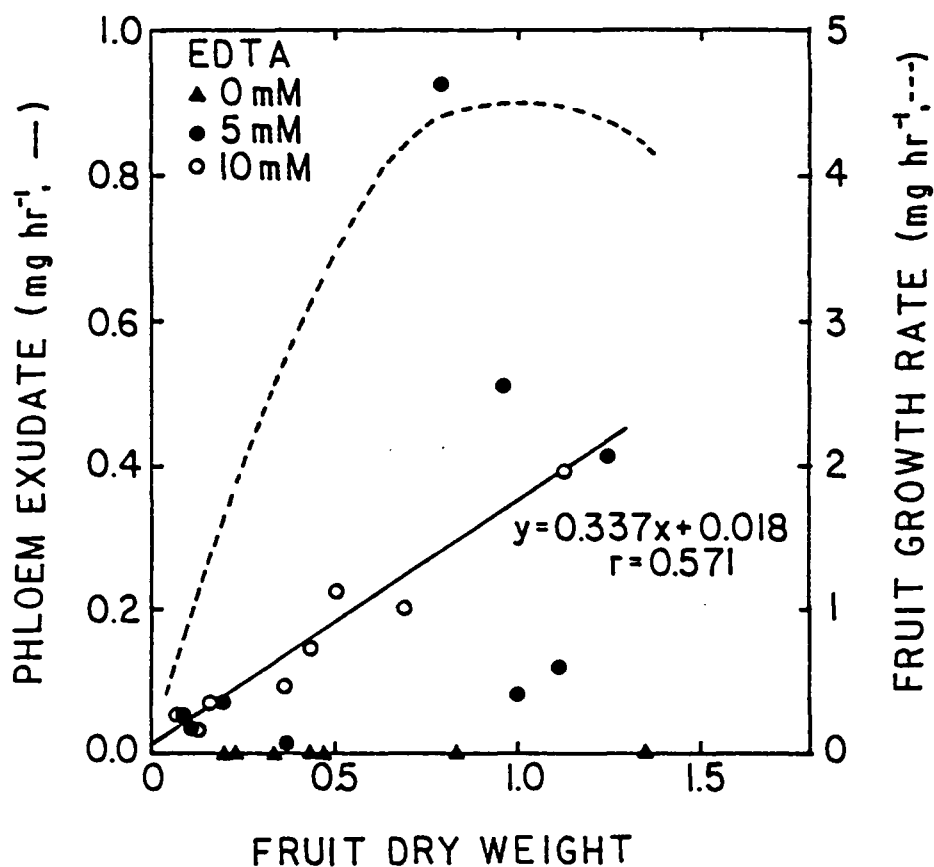


Fig. 4.1. The effect of fruit size on rate of phloem exudation expressed as sucrose equivalents into 0, 5, and 10 mM EDTA. The straight line represents the regression of phloem exudation rate into both 5 and 10 mM EDTA vs dry weight of the fruit at removal. The rate of primary fruit growth at various fruit sizes is plotted for comparison.

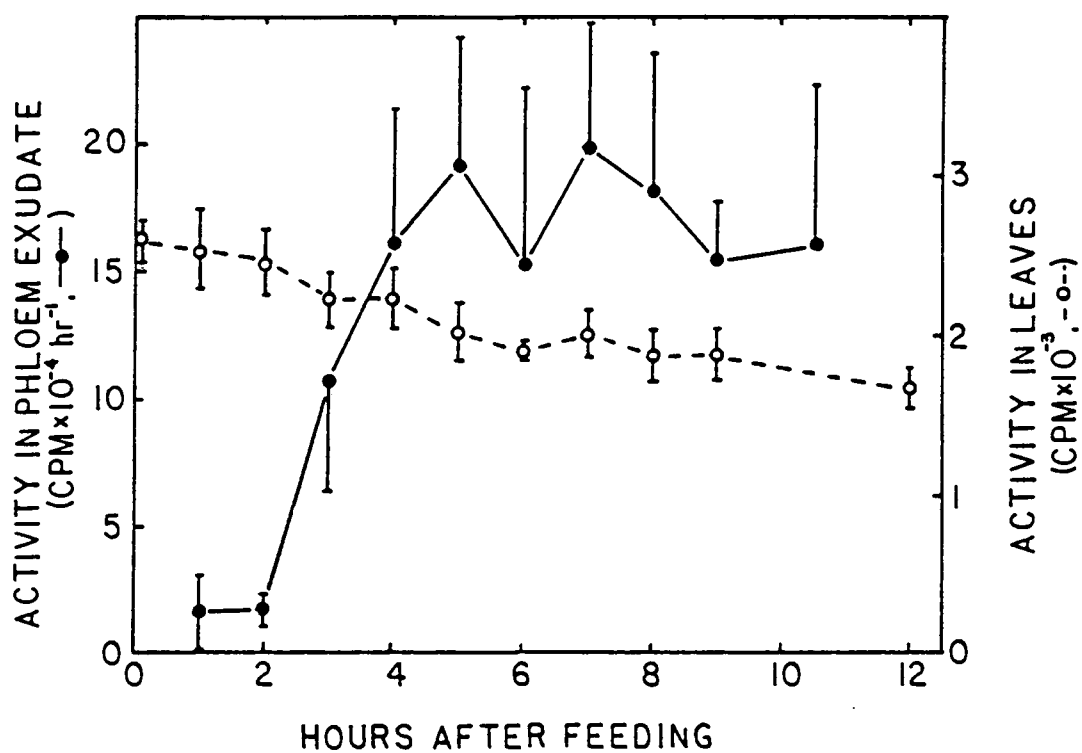


Fig.4.2. Rate of ^{14}C -assimilate exudation from strawberry pedicels into 5 mM EDTA and export of ^{14}C -assimilates from leaf blades. Values represent means of 3 replicates \pm standard error.

Table 4.1 shows the distribution of ^{14}C -activity in phloem exudate. The partitioning of activity in the exudate was the same at all sampling times. More than 96% of the label in the phloem exudate was found in the neutral fraction and about 95% of this was recovered as sucrose. Only 1-2% of activity in the neutral fraction was found in glucose and fructose. The presence of glucose and fructose may be an artifact from the breakdown of sucrose by invertase from injured cells and/or the free space during exudate collection (18). Although some species in the Rosaceae family translocate the sugar alcohol sorbitol, it has not been found in exudates from members of the subfamily Rosoideae, to which the strawberry belongs (Kluge, as cited by 18). Plouvier (10) failed to detect sorbitol in the stems and leaves of the wood strawberry (Fragaria vesca L.). In the present study no sugar alcohols or oligosaccharides were found in the phloem exudate. A small portion of the exudate was found in the acid and basic fractions. These fractions correspond to organic acids, sugar phosphates, amino acids and other charged compounds that have been shown to be translocated in the phloem (18).

The results presented here indicate sucrose is the major translocated assimilate arriving at the strawberry fruit. During fruit development, however, glucose and fructose are accumulated in greater amounts than sucrose (13). Sucrose arriving in the fruit must be hydrolyzed at some point. Cell wall bound invertase is active in the strawberry fruit (6) and has been shown to be involved in phloem unloading and sugar uptake in other plants (7).

Table 4.1. Distribution of radioactivity in phloem exudate collected in 5 mM EDTA from strawberry pedicels 6 hr after feeding plants $^{14}\text{CO}_2$.

Fraction	Distribution of activity (%) ^z
Acid	1.9 \pm 0.4
Basic	1.6 \pm 0.3
Neutral	96.5 \pm 0.7
Sucrose ^y	95.3 \pm 0.4
Glucose	1.1 \pm 0.1
Fructose	2.1 \pm 0.3

^z Each value represents mean of 3 replications \pm standard error

^y Percentages of neutral fraction

Determining how sucrose is unloaded from the phloem in the strawberry fruit may lead to a better understanding of fruit growth.

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Chapter 5

GROWTH AND SUGAR UPTAKE OF STRAWBERRY FRUIT
AT DIFFERENT INFLORESCENCE POSITIONS

Abstract

Fruit were harvested from greenhouse grown 'Brighton' plants and grouped according to their position on the inflorescence. Fruit growth was analyzed from anthesis through ripening. Absolute growth rates reached a maximum at the onset of ripening with primary fruit having the highest rates reaching $106 \text{ mg dry weight} \cdot \text{day}^{-1}$. Secondary, tertiary and quaternary fruit had maximum growth rates of 53, 22 and 3 $\text{mg dry weight} \cdot \text{day}^{-1}$, respectively. Fruit size measured as length, diameter, fresh weight or dry weight was greatest in primary fruit throughout development and decreased with fruit at lower rankings on the inflorescence. At 15 days after anthesis fresh weight of secondary, tertiary and quaternary fruit were, respectively, 55, 13 and 3% of primary fruit weight. The rate of fruit expansion, estimated from achene separation, was greatest in primary fruit and at the onset of ripening reached $0.93 \text{ mm}^2 \cdot \text{achene}^{-1} \text{ day}^{-1}$. Lower ranking fruit expanded more slowly and to a lesser extent than primary fruit. Relative growth rates were also highest in primary fruit from anthesis through red ripe. To relate fruit growth to rates of sucrose uptake, discs of fruit cortex tissue were incubated in $10 \text{ mM } ^{14}\text{C}$ -sucrose solutions. Sucrose uptake rates into tissue discs from primary, secondary and tertiary tissue decreased

parallel to their decline in the rate dry matter accumulated in the fruit. In primary fruit, sucrose uptake reflected dry matter accumulation at increasing ages, the rate of both activities decreasing 60% from 9-17 days after anthesis.

Introduction

The fruit is a strong sink for dry matter in the strawberry plant. During fruiting, 40% of the total dry weight of greenhouse (Chapter 3) or field grown plants (12) is in the fruit. The growth rate of a fruit largely determines its size, a characteristic important to yield and quality in the strawberry. The strawberry inflorescence is a cyme giving rise to a hierarchy of fruit decreasing in size with inferior blossom position (8, 18). Under normal growing conditions, fresh weight of well formed fruit is associated with 2 factors: the total number of achenes per fruit and the separation of achenes on the fruit surface (achenes per cm^2) (1). Within a cultivar, the number of achenes on a fruit at a given inflorescence position appears to be fairly constant, but declines with lower fruit ranking. Fruit expansion, measured in achenes per cm^2 , however, is more variable depending on fruit ranking (8) as well as growing conditions during fruit development (19).

One factor that may limit fruit growth, and contribute to the reduced size at lower rankings within an inflorescence, is the rate at which translocated assimilates are accumulated in fruit tissues. Sucrose was shown to be the major assimilate transported to the strawberry fruit (Chapter 4). The ability of a fruit to remove sucrose from the phloem may determine the partitioning of assimilates to that fruit. In an apoplastic unloading system, the ability of sink cells to remove sugar from the free space may be the limiting step of assimilate import (4). Similarly, a lower capacity

for sugar uptake might restrict the growth of strawberry fruit of inferior blossom positions.

