Leaf Carbohydrate Status and Enzymes of Translocate Synthesis in Fruiting and Vegetative Plants of *Cucumis sativus* L.[1]

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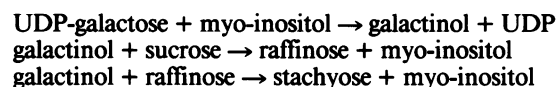
ABSTRACT

Carbon partitioning in the leaves of *Cucumis sativus* L., a stachyose translocating plant, was influenced by the presence or absence of a single growing fruit on the plant. Fruit growth was very rapid with rates of fresh weight gain as high as 3.3 grams per hour. Fruit growth was highly competitive with vegetative growth as indicated by lower fresh weights of leaf blades, petioles, stem internodes and root systems on plants bearing a single growing fruit compared to plants not bearing a fruit. Carbon exchange rates, starch accumulation rates and carbon export rates were higher in leaves of plants bearing a fruit. Dry weight loss from leaves was higher at night from fruiting plants, and morning starch levels were consistently lower in leaves of fruiting than in leaves of vegetative plants indicating rapid starch mobilization at night from the leaves of fruiting plants. Galactinol, the galactosyl donor for stachyose biosynthesis, was present in the leaves of fruit-bearing plants at consistently lower concentration than in leaves of vegetative plants. Galactinol synthase, and sucrose phosphate synthase activities were not different on a per gram fresh weight basis in leaves from the two plant types; however, stachyose synthase activity was twice as high in leaves from fruiting plants. Thus, the lower galactinol pools may be associated with an activation of the terminal step in stachyose biosynthesis in leaves in response to the high sink demand of a growing cucumber fruit.

The presence on a cucumber plant of a single fertilized growing fruit is known to strongly suppress the growth of subsequently fertilized fruit (17) as well as the growth of parthenocarpically induced fruit (4). When the fruit ceases growth due to maturation, or if the fruit is harvested prematurely, a subsequently fertilized ovary will begin to grow within a few days. The strong sink demand imposed by developing fruits is well recognized. In numerous species, including cucumber (1), accelerated leaf carbon exchange rates have been associated with fruit and seed growth (3, 7, 11, 18) and this suggests an important role of leaf photosynthesis which occurs concurrently with fruit growth. For instance, photosynthesis by developing soybean pods was found to make a negligible contribution to the total import of dry weight of the pod (23), and rapid movement of ^{14}C from leaves using $^{14}CO_2$ to growing reproductive structures has often been observed (1, 9, 15, 16, 28).

In vegetatively growing sucrose translocators, the rate of export of assimilates from the leaf has been correlated positively with photosynthetic rate (5, 10, 14) or with leaf sucrose concentration (2, 5, 14, 22) which in turn are correlated positively with leaf SPS[2] (14, 22, 24, 25). Recently it was found that SPS activity was higher in leaves of fruit-bearing eggplants than in plants from which the fruit was removed at an early stage of development (3). A similar relationship between leaf SPS and pod growth was previously demonstrated during the flowering and fruiting period through the use of male-sterile and male-fertile soybean genotypes (11).

In cucumber plants, where stachyose is a major carbohydrate translocated (27), it is not clear that the rate of translocate synthesis is regulated at the level of SPS activity, or at one of the reactions of raffinose saccharide biosynthesis. Stachyose biosynthesis involves the following three steps beyond sucrose formation (13).

UDP-galactose + myo-inositol → galactinol + UDP
galactinol + sucrose → raffinose + myo-inositol
galactinol + raffinose → stachyose + myo-inositol

Gal Syn, the enzyme which catalyzes the first reaction above, has recently been proposed to be important in regulating carbon partitioning between sucrose and the raffinose saccharides (8, 19). The present experiments were conducted to examine the effects of a single growing cucumber fruit on certain aspects of plant growth, leaf carbohydrates, export rates, and the enzymic capacity of leaves for translocate synthesis.

MATERIALS AND METHODS

Growth of Plants. Seeds of *Cucumis sativus* L. cv Calypso were germinated and grown in a glass greenhouse in plastic pots that were 30 cm in diameter by 18 cm in depth. The growth medium was a mixture of equal volumes of sand, soil, and peat moss. The vines were grown upright on strings suspended from the superstructure of the greenhouse. Pots were watered daily, or more frequently if needed, and fertilized weekly with a complete soluble fertilizer. Minimum day and night temperatures in the greenhouse were 27 and 21°C, respectively. Plants for all experiments were grown between the months of September and March with natural photoperiods and light intensities. Cloudy weather was prevalent during much of the growth period.