In this study the growth of strawberry fruit from different positions on an inflorescence was compared throughout development. The accumulation of ^{14}C -sucrose by fruit cortex tissue was measured to determine if sucrose uptake was associated with the rate of fruit growth. A range of growth rates was obtained by using fruit at different developmental stages and inflorescence positions.

Material and Methods

Plant material. Crowns of 'Brighton' strawberry (*Fragaria* X *ananassa* Duch.), a day-neutral cultivar, were planted in 3 liter pots containing a mix of soil: coarse sand: peat (1:1:1, by volume). Plants were grown in a greenhouse during summer at $30 \pm 5^\circ \text{C}$ (day) and $18 \pm 2^\circ$ (night). Supplemental lighting from high pressure sodium lamps ($175 \pm 25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 400-700 nm) was provided in the morning and evening to maintain a 16 hr photoperiod. During the first 30 days after planting, flower buds were continuously removed to allow plant establishment. Thereafter plants were allowed to flower, and these were tagged and dated at anthesis. Flowers were pollinated with a camel hair brush to ensure good fruit set. Every 3 days, starting at first flowering and continuing for 36 days, fruit from the first 2 inflorescences to emerge of 4 randomly choosen plants were harvested. Fruit were grouped according to their position on the inflorescence. Calyxes were removed and fruit fresh weight, length, diameter (at the widest point) and achenes per cm^2 measured. Fruit dry weight was determined after drying at 80° for 48 hr, or after freeze drying.

Fruit growth analysis. Fruit length, diameter, fruit surface area per achene and natural log transformations of fresh weight and dry weight were fitted to polynomial functions of increasing degree up to a cubic function using regression analysis (6, 7). Terms of higher order than cubic did not add significantly to any of the models. Fruit growth rates ($\text{g dry weight}\cdot\text{day}^{-1}$) and relative growth

rates ($\text{g dry weight} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{day}^{-1}$) were derived from fitted functions for dry weight (14). Rate of fruit expansion ($\text{mm}^2 \text{ achene}^{-1} \cdot \text{day}^{-1}$) was calculated from the fitted function for fruit surface area per achene, and the rate of dry matter accumulation ($\text{mg dry weight} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$) was derived from functions of fruit fresh and dry weight.

Sucrose uptake. Tissue discs (7 mm diameter) were cut from fruit cortex with a cork borer and razor blade using a Plexiglas jig to obtain a uniform thickness of 1.3 mm. Discs were pooled in 25 ml of solution containing 10 mM sucrose, 50 mM CaCl_2 and 20 mM citrate buffer (pH 5.0) and then incubated 30 min at 25°C and 80 rotations per minute in a gyratory water bath. Ten discs (0.52g fresh weight) were placed into 5 ml of incubation solution containing ^{14}C -sucrose ($20 \text{ nCi} \cdot \text{mmol}^{-1}$) for 2 hr, then rinsed for 30 min in ice-cold unlabeled solution to remove activity from the free space. Radioactivity remaining in discs was used as a measure of sucrose uptake and was determined by liquid scintillation counting after combustion in a Packard Oxidizer.

Results and Discussion

Sink strength of a strawberry fruit can be expressed as the fruit growth rate (dry matter accumulated per unit time). The change of the growth rates over time of primary, secondary, tertiary and quaternary berries is seen in Figure 5.1A. The growth rate of all fruit increased from anthesis until it peaked during ripening and then began to decline. Negative growth rates were observed as fruit became over ripe. Primary fruit had the greatest growth rate which peaked 19 days after anthesis at $106 \text{ mg} \cdot \text{day}^{-1}$. Rates declined with fruit of inferior position (Table 5.1, Fig. 5.1A). Some tertiary and most quaternary fruit failed to expand.

The growth rate of individual fruit was divided into 2 multiplicative components, fruit size and fruit activity. Size was measured as length, diameter or weight. Activity was expressed as rate of relative expansion ($\text{mm}^2 \cdot \text{achene}^{-1} \cdot \text{day}^{-1}$) or relative growth rate ($\text{g dry weight} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{day}^{-1}$). Both size and activity of fruit were studied to understand the differences in fruit growth rate.

Diameter of fruit increased linearly until fruit began to ripen (Fig. 5.2A). The increase slowed and then stopped as the fruit became red ripe. A linear increase in fruit diameter was also observed by Crane and Baker (3). Throughout fruit development primary fruit of 'Brighton' had a greater diameter than fruit at lower rankings (Table 5.1, Fig. 5.2A). Fruit length followed the same pattern (data not shown). Fresh weight increased

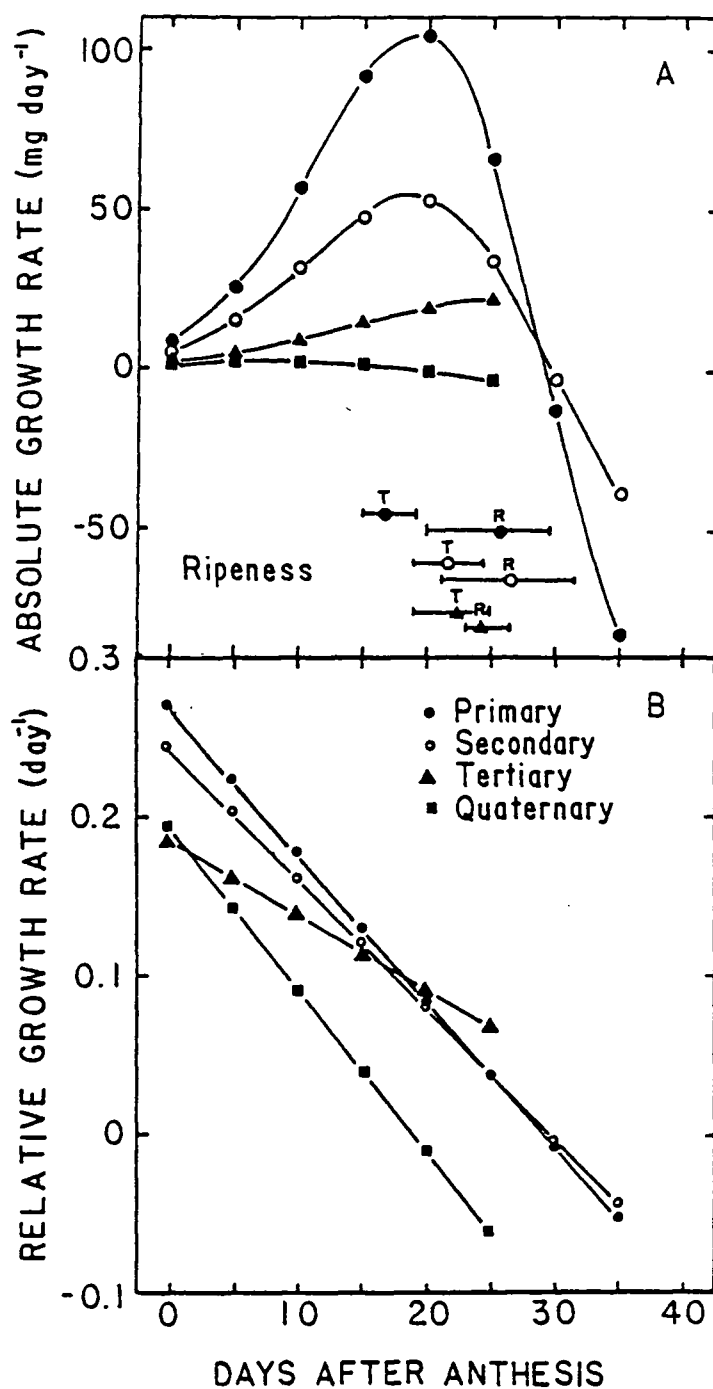


Fig. 5.1. Absolute growth rate (A) and relative growth rate (B) of 'Brighton' strawberry fruit. Points adjacent to 'Ripeness' indicate the mean date of color turning (T) and full red ripe (R) with bars representing the range of dates.

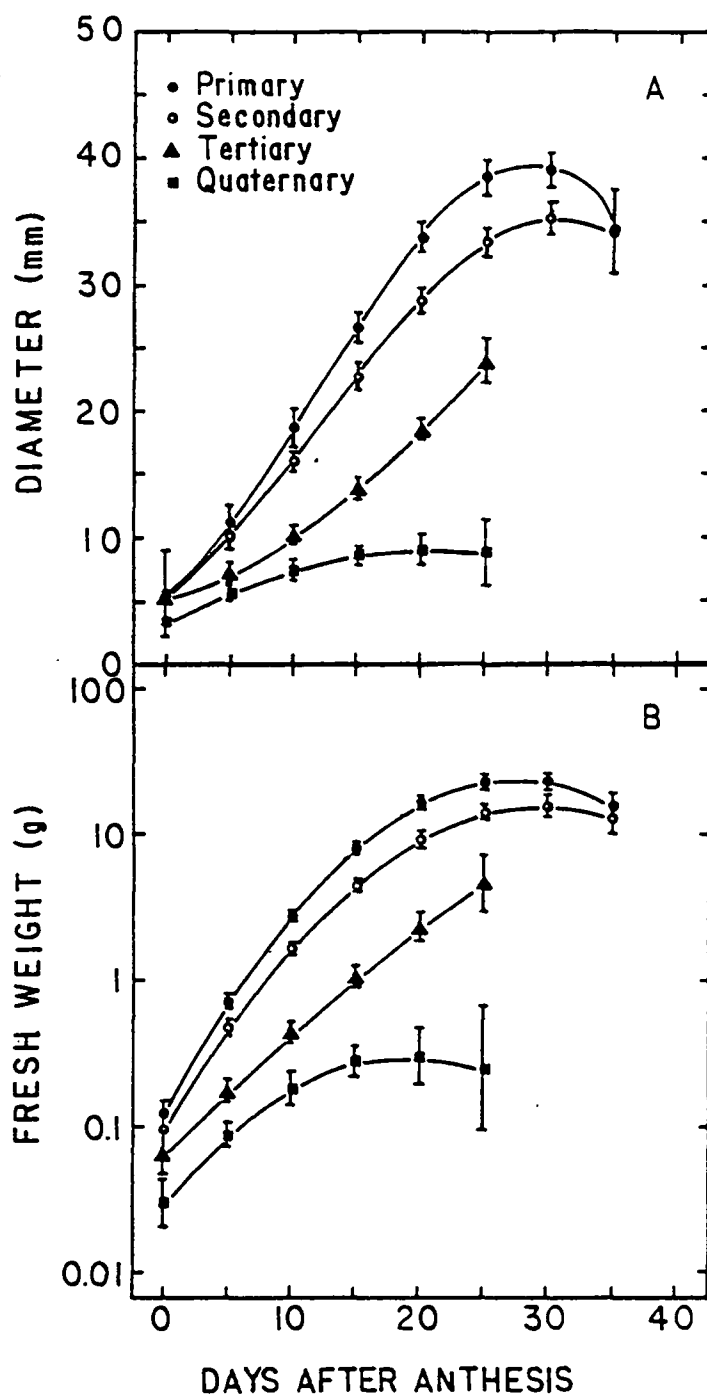


Fig. 5.2. Diameter (A) and fresh weight (B) of 'Brighton' strawberry fruit throughout development. Curves represent fitted regression curves, points are fitted values on the curves and error bars are 95% confidence intervals around the curves.

logarithmically for the first 10 days after anthesis (Fig. 5.2 B). Ripening slowed weight gains and no increases in weight occurred after fruit became red ripe. 'Red Gauntlet' and 'Prize Winner' both showed patterns of fresh weight gain similar to 'Brighton' (9,20). As 'Brighton' fruit became over ripe, 30 to 35 days after anthesis, a slight drop in fresh weight was observed. This decline may have resulted from the increased rate of water loss associated with ripening (17). The gain in dry weight followed a pattern similar to fresh weight, but the rate of weight increase was not as great (data not shown). As seen with diameter, fresh and dry weight of primary fruit were about 45% larger than secondary fruit (Table 5.1). This follows a trend similar to that reported by Sherman and Janick (16) in which the weight of 1 primary fruit was equal to 2 secondary or 4 tertiary fruits.