The general procedure for comparison of fruiting and vegetative plants involved maintaining plants in a vegetative, single stem condition by mechanical removal of developing vegetative

[2] Abbreviations: SPS, sucrose phosphate synthase; Gal Syn, galactinol synthase; St Syn, stachyose synthase.

and reproductive axillary buds until plants had grown to 18 to 20 nodes. At this time, some of the plants were allowed to flower over a 2- to 3-d period, and a single female flower was pollinated on 8 to 10 plants at the 10th to 13th node. Typically, fruit were allowed to grow for 12 to 14 d prior to sampling. For comparison, other plants were maintained in a vegetative condition as described above. At the time of sampling, leaves from identical node positions were sampled from vegetative and fruiting plants. In this way, it was possible to observe the influence of a single growing fruit on various parameters in plants that were maintained under otherwise identical cultural conditions.

Fruit Growth Measurements. Nondestructive estimates of fruit fresh weight were obtained by measuring the length and central diameter of fruit. Approximate volume, calculated as a cylinder, was used to estimate fruit fresh weight from a regression equation established previously (12). The equation is, fruit fresh weight in g = 0.76 (approximate volume, cm^3) −1.19.

Carbohydrate Extraction and Analysis. Leaf tissue was sampled by removing 1.13 cm^2 discs with a cork borer. Typically, eight discs were removed from two leaves to comprise a sample. Fresh weight of the sample was recorded and the tissue was either killed by boiling in 5 ml of 80% aqueous ethanol or freeze-dried, after which dry weight was recorded. The sample was homogenized with a Brinkmann Polytron Homogenizer[3] (Brinkmann Instruments, Westbury, NY). After centrifugation, the residue was extracted twice more with 5 ml portions of 80% ethanol and the supernatants were combined and taken to dryness *in vacuo* with a Buchler Evapo-Mix (Buchler Instruments, Fort Lee, NJ) in 16 × 150 mm tubes. The contents were dissolved in 1 ml of water, centrifuged, and subjected to analysis using HPLC.

The HPLC system consisted of a pump set to deliver water, the solvent, at a rate 0.5 ml min^{-1}. The column was a Sugar Pak I (Waters Associates, Milford, MA) held at 75°C and was preceded by microguard anion and cation exchange guards (Bio Rad Laboratories) maintained at 22°C. Sugars were detected with a refractive index detector and the output was recorded with a strip chart recorder. Sample size was 20 µl. Stachyose, raffinose, galactinol, and sucrose were identified by comparison to retention times of sugar standards.

The leaf residue left after ethanolic extraction was resuspended in 2.0 ml of 0.2 M KOH and boiled for 30 min. After cooling, the pH of the mixture was adjusted with 1 M acetic acid to about pH 5.5 and the gelatinized starch was digested to glucose using 36 units of amyloglucosidase (Sigma Chemical, grade V) which had previously been dialyzed against 50 mM Na-acetate buffer (pH 4.5). After 60 min of digestion the samples were boiled. The released glucose was detected with an enzymic link of hexokinase and glucose-6-P dehydrogenase. Starch is reported as glucose equivalents.

Carbon Exchange Rates. Carbon exchange rate was measured on leaves by means of an IR gas analyzer equipped with 10 cm^2 clamp-on leaf cuvette. Ambient air containing about 350 µl/l CO_2 was passed through the cuvette at a flow rate of 1.5 l/min and differences in CO_2 concentration between incoming and exhaust air were measured. Carbon exchange rates were measured as mg CO_2 fixed/dm^2 h^{-1}.

Mass Carbon Export. For estimation of assimilate export from leaves, two sets of discs were removed from each leaf. One set was removed at 0900 h and another at 1500 h on the same day. These were subsequently freeze-dried and weighed, which provided an estimate of the rate of dry weight gain per unit of leaf

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[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the North Carolina Agricultural Research Service or by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

area. Carbon exchange rate was measured during the interval between removal of the discs. Export was calculated from the following equation: mass carbon export = mg CO_2/dm^2 h^{-1} (0.68) − dry weight accumulation in mg/dm^2 h^{-1}. This method has been described elsewhere (26).

Extraction and Assay of Enzymes. SPS was extracted and assayed from cucumber leaf tissue as described elsewhere (11). Gal Syn was extracted and assayed utilizing an isotopic assay (8). For extraction of St Syn, 2 g of finely chopped leaf tissue was homogenized at 0°C in 10 ml, 50 mM Hepes-NaOH buffer (pH 7.0) containing 20 mM mercaptoethanol. A 2 ml portion of extract was desalted on a Sephadex G-25 column into 20 mM Hepes-NaOH buffer (pH 7.0) containing 20 mM mercaptoethanol. One hundred µl of the extract was incubated for 30 min in a reaction volume of 200 µl containing 5 mM galactinol, 10 mM raffinose, 10 mM mercaptoethanol, and 10 mM Hepes-NaOH buffer (pH 7.0). Minus galactinol blanks were incubated as controls. Reactions were terminated by addition of 0.8 ml of ethanol and immersion in a boiling water bath for 30 s. Contents of the tubes were passed through 0.45 mµ filters, evaporated to dryness, and the residue was redissolved in 200 µl of water. Stachyose was detected by analysis of 100 µl of this sample with the HPLC system described above. Rates of stachyose formation were linear up to 1 h, and the formation of stachyose was totally dependent upon the presence of galactinol in the assay mixture.

Galactinol Purification. Galactinol, which is not commercially available, was purified from fully expanded cucumber leaves (200 g) that were thoroughly homogenized and extracted with boiling 80% ethanol. The extract was evaporated to dryness *in vacuo* and prepared for paper chromatographic separation of sugars as described elsewhere (21). The extract was streaked onto Whatman No. 17 preparative paper and developed descendingly with a solvent of *n*-propanol:ethyl acetate:water (7:1:2 v/v) for 7 h. Carbohydrates on the papers were visualized on a test strip with Tollen's reagent. The region of the paper corresponding to galactinol was eluted with water and concentrated *in vacuo*. Galactinol was then further purified by repetitive injections onto the HPLC column described above. The single peak recorded was collected from the effluent line of the detector. The galactinol was concentrated and stored at −20°C. Upon acid hydrolysis, the compound yielded equimolar amounts of galactose and myo-inositol. The purified compound exhibited the same mobility in paper chromatographic systems as ^{14}C-labeled galactinol prepared enzymically (20). Galactinol in the concentrated solution was quantitated by means of an anthrone assay to determine the galactosyl content of the solution using galactose as a standard. Anthrone reagent did not react with the inositol portion of galactinol.

RESULTS

Cucumber fruit were found to grow both during the day and night (Fig. 1). Growth rate during the day was consistently slower than during the night. In a parallel study, 10 plants bearing fruit with a fresh weight of 265 ± 23 g were divided into two groups. At the end of a photoperiod, all the leaves and petioles were removed from five of the plants and the remainder of the plants served as controls. The overnight growth rate of fruit on defoliated plants was 1.1 ± 0.6 g fresh weight h^{-1} as compared to 3.3 ± 0.5 g fresh weight h^{-1} on the control plants, which strongly suggests that fruit growth at night was partially dependent upon assimilate mobilization from leaves. The expansion of young sink leaves, as measured by increase in length and width at 3-h intervals over a single 24-h period, was found to occur linearly without diurnal variation (data not shown). Thus, no evidence was found to suggest that fruit and leaf growth avoid competition by growth at different periods of the diurnal cycle.