Fruit activity decreased with inferior blossom position. Fruit expansion, measured in $\text{mm}^2 \cdot \text{achene}^{-1}$, the inverse of $\text{achene} \cdot \text{cm}^{-2} \times 100$, was greatest in primary fruit (Fig. 5.3A). Maximum expansion values, which occurred when fruit were red ripe, were 10.4, 7.1 and 5.4 $\text{mm}^2 \cdot \text{achene}^{-1}$ for primary, secondary and tertiary fruit, respectively. Abbott et al. (1) proposed that the equivalent of 17 $\text{mm}^2 \cdot \text{achene}^{-1}$ (6 achenes $\cdot \text{cm}^{-2}$) is the maximum expansion a fruit can reach and this value can be used to determine the amount of shortfall in fruit size. In the present study primary, secondary and tertiary fruit reached 62, 43 and 33%, respectively, of their 'potential' size. Primary fruit expanded faster than fruit at lower rankings (Table 5.1), peaking at the time of red color formation and

Table 5.1. 'Brighton' strawberry fruit growth rate, diameter, fresh weight, dry weight, rate of fruit expansion and relative growth rate (RGR) 15 days after anthesis.

Fruit position	Percent of primary fruit					RGR
	Growth rate	Diameter	Fresh weight	Dry weight	Rate of expansion	
Secondary	52	85	55	56	46	92
Tertiary	16	52	13	18	27	86
Quaternary	2	32	3	6	--	32

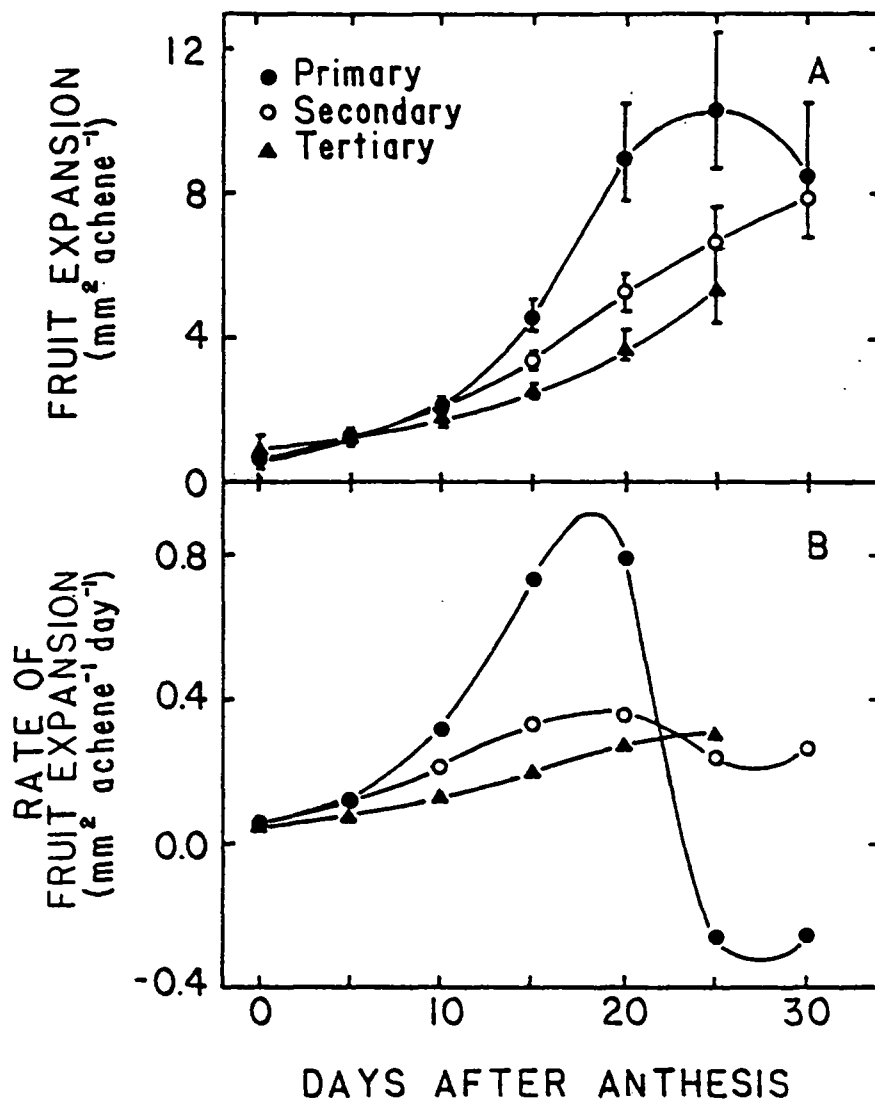


Fig. 5.3. Fruit expansion (A) and rate of fruit expansion (B) of 'Brighton' strawberry fruit throughout development. Curves representing fruit expansion are fitted regression lines, points are fitted values and error bars are 95% confidence intervals around the curves. Curves representing rates of fruit expansion are derivatives of those in A.

declining during ripening (Fig. 5.3B). Janick and Eggert (8) also measured a decrease in the rate of fruit expansion, measured as fresh weight per achene, with inferior blossom position.

Relative growth rate of fruit decreased linearly throughout development as less of the fruit weight was involved in dry matter import and more was tied up in structural and storage components of the fruit (Fig. 5.1B). Relative growth rate was greatest in primary fruit through the time of red ripe and decreased at inferior fruit rankings (Table 5.1). Relative growth rate of tertiary fruit decreased more slowly than other fruit. The development of tertiary fruit was variable, with the first to bloom developing fully whereas later fruit grew more slowly and some failed to expand past anthesis. The reduced competition resulting as primary and secondary fruit ripened and stopped growing, may have helped to maintain the relative growth rate of these slowly growing fruit.

Sucrose uptake into the strawberry fruit cortex tissue may be a process limiting growth of the fruit and a determinant of the competitiveness of fruit within an inflorescence. To determine if sucrose uptake was related to dry matter accumulation in the fruit, tissue discs were cut from primary, secondary and tertiary fruit that were all at the same developmental stage, a day or two before color change. Sucrose uptake rates were greatest in discs from primary fruit (Table 5.2). The lower rates observed with secondary and tertiary fruit reflected the lower rates of dry matter accumulation in these fruit. Lis and Antoszewski (10) observed that primary fruit accumulated twice as much ^{14}C -sucrose and $^{32}\text{P}\text{O}_4^{-3}$ fed

Table 5.2. ^{14}C -sucrose uptake from 10 mM sucrose solutions into 'Brighton' strawberry fruit tissue discs and dry matter accumulation in primary, secondary and tertiary fruit at the same stage of development. Sucrose uptake rates are the means of 3 replicates and dry matter accumulation rates are from regression curves of the corresponding fruit.

Fruit position	Sucrose uptake ²		Dry matter accumulation	
	(mg·g ⁻¹ FW·hr ⁻¹)	%	(mg·g ⁻¹ FW·hr ⁻¹)	%
Primary	0.201 a	100	0.471	100
Secondary	0.159 b	79	0.334	71
Tertiary	0.154 b	77	0.316	67

²Mean separation by Duncan's multiple range test, 5% level

to leaves as secondary fruit. In the present study absolute rates of sucrose uptake in tissue discs were less than half the rates of dry matter accumulation observed in the intact fruit. Sucrose uptake into discs increases with higher sucrose concentrations in the bathing solution (Chapter 6). Rates of uptake in the present study were measured at 10 mM sucrose, but at 50 mM sucrose the observed uptake rate of $0.468 \text{ mg} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$ in primary fruit equaled the growth of intact fruit. Although sucrose levels in the strawberry fruit free space have not been measured, a concentration of 50 mM would not appear to be excessive since the concentrations in other sink tissues range from 8-60 mM (5, 15).

Sucrose uptake by discs from primary fruit ranging from 9 to 17 days old was also measured (Table 5.3). From 9 to 15 days after anthesis sucrose uptake rates decreased, reflecting to the decline in the rate of dry matter accumulation in intact fruit. A slight increase in the rate of uptake, however, was observed in fruit tissue 17 days after anthesis which did not correspond to the decrease in fruit growth. At this time fruit were beginning to turn color and rapidly soften. A rise in apparent sucrose uptake may have been caused by an increase in membrane permeability because of injury and/or rapid senescence of the tissue. Sorbitol uptake into apple tissue follows a similar pattern during fruit development, decreasing with fruit age but leveling off and then increasing slightly with ripening (2).

The observed association between sucrose uptake and strawberry fruit growth may indicate this process limits fruit growth, however,

Table 5.3. ^{14}C -sucrose uptake from 10 mM sucrose solutions into 'Brighton' strawberry fruit tissue discs and dry matter accumulation in primary fruit 9 to 17 days after anthesis. Sucrose uptake rates are the means of 3 replicates and dry matter accumulation rates are from regression curves of primary fruit.

Fruit age	Sucrose uptake ^z		Dry matter accumulation	
	(mg.g ⁻¹ FW.hr ⁻¹)	%	(mg.g ⁻¹ FW.hr ⁻¹)	%
9	0.457 a	100	0.938	100
13	0.229 b	50	0.589	63
15	0.172 c	38	0.470	50
17	0.183 c	40	0.375	40

^zMean separation by Duncan's multiple range test, 5% level

it is not possible to specify the cause and effect relationship. In an apoplastic route for phloem unloading, sugar uptake into sink cells could control fruit growth and expansion. In sugar cane, higher rates of sucrose uptake into internode tissue were associated with higher rates of translocation to the internode (11) as well as higher sucrose content (13). A better understanding of sugar uptake and phloem unloading may be helpful in interpreting the performance of strawberry fruit at different inflorescence positions.

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Chapter 6

SUGAR UPTAKE IN THE STRAWBERRY FRUIT

Abstract

Tissue discs cut from 'Brighton' strawberry fruit 15-17 days after anthesis were incubated in ^{14}C -labeled sucrose, glucose or fructose. The presence of CaCl_2 in the incubation solution was necessary to maintain respiration of fruit tissue. Sucrose uptake into tissue discs was nearly constant over 4 hr, and had a pH optimum of 5.0. Total ^{14}C -sucrose uptake was greatest at 40°C , although incorporation of label into the insoluble fraction peaked at 30° . The metabolic inhibitors NaCN (5 mM), dinitrophenol (DNP, 3 mM) and CCCP (100 μM) stimulated sucrose uptake 34, 94 and 54%, respectively. Uptake of sucrose was inhibited by 10^{-5}M DNP (36%) 5 mM glucose (16%) and N_2 (16%). Kinetic analysis of sucrose uptake revealed both a linear component with a first order rate coefficient (k) of $0.018 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1} \cdot \text{mmol}^{-1}$ and a saturable component having a V_{max} of $0.65 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$ and a K_M of 12 mM. The kinetic characteristics of fructose uptake were similar to those of sucrose. Glucose, however, was taken up much more rapidly than either sucrose or fructose and only demonstrated saturation kinetics, showing a V_{max} of $8.3 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$ and K_M of 88 mM. To determine if sucrose was hydrolyzed in the free space before being accumulated, tissue discs were incubated in (^{14}C -fructosyl)sucrose, ($\text{U-}^{14}\text{C}$)sucrose, ^{14}C -fructose or ^{14}C -glucose

and distribution of ^{14}C in metabolite fractions measured. After 2 hr over 50% of the label was found in glucose or fructose. The distribution of ^{14}C between glucose and fructose moieties of sucrose isolated from tissue fed (^{14}C -fructosyl)sucrose indicated that a considerable portion of the sucrose recovered underwent hydrolysis and randomization. Eight hr after labeled sugars were fed to abraded leaves, sucrose isolated from fruit had been randomized, indicating sucrose had undergone hydrolysis, possibly before entering the fruit tissue.

Introduction

The process of phloem unloading and the subsequent movement of sugars into sink cells appears to be important in controlling the availability of metabolites needed to maintain sink growth. Identification of the pathway and mechanism by which sugars are accumulated by sink organs may point to ways of increasing and/or regulating partitioning of dry matter to economically important sinks.

Transport of sugars from the phloem to individual sink cells may be symplastic, through plasmodesmata, or apoplastic, in which sugars pass through the free space. Both of these pathways have been shown to occur in different plants and plant organs. Sugars move via the symplast in roots (5, 9) and immature organs (8) whereas an apoplastic system may operate in reproductive organs (7, 23) and vegetative storage organs (8, 11, 25). In plant tissues having apoplastic phloem unloading, sugar uptake is, at least in part, an active carrier-mediated process (11, 18, 25).

In the strawberry, as well as other fleshy fruit, little is known of the processes involved in the accumulation of sugars. Sucrose has been shown to be the major translocated sugar in the strawberry plant (Chapter 4), and rates of sucrose uptake in strawberry fruit cortex tissue correlate with fruit growth rates (Chapter 5). This study investigates the means by which sucrose is taken up from the free space of the strawberry fruit cortex and how this

process relates to phloem unloading and sugar accumulation in intact fruit.

Material and Methods

Plant material. Day neutral strawberry plants (Fragaria X ananassa Duch. cv Brighton) were grown in a greenhouse under a 16 hr photoperiod. Unripe primary fruit at 15-17 days post anthesis were used in all experiments. Using a cork borer, a 7 mm diameter plug of tissue was removed from the fruit cortex and cut into 1.3 mm thick discs with a razor blade and Plexiglas jig. Discs were pooled in a buffer solution and allowed to equilibrate for 30 min at 25°C and constant agitation.

Tissue incubation. Ten discs (0.52 g fresh weight) were placed in 5 ml of solution in a 50 ml Erlenmeyer flask and incubated at 25°C and 80 rotations per min in a gyratory water bath shaker. The solution, unless otherwise noted, contained 10 mM sucrose, 50 mM CaCl₂ and 20 mM citrate buffer (pH 5.0).

To measure tissue respiration, flasks were sealed with serum caps and the internal atmosphere sampled immediately and again after 60 min. Air samples (2 ml) were analyzed for CO₂ using a flow through infrared gas analyzer system (3). Between measurements flasks were flushed with air and resealed.

Sugar uptake into tissue discs was determined by incubating discs in ¹⁴C-labeled sugars (20 nCi/mmol) for 2 hr followed by a 30 min rinse in 4 changes of ice-cold unlabeled solution to remove labeled sugar from the free space. Rinsed discs were extracted overnight in 80% ethanol at 80°C. The ethanol soluble fraction was dried at 50°, taken up into 1 ml of H₂O and radioactivity determined

by liquid scintillation spectroscopy. The insoluble fraction was combusted in a Packard Oxidizer and assayed. Kinetic analysis was performed using Lineweaver-Burk plots. Where two components were present, the first order rate coefficient k was calculated as the slope of the linear regression equation for points on the total uptake curve at or above 25 mM substrate concentrations. The contribution of the linear component to total uptake was estimated as k multiplied by the substrate concentration at each point. The linear component was subtracted from total uptake to give rates due to the saturable component.

Distribution of ^{14}C -labeled metabolites. Strawberry fruit tissue discs were incubated 2 hr in solutions containing 10 mM ($\text{U-}^{14}\text{C}$) sucrose, (^{14}C -fructosyl) sucrose, ^{14}C -fructose or ^{14}C -glucose. Discs were separated into soluble and insoluble fractions as described previously. Soluble fractions were passed through coupled Dowex 50-X8 (H^+) and Dowex 1-X8 (formate) cation and anion exchange columns, resulting in a neutral fraction (1). Basic and acidic fractions were eluted from the cation and anion resin, respectively, with 2 N NH_4OH or 2 N HCl . Fractions were dried under reduced pressure, the residue dissolved in a known volume of 50% ethanol and radioactivity determined. The neutral fraction was analyzed for individual sugars by descending paper chromatography. Known sugars were co-chromatographed with samples on Whatman No. 1 filter paper and developed 72 hr in 2-butanone: acetic acid: boric acid saturated water (9:1:1) (21). Radioactivity of individual sugars was measured

by cutting 18 mm squares from the chromatograms and counting activity according to Wang and Jones (24). Sucrose was eluted from the chromatograms and hydrolyzed with 10-20 units of invertase (Sigma) per μmol sucrose for 2 hr at 50°C . Sugars were rechromatographed to determine the ratio of ^{14}C -glucose to ^{14}C -fructose.

To compare sugar metabolism in fruit tissue discs with intact fruit, strawberry plants were reduced to one leaflet and one primary fruit. Leaflets were lightly abraded with 400 carborundum paper to facilitate sugar uptake. The following day, 2 μCi of ($\text{U-}^{14}\text{C}$) sucrose, (^{14}C -fructosyl) sucrose or ^{14}C -fructose were fed to abraded leaflets. Fruit were harvested 8 hr after feeding, frozen and freeze-dried. Fruit tissue was ground and analyzed in the same manner described for tissue discs.

Results

Strawberry fruit tissue incubated in 10 mM sucrose and 20 mM MOPS buffer (pH 7.0) initially had a high rate of respiration which dropped rapidly during the first 4 hr of incubation (Fig. 6.1, control). Addition of sorbitol as an osmoticum decreased but did not eliminate the decline in respiration. CaCl_2 , at the same osmotic potential as the sorbitol solutions, had a greater stabilizing effect, and 50 mM CaCl_2 maintained tissue respiration at rates similar to those of intact fruit. Addition of CaCl_2 also decreased the rate of sucrose uptake. Fruit discs incubated in 10mM ^{14}C -sucrose in the absence of CaCl_2 accumulated twice as much radioactivity as those incubated with 50 mM CaCl_2 (data not shown).

The pH of the bathing solution has been shown to affect sugar uptake (11, 12, 17). Measurement of the uptake of ^{14}C -sucrose into strawberry fruit discs over the pH range of 4-8, showed that uptake was greatest at pH 5.0 (Fig. 6.2). Sucrose uptake at pH 8 was 40% lower. Some browning of the tissue was observed at pH 7.0 and above.

Sucrose uptake by tissue discs was relatively linear over 4 hr (Fig. 6.3). Incorporation of ^{14}C -sucrose into the insoluble fraction also occurred at a constant rate comprising 16-22% of the radioactivity after 2-4 hr.

Temperature strongly influenced sucrose uptake from a 10 mM sucrose solution (Fig. 6.4), showing an optimum at about 40 °C.

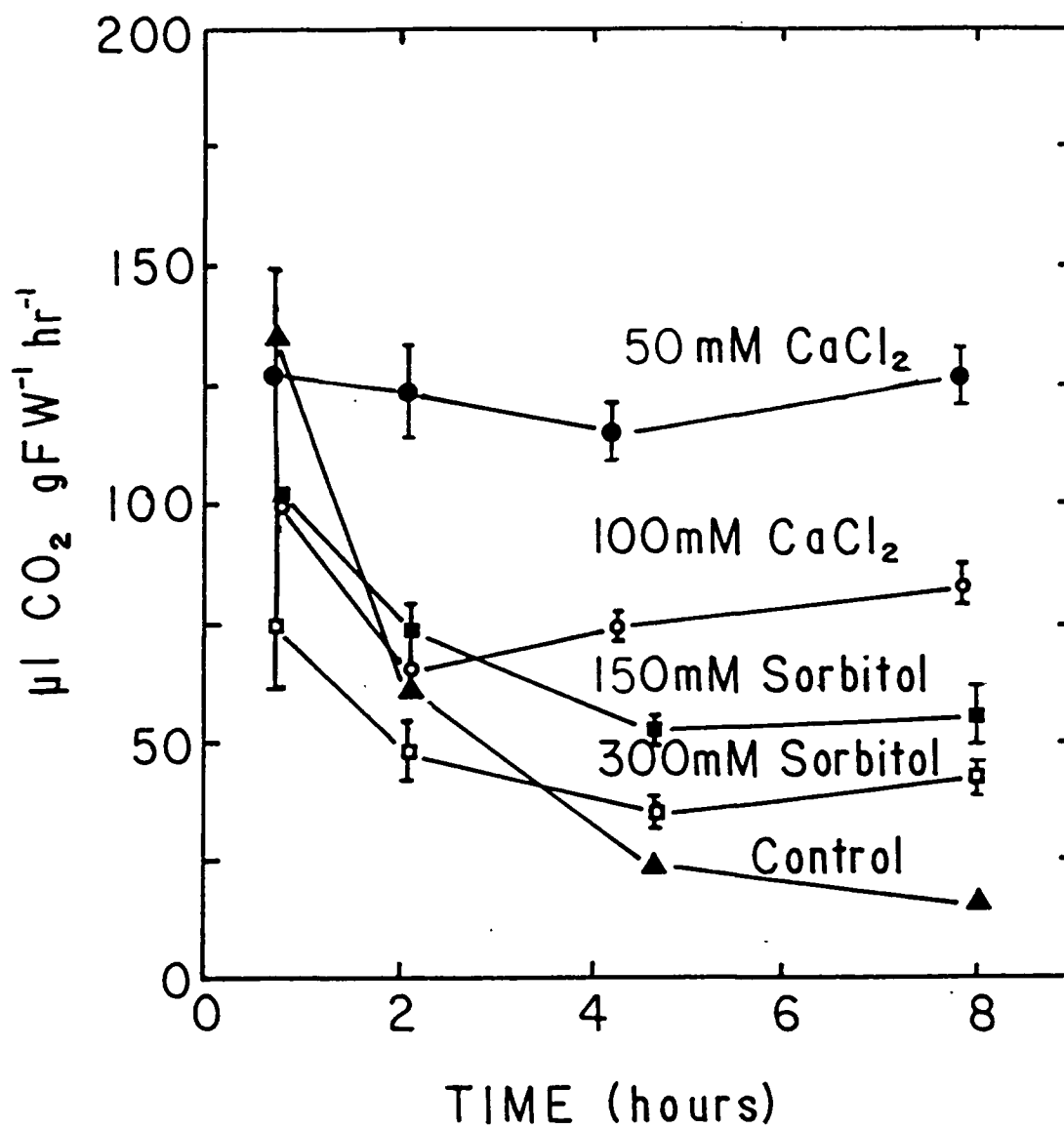


Fig. 6.1. Respiration of strawberry fruit discs over time in various concentrations of CaCl_2 and sorbitol. Control contained 10 mM sucrose and 20 mM MOPS buffer (pH 7.0). Points represent the mean \pm standard error of three replicates.