Data in Figure 2 document that vegetative growth of cucumber
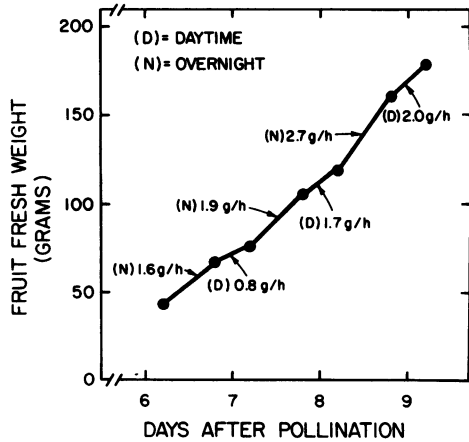
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FIG. 1. Cucumber fruit growth rate during day and night. Numbers to the sides of the curve are growth rates expressed as g fresh weight/h. Data are the average of three fruit.
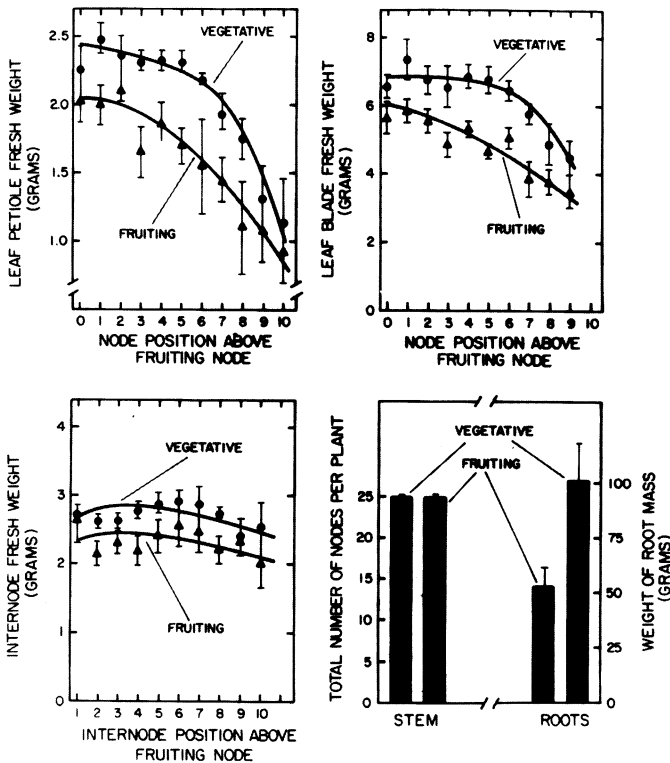

FIG. 2. Fresh weights of vegetative parts of cucumber plants and number of nodes per plant as influenced by the presence of a fruit growing on the plant for 11 d. Fruit were present on the 12th node and had an average fresh weight of 195 g at the time of harvest. Data are the average of five plants. Vertical bars are SD.
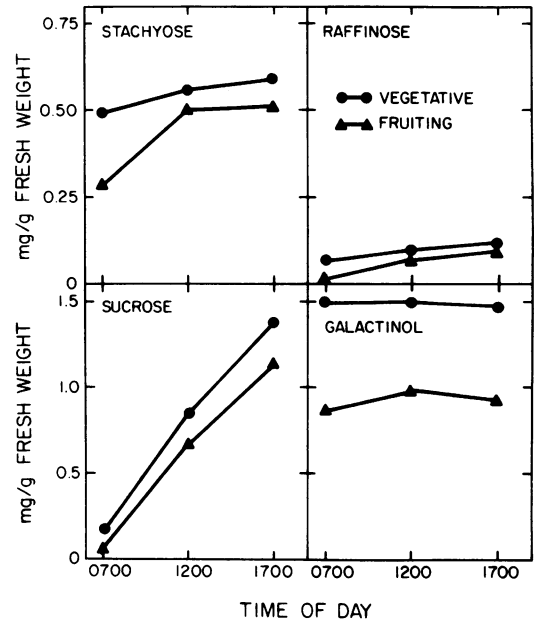

FIG. 3. Change in leaf oligosaccharide concentration during the photoperiod in fruiting and vegetative cucumber plants. Leaf samples represent punches combined from two leaves at and immediately above the fruiting node. Fruit weight at the time leaves were sampled was about 300 g. Data are the mean of two replications of five plants each.
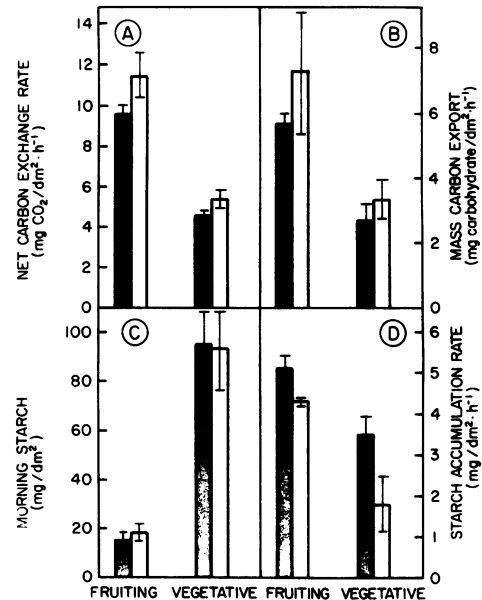