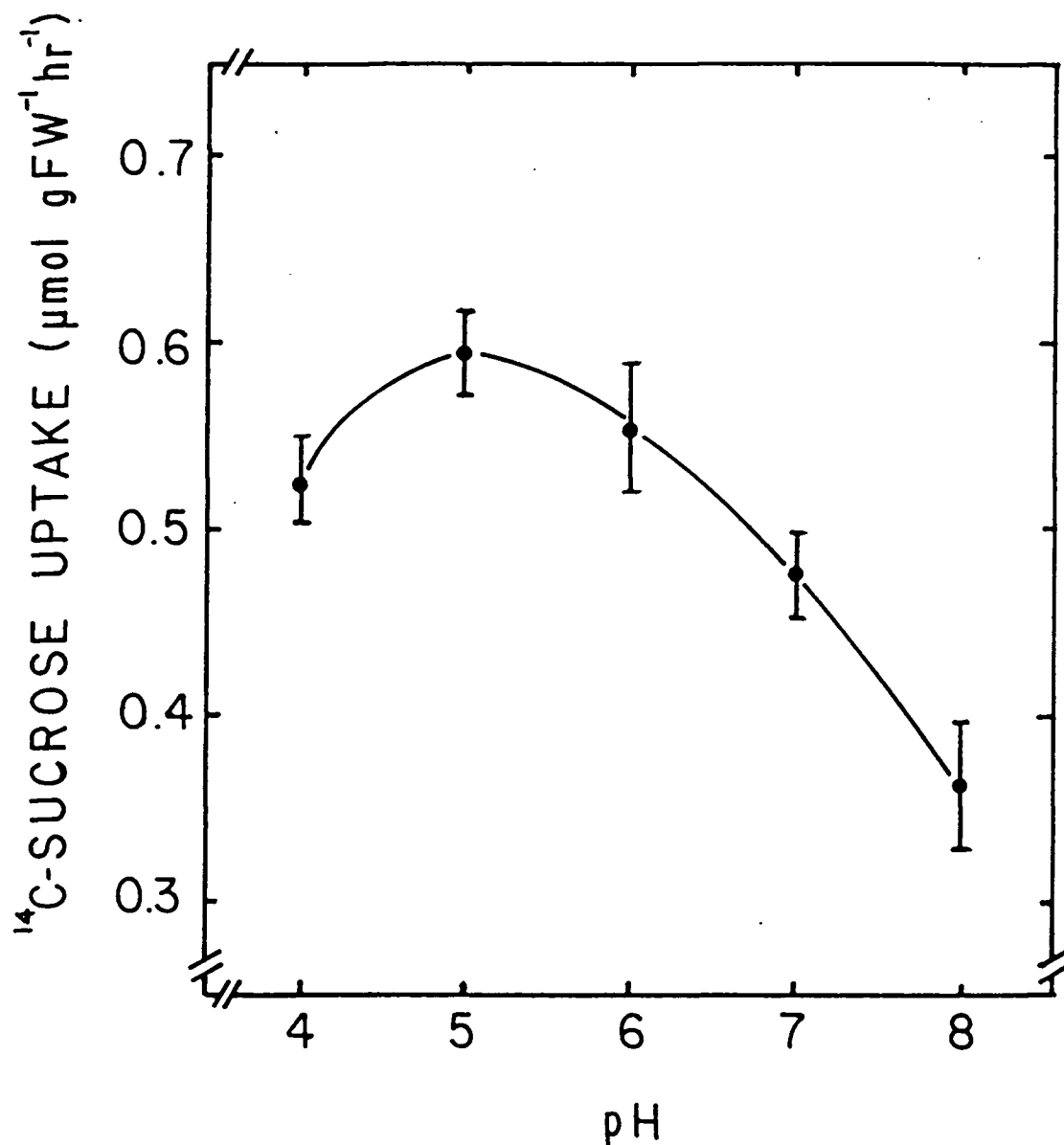


Fig. 6.2. Uptake of ^{14}C -sucrose in 20 mM citrate-phosphate (pH 4 and 5), 2(N-morpholino) ethanesulfonic acid (MES, pH 6), 3(N-morpholino) propanesulfonic acid (MOPS, pH 7) and N-2-hydroxyethylpiperazine propane sulfonic acid (EPPS, pH 8) buffers. Points represent the means \pm the standard error of three replicates.

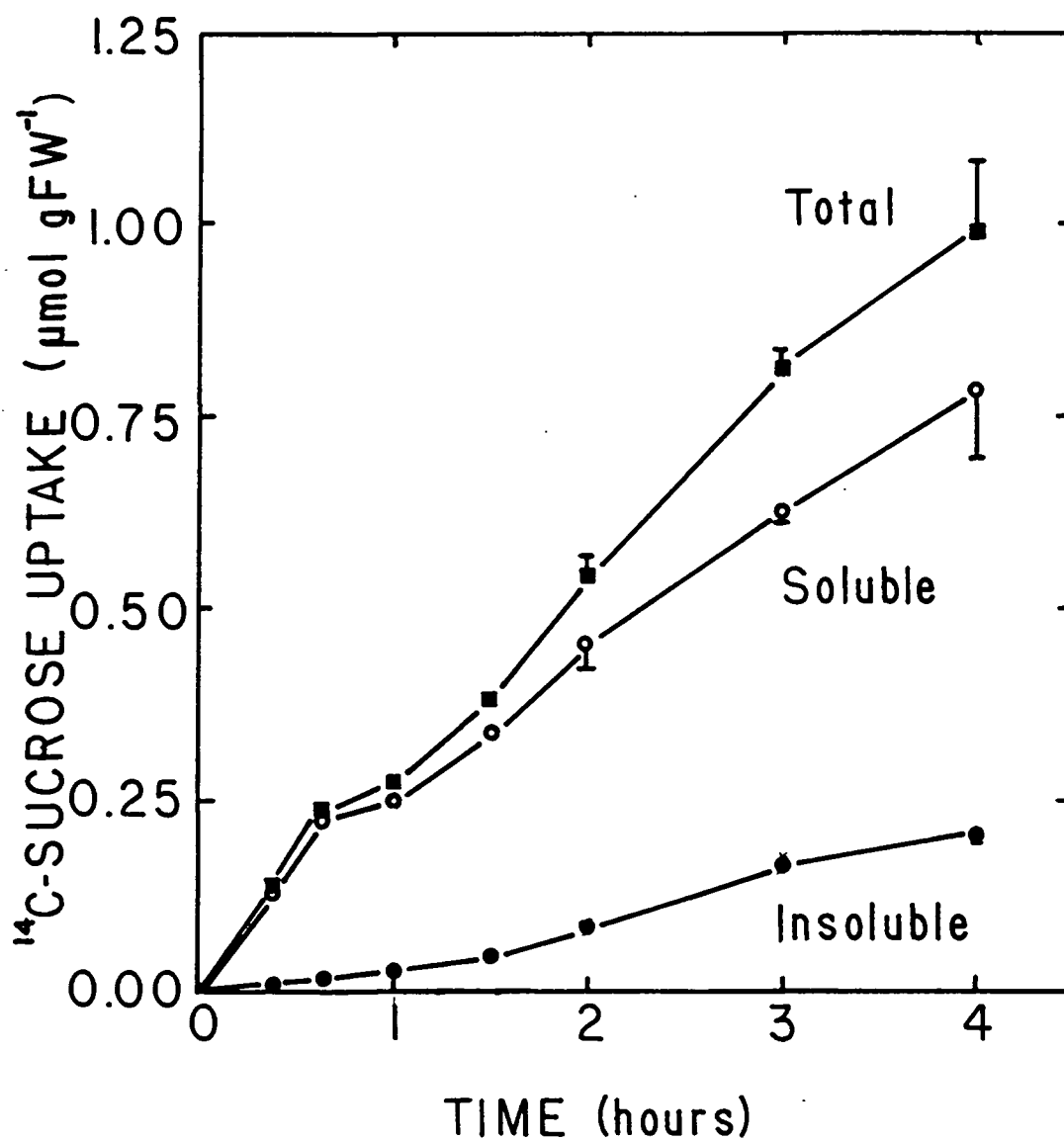


Fig. 6.3. Uptake and incorporation of ^{14}C -sucrose by strawberry fruit tissue in 10 mM sucrose over 4 hr. Points represent the mean \pm the standard error of two replicates.

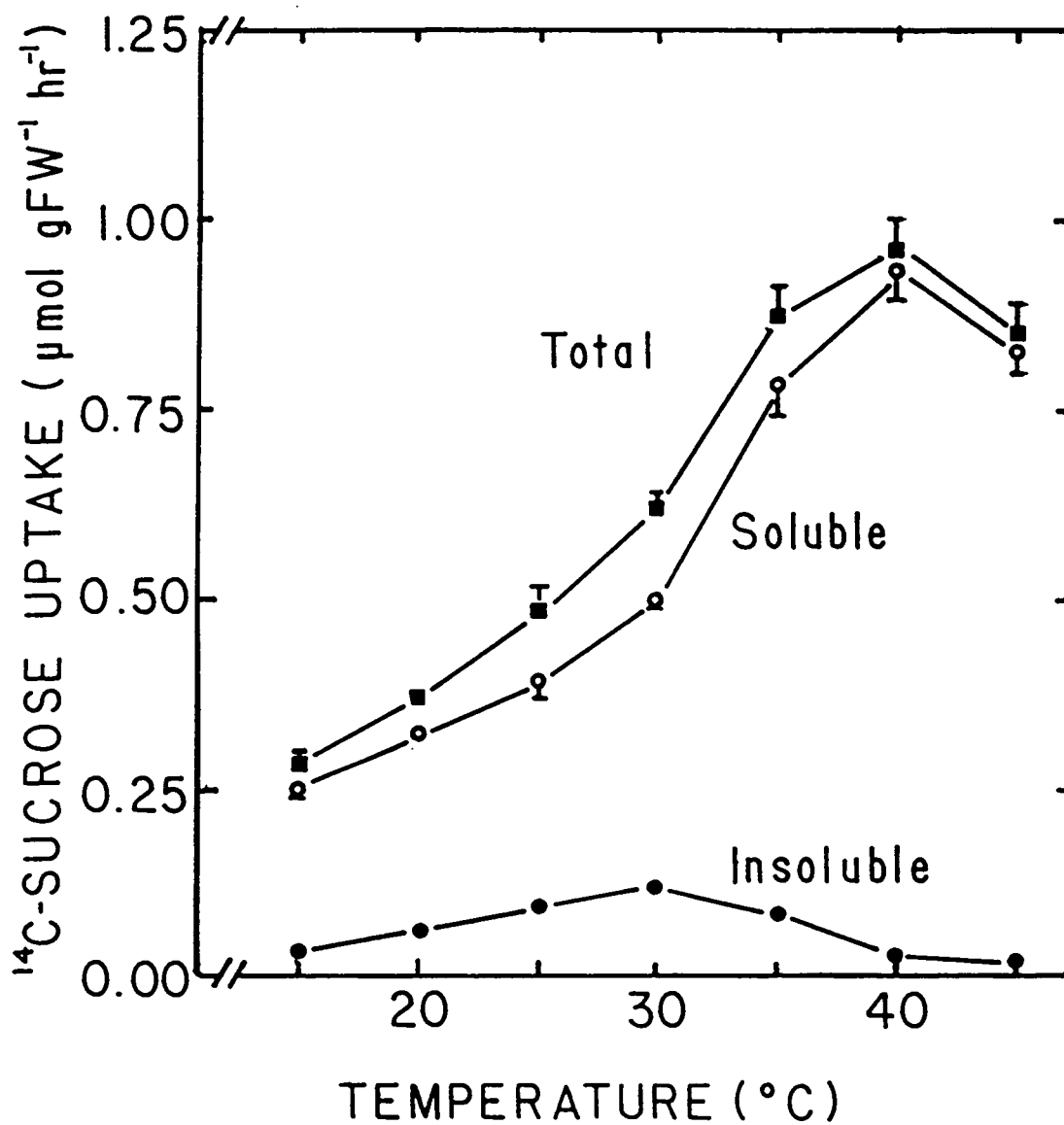


Fig. 6.4. Effect of temperature on uptake and incorporation of ^{14}C -sucrose. Points represent the mean \pm the standard error of 4 replicates.

Incorporation of radioactivity into insoluble components peaked at about 30 ° declining to near zero at 40 °.

Kinetic analysis of sucrose uptake revealed 2 components (Fig. 6.5). At low sucrose concentrations a saturable component following Michaelis-Menton kinetics was predominate, whereas above 25 mM sucrose, a linear component following first order kinetics dominated. Sucrose uptake can be described by the equation $v = [V_{\max} S / (S + K_M)] + kS$ (19), where v is the rate of sucrose uptake, S is the sucrose concentration in the medium, V_{\max} and K_M are Michaelis-Menton constants and k is the first order rate coefficient. For sucrose uptake, the values of V_{\max} , K_M and k are $0.65 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$, 12.5 mM and $0.018 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1} \cdot \text{mmol}^{-1}$, respectively.

The metabolic inhibitors NaCN (5 mM), dinitrophenol (DNP, 3 mM) and carbonyl cyanide -*m*-chlorophenyl hydrazone (CCCP, 100 μM) all increased sucrose uptake while inhibiting respiration and incorporation of ^{14}C -label into the insoluble fraction (Table 6.1). Sucrose uptake in the presence of 3 mM DNP showed first order kinetics with a rate coefficient of $0.103 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1} \text{ mmol}^{-1}$ which was 6 fold greater than in control tissue. The saturable component of sucrose uptake was not apparent in tissue bathed in 3 mM DNP (data not shown). When tissue discs were incubated for 2 hr in (^{14}C)-sucrose prior to placement in 3 mM DNP, efflux of radioactivity after 1 hr was 11% higher than the loss from control discs. The greater uptake of sucrose in the presence of DNP was similar to that of discs in solutions without calcium. When all

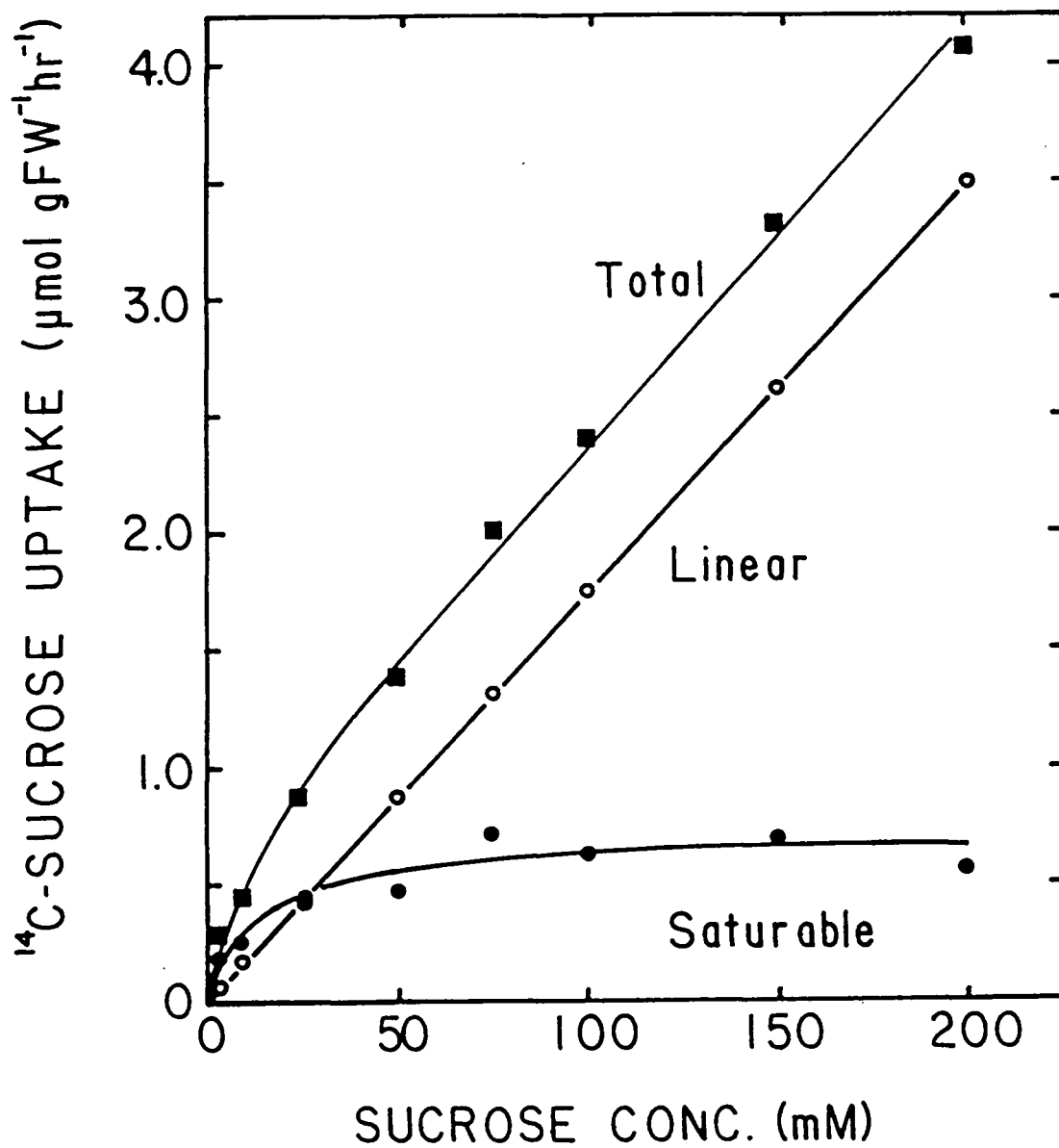


Fig. 6.5. Dependence of the total, linear and saturable components of ^{14}C -sucrose uptake on sucrose concentration.

Table 6.1. Effect of metabolic inhibitors, glucose and N_2 , on sucrose uptake, metabolism and respiration in strawberry fruit discs. Tissue was incubated in 10 mM sucrose for 2 hr. Control values for sucrose uptake, insoluble fraction and respiration were $0.57 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$, $0.095 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$ and $117 \mu\text{l CO}_2 \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$, respectively.

Inhibitor	Sucrose uptake (% control)	Insoluble fraction (% control)	Respiration (% control)
NaCN, 5mM	134	15	6
DNP ^z , 3mM	194	19	2
CCCP, 100 μM	154	22	6
Glucose, 5mM	84	47	--
N_2 , 100%	84	11	--

^zDNP, 2, 4-dinitrophenol, CCCP, carbonyl cyanide-m-chlorophenyl hydrazone

discs were incubated without calcium, 3 mM DNP only stimulated sucrose uptake 30%. At lower concentrations, DNP inhibited sucrose uptake (Table 6.2). Uptake from 10 mM sucrose was reduced 36% by 10^{-5} M DNP. At this concentration incorporation of radioactivity into the insoluble fraction was strongly inhibited (78%), whereas respiration was slightly stimulated (7%). Both a N_2 atmosphere and 5 mM glucose inhibited sucrose uptake 16% while incorporation of ^{14}C into insoluble substances decreased by over 50% (Table 6.1).

Because of the reported presence of cell wall bound acid invertase in strawberry fruit (13), the kinetics of glucose and fructose uptake into strawberry fruit discs were measured and compared with that of sucrose (Fig. 6.6). Fructose uptake was very similar to that of sucrose, having both a linear and a saturable component. For fructose, the values of V_{max} , K_M and k were $0.55 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$, 13.2 mM and $0.017 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1} \cdot \text{mmol}^{-1}$, respectively. Glucose uptake was much more rapid than that of either sucrose or fructose at all concentrations tested. Unlike sucrose and fructose, glucose exhibited only a saturable component having a V_{max} and K_M of $8.33 \mu\text{mol} \cdot \text{g}^{-1} \text{ fresh weight} \cdot \text{hr}^{-1}$ and 88 mM, respectively.

Sucrose and hexoses were fed to strawberry fruit cortex discs to determine how these sugars are metabolized by the fruit tissue. After 2 hr incubation in labeled sugars, over 80% of all radioactivity was recovered in the soluble fraction, 5-8% in the acid fraction and 2-3% in the basic fraction regardless of the labeled sugar fed (Table 6.3). The distribution of sugars within

Table 6.2. Effect of dinitrophenol (DNP) concentration on sucrose uptake, incorporation in the insoluble fraction and respiration of strawberry fruit discs. Discs were incubated in 10 mM ^{14}C -sucrose for 2 hr. Control values for sucrose uptake, insoluble fraction and respiration were $0.48 \mu\text{mol} \cdot \text{g}^{-1}$ fresh weight $\cdot \text{hr}^{-1}$, $0.07 \mu\text{mol} \cdot \text{g}^{-1}$ fresh weight $\cdot \text{hr}^{-1}$ and $128 \mu\text{l CO}_2 \cdot \text{g}^{-1}$ fresh weight $\cdot \text{hr}^{-1}$, respectively.

DNP conc (M)	Sucrose uptake (% control)	Insoluble fraction (% control)	Respiration (% control)
10^{-6}	100	91	122
10^{-5}	64	22	107
10^{-4}	86	19	5
10^{-3}	176	17	1

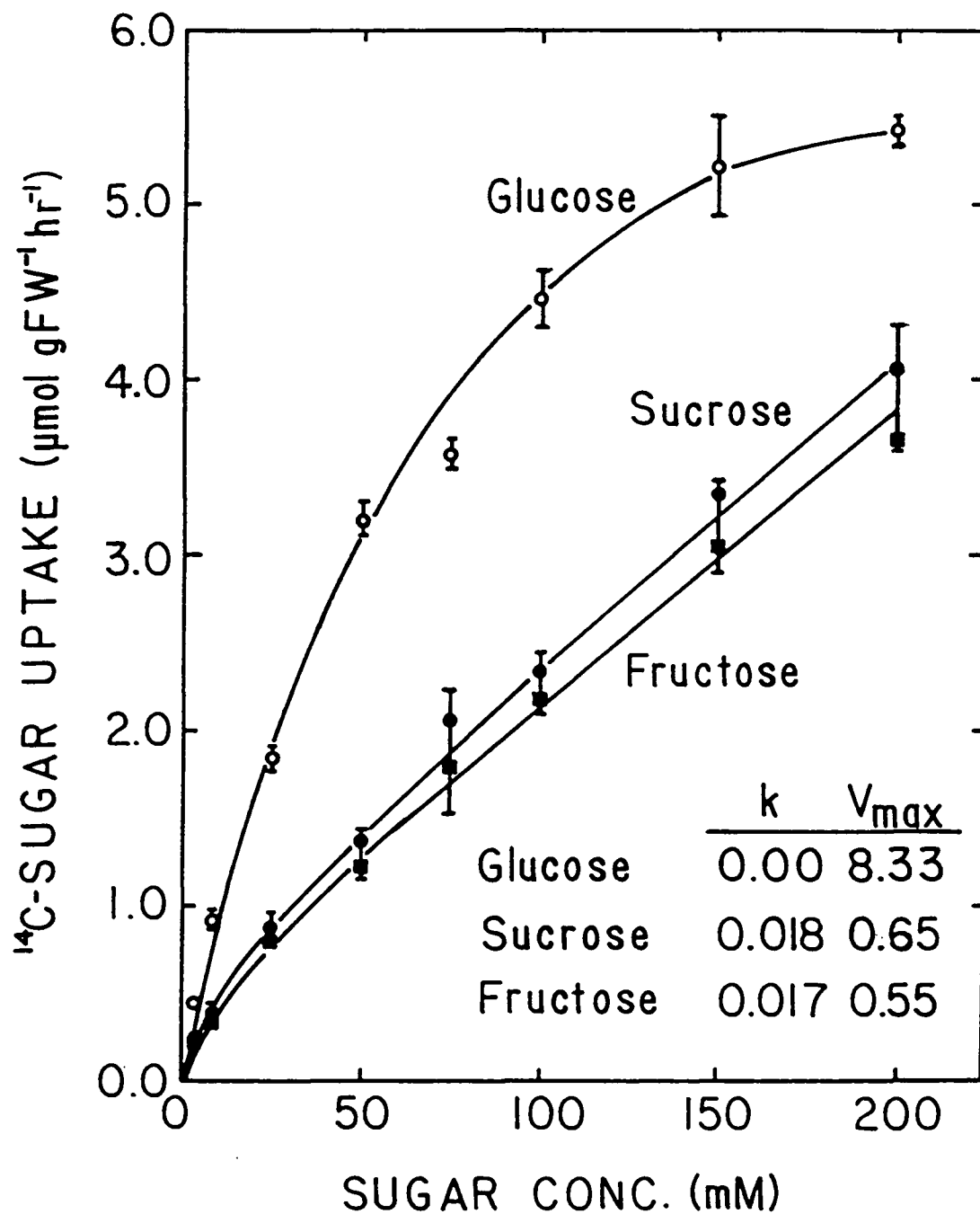


Fig. 6.6. Concentration dependence of ^{14}C -glucose, ^{14}C -sucrose and ^{14}C -fructose uptake by strawberry fruit discs. Points represent the means \pm the standard error of two replicates.

Table 6.3. ^{14}C -Metabolite distribution in strawberry fruit cortex tissue following the accumulation of various ^{14}C -sugars. Fruit discs were incubated in 10 mM sugar solutions for 2 hr.

Fraction	Supplied sugar			
	$(^{14}\text{C}\text{-fructosyl})\text{sucrose}$	$(\text{U-}^{14}\text{C})\text{sucrose}$	$^{14}\text{C}\text{-fructose}$	$^{14}\text{C}\text{-glucose}$
	% distribution			
Insoluble	12 ± 2^z	18 ± 1	19 ± 1	16 ± 1
H_2O Soluble	88 ± 2	82 ± 1	82 ± 1	84 ± 1
Acidic	5 ± 1	8 ± 1	7 ± 1	7 ± 1
Basic	3 ± 1	2 ± 1	3 ± 1	2 ± 1
Neutral	93 ± 1	90 ± 1	90 ± 1	91 ± 1
Glucose	14 ± 2	48 ± 2	16 ± 2	69 ± 2
Fructose	54 ± 2	25 ± 1	59 ± 1	10 ± 5
Sucrose	17 ± 1	19 ± 1	11 ± 1	16 ± 2
G/F	0.10 ± 0.02	0.49 ± 0.05	0.35 ± 0.05	0.59 ± 0.03
Total Uptake ($\mu\text{mol sugar} \cdot 2 \text{ hr}^{-1}$)	0.417	0.671	0.671	1.563

z values represent the means of 3 replicates \pm standard error

the neutral fraction, however, depended on the labeled sugar supplied to the tissue. Together, glucose and fructose accounted for 68-79% of the radioactivity in the neutral fraction. In discs fed (U- ^{14}C)sucrose or ^{14}C -glucose, half of the radioactivity in the neutral fraction was in glucose, whereas fructose accounted for over half of the radioactivity when discs were fed (^{14}C -fructosyl)sucrose or ^{14}C -fructose.

If sucrose is hydrolyzed and subsequently resynthesized, the distribution of radioactivity between its glucose and fructose moieties would, because of isomerase activity, be different than that of the ^{14}C -sucrose offered the tissue. Glucose/fructose (G/F) ratios of sucrose isolated from tissue discs were all below 1.0 regardless of the sugar fed. The G/F ratio of sucrose from tissues fed (^{14}C -fructosyl) sucrose was significantly higher than the ratio of the original sugar (i.e., 0.01) but lower than that from discs incubated in ^{14}C -fructose (0.10 vs 0.35). This suggests that some hydrolysis and randomization of exogenously supplied sucrose had taken place.

To compare the metabolite distribution in tissue discs with that in intact fruit, labeled sugars were fed to source leaves and metabolites in fruit were analyzed 8 hr later. The distribution of radioactivity among metabolites was similar regardless of the sugar fed (Table 6.4). Intact fruit had a 2-3 times higher proportion of activity in the insoluble fraction than was found in tissue discs. Also 50% of the radioactivity in the neutral fraction was in sucrose with the remaining equally distributed between glucose and fructose.

Table 6.4. ^{14}C -Metabolite distribution in strawberry fruit 8 hr after leaves were fed ^{14}C -sugars.

Supplied sugar			
Fraction	(^{14}C -Fructosyl)sucrose	(U- ^{14}C)sucrose	^{14}C -fructose
% distribution			
Insoluble	40 \pm 3 ^z	37 \pm 7	41 \pm 3
H ₂ O Soluble	60 \pm 3	63 \pm 7	59 \pm 3
Acidic	6 \pm 1	6 \pm 1	6 \pm 1
Basic	5 \pm 1	6 \pm 1	5 \pm 1
Neutral	89 \pm 1	88 \pm 2	89 \pm 1
Glucose	25 \pm 4	28 \pm 5	25 \pm 3
Fructose	23 \pm 1	22 \pm 3	23 \pm 2
Sucrose	49 \pm 3	47 \pm 0	51 \pm 5
G/F	0.55 \pm 0.04	0.51 \pm 0.05	0.47 \pm 0.01

^zvalues represent the means of 3 replicates \pm standard error

Sucrose isolated from plants fed (^{14}C -fructosyl)sucrose or (U- ^{14}C)sucrose had a G/F ratio of approximately 0.5 indicating that sucrose underwent considerable hydrolysis.

Discussion

The strawberry fruit at 15 days after anthesis is undergoing many rapid changes. At about this time the rate of fruit expansion is at its peak (Chapter 5) and Knee et al. (15) have reported the middle lamella of cortex parenchyma has begun to breakdown. The ability of calcium to maintain respiration in discs cut from fruit of this age may be through the prevention of cell separation and maintenance of tissue integrity. Neal (20) demonstrated the importance of divalent cations, such as Ca^{+2} , in preserving cell adhesion in ripening strawberry fruit.

While maintaining respiration, calcium appeared to inhibit sucrose uptake. Ehwald et al. (6) found calcium reduced sugar efflux from sugar beet root discs. They proposed that calcium caused a decrease in membrane permeability, or that it mechanically stabilized the structure of the cell wall-membrane complex, enabling the cells to tolerate the higher turgor pressures that result from incubation in dilute solutions. ATPase, which appears to be involved in active sugar uptake (17), was inhibited by calcium in strawberry tissue extracts (2). Since respiration, which would be necessary for ATP production, required the addition of calcium, it appears that increased sucrose uptake in the absence of CaCl_2 was a result of greater membrane permeability and leakage of sucrose into the tissue.

The two kinetic components of sucrose uptake observed in strawberry fruit tissue have been reported in other plant tissues

(18, 19, 22). The biphasic nature of sucrose uptake could indicate accumulation into two cell types. The strawberry cortex tissue used in these experiments is not homogeneous, but contains several cell types including parenchyma cells as well as vascular tissue. In cotyledons of Ricinus communis, Komor (16) suggested the two components of sucrose uptake were due to a linear diffusional component superimposed upon a saturable, carrier mediated transport system. However, in sugar beet roots, the linear component was inhibited by reduced temperatures and metabolic inhibitors (22), indicating an energy dependent mechanism that has not been adequately explained.

The effect of metabolic inhibitors and temperature on sucrose uptake were measured to determine if uptake is dependent on metabolic energy. The increased rates of sucrose uptake observed in 3 mM DNP, 100 μ M CCCP and 5 mM NaCN were opposite of effects observed on sucrose uptake in tissue discs from the taproot of sugar beet (25). Sucrose uptake into sugar beet tap root tissue was measured under the system used for strawberry tissue and the decrease in uptake by the inhibitors was similar to that reported by Wyse (25). In strawberry fruit tissue these relatively high inhibitor concentrations may cause an increase in the permeability of the sucrose limiting membrane. Jackson (14) demonstrated with barley roots that at acid pH, DNP increased membrane permeability to ions. The increase in the linear component of sucrose uptake as well as larger efflux caused by 3 mM DNP in strawberry tissue is consistent with a greater permeability

of the tissue to sucrose. At lower DNP concentrations, membrane permeability may be affected to a lesser extent, and reduced sucrose accumulation may indicate the involvement of an active uptake system.

Although higher temperature generally enhanced sucrose uptake, above 30° C, incorporation of radioactivity into insoluble components decreased indicating reduced synthetic activity. Increased membrane permeability caused by higher temperature (4) may partially account for this greater uptake of sucrose.

In some plant tissues sucrose is believed to be hydrolyzed by an invertase in the free space before uptake (11). Harikrishnan et al. (13) reported the presence of a cell wall bound invertase with a pH optimum of 4.6 in strawberry fruit. The similar pH optimum observed for sucrose uptake may indicate the involvement of invertase. If sucrose is hydrolyzed before uptake, strawberry fruit tissue might be expected to accumulate the resulting hexoses more rapidly than sucrose. This was found to be the case with the rate of glucose uptake being much higher than that of sucrose. The saturable nature of glucose uptake indicates a different mechanism than that involved in sucrose accumulation. In sugar cane internodes, in which sucrose is hydrolyzed in the free space, glucose is also taken up more rapidly than sucrose (10). In the present study the inhibition of sucrose uptake by glucose may also indicate inversion of sucrose before it is accumulated in the tissue.

The ^{14}C -metabolite distribution in fruit discs fed (U- 14) sucrose was very similar to the expected distribution if fruit had been

fed equal amounts of ^{14}C -fructose and ^{14}C -glucose. Because of the greater rate of glucose uptake, higher accumulation of glucose than fructose would be expected. This probably contributed to the greater proportion of radioactivity in glucose than fructose in tissues fed (U- ^{14}C)-sucrose. Contrariwise, metabolite distribution in tissues fed (^{14}C -fructosyl) sucrose was similar to those fed ^{14}C -fructose, both having high levels of labeled fructose. The alteration of the G/F ratio of (^{14}C -fructosyl)sucrose fed to tissue discs indicates that some sucrose underwent hydrolysis, but that a portion may have entered the fruit tissue intact. The fact that the G/F ratio of sucrose from (U- ^{14}C) sucrose fed tissue is not 1.0 also suggests hydrolysis. ^{14}C -glucose may be diluted by a pool of unlabeled glucose resulting in a G/F ratio of less than 1.0 when sucrose is resynthesized. From the above observations it appears that sucrose may be hydrolyzed and taken up as hexoses, with some sucrose entering the tissue intact, possibly by a passive leakage.

In intact fruit asymmetrically labeled sucrose was randomized similarly to uniformly labeled sucrose which would be consistent with apoplastic unloading and hydrolysis in the free space. Because sucrose fed to leaves may have taken 3-4 hr to arrive at the fruit (Chapter 4), labeled sucrose may have had a longer period of time to be metabolized by fruit than ^{14}C -sugars fed to fruit discs. This may explain why there was a larger portion of the activity in the insoluble fraction and why the levels of radioactivity recovered in sucrose were also higher than in tissue discs. 'Brighton' strawberry fruit at this stage of development are accumulating

sucrose more rapidly than glucose and fructose (Appendix). Sucrose could also have been accumulated in intact fruit without hydrolysis with hydrolysis and randomization occurring in the tissue after uptake.

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Chapter 7

EPILOGUE

Fruiting in strawberry has a profound effect on the initiation and growth of other plant organs. Much of the effect is attributed to the strawberry fruit's ability to out compete other plant parts for available assimilates. One factor that may determine this competitiveness is the system in the fruit which unloads sucrose from the phloem. Sucrose uptake from the free space of cortex tissue may be part of this system. The rates of sucrose uptake into fruit tissue correlate well with growth of fruit and sucrose uptake may be a process limiting growth of lower ranking fruit. In this study an attempt was made to understand the mechanism by which sucrose is accumulated in fruit tissue and how this relates to phloem unloading in the fruit. While we have a better understanding of the process of sucrose uptake, more work is needed to elucidate the mechanism by which sucrose is unloaded from the phloem.

The two component system observed in sucrose uptake indicates that more than one mechanism may be involved in the accumulation of sucrose in strawberry fruit discs. The linear component of uptake may reflect a passive diffusion resulting from the cutting and bathing of the fruit tissue. The strawberry fruit is a senescing organ and parenchyma cells in the fruit at this stage of development are large and may not be able to handle incubation without becoming

'leaky'. When tissue was treated with 3 mM DNP, the linear component increased while the saturable component was no longer apparent. Since total uptake was reduced at lower inhibitor concentrations (10-100 μ M), determining the effect of inhibitors on these two components may give some indication of whether the linear component is dependent on metabolic energy. In young fruit tissue, where sucrose uptake is higher and cells have not fully expanded, fruit tissue may not be as 'leaky' when incubated in solutions. Such tissue could be used to test the idea that the linear component results from mostly passive uptake.

The effect of nonpermeant protein inhibitors on sucrose uptake could help to describe the nature of the uptake system and differentiate between carriers located on the plasmalemma and the tonoplast. Because these inhibitors only react with proteins on the cell surface, inhibition caused by these compounds is associated with the cell wall-plasma membrane complex.

While cell wall bound invertase activity has been reported to occur in strawberry fruit, determining invertase activity in 'Brighton' fruit throughout development would help to better understand sucrose uptake. Induction of invertase activity in fruit discs should also be studied to compare invertase activity in discs with intact fruit. The involvement of a free space invertase in sucrose uptake would indicate the importance of glucose and fructose uptake in the fruit tissue. The accumulation of these sugars in the fruit deserves additional study.

In the intact fruit asymmetrically labeled sucrose was hydrolyzed and randomized after being fed to abraded leaves. This experiment needs to be repeated, with the fruit being analyzed after shorter periods of time following feeding of labeled sugars. If considerable randomization still occurred after a shorter time period, hydrolysis of sucrose prior to its uptake would be strongly implicated.

Determining if sucrose unloading is apoplastic or symplastic is important in deciding whether sucrose uptake is part of the natural unloading mechanism. When the mechanism by which sucrose is unloaded from the phloem is understood, work can then commence on the regulation of this process. Research in this area could identify new methods for increasing future yields of strawberry as well as that of other crops.

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APPENDIX

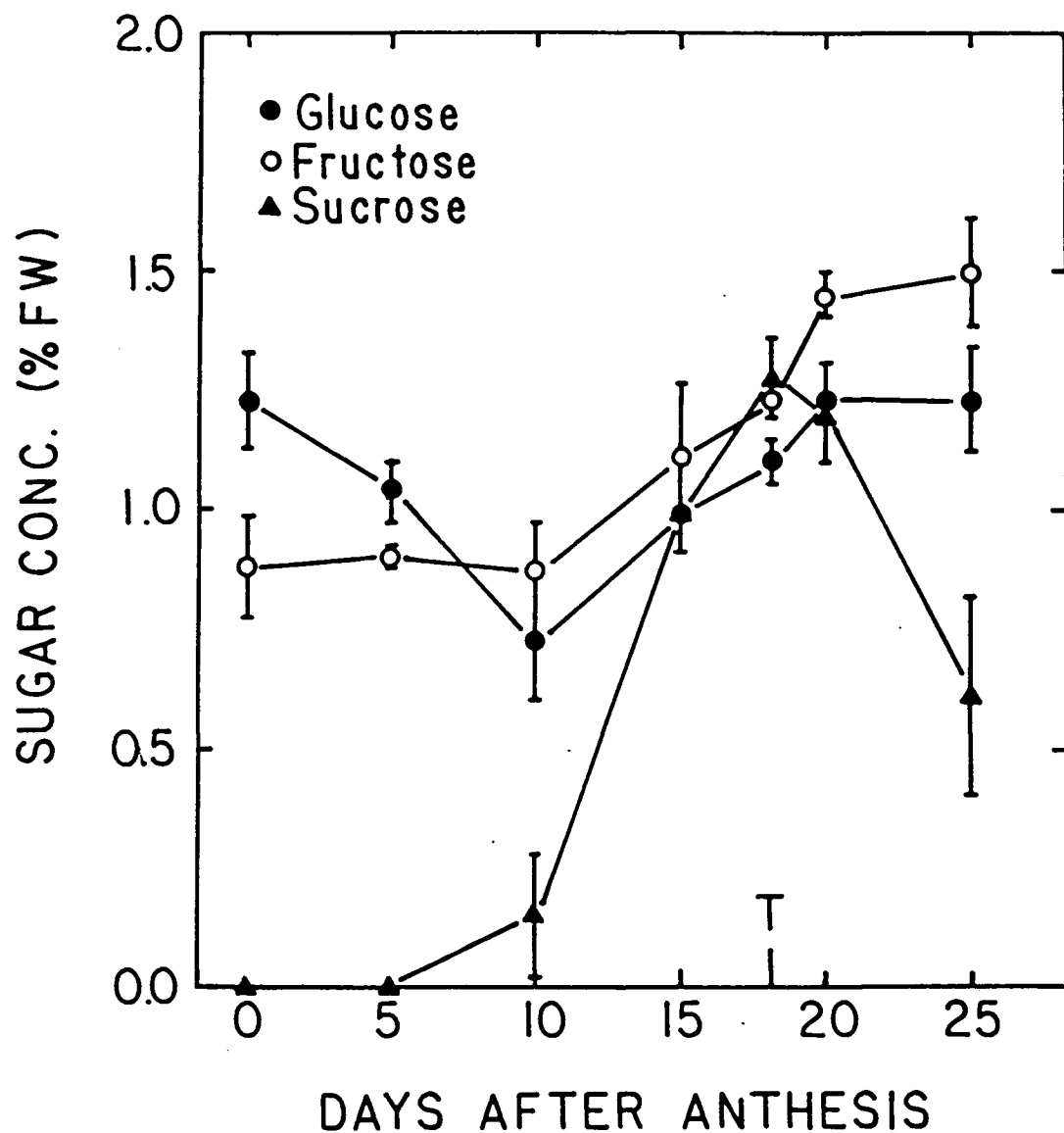


Fig. A.1. Concentration of glucose, fructose and sucrose in 'Brighton' strawberry fruit throughout development. 'T' indicates the time fruit began to turn color. Points represent the means \pm standard error of three replicates.