FIG. 4. Differences in (A) carbon exchange rate, (B) export rate, (C) morning starch concentration, and (D) starch accumulation rate in leaves of fruiting and vegetative cucumber plants. Open bars are two leaves at and immediately above the fruiting node. Shaded bars are two leaves at nodes 3 and 5 below the fruiting node. Mass carbon export and starch accumulation rate were estimated from leaf punches taken at the beginning and end of a 6-h interval beginning at 0900. Data are from four single plant replications. Vertical bars are SD. Fruit weight at the time of sampling averaged 380 g.

plants was strongly suppressed by the presence of a growing fruit on the plant for an 11-d period. The total number of nodes attained by vegetative and fruiting plants was equal, but fresh weights of all plant parts above the fruiting node were less on fruiting plants. Fruit growth was apparently highly competitive with root growth. Concentrations of stachyose, raffinose, and sucrose were slightly lower in leaves of fruiting plants (Fig. 3). The most striking difference observed was that of galactinol concentration. In several other samplings for leaf sugar concentration during the photoperiod with other sets of cucumber plants, stachyose, raffinose, and sucrose pools were found to vary in a small but inconsistent manner between fruiting and vegeta-

tive plants (data not shown). However, galactinol levels were consistently observed to be between 30 to 35% lower in leaves of fruiting plants as compared to the leaves of vegetative plants. The marked increase in sucrose concentration during the photoperiod was also found consistently in several experiments not

reported.

Carbon exchange rates, assimilate export rates, and starch accumulation rates were about twice as high in leaves of fruiting cucumber plants as in leaves of vegetative plants (Fig. 4). These data were collected on an overcast day in the greenhouse when light intensity at canopy level was about 200 $\mu E/m^2$ s^{-1}. On a day of very high light intensity carbon exchange rates near midday were found to be 29.4 ± 8.0 and 15.0 ± 5.2 mg CO_2/dm^2 h^{-1} for leaves of five fruiting and five vegetative plants, respectively, on the 14th d after fruit were pollinated.

Low morning starch levels in the leaves of fruiting cucumber plants associated with rapid starch accumulation rates during the photosynthetic period suggested that assimilates accumulated during the photoperiod might be lost more rapidly at night from the leaves of fruiting than from the leaves of vegetative plants. The rate of dry weight loss from cucumber leaves at night was estimated from dried leaf punches taken between 2100 and 0300 h. Dry weight was lost at a linear rate of 4.3 and 2.3 mg/dm^2 h^{-1} from leaves of fruiting and vegetative plants, respectively (Fig. 5). Galactinol concentrations in the leaves of those plants at midnight were 0.9 and 1.5 mg/g fresh weight for fruiting and vegetative plants, respectively, which is similar to that observed in other plants during the photoperiod (Fig. 3).

Gal Syn activity was substantially higher in cucumber leaves than either SPS or St Syn (Table I). St Syn, the terminal transferase in stachyose formation (6, 13), was found to be present at about twice the activity level in fruiting leaves as in vegetative leaves on a per gram fresh weight basis.
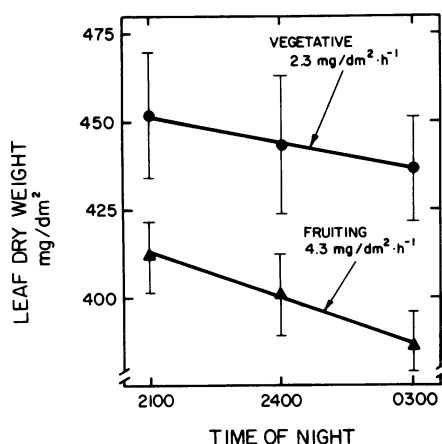

FIG. 5. Loss in leaf dry weight at night from leaves of fruiting and vegetative cucumber plants. Data are from leaf punches taken repetitively from four leaves including leaves at the fruiting node and three additional nodes above the fruiting node. Fruit weight at the time leaves were sampled was about 154 g. Data represent the average of five plants. Vertical bars are SD.

Table I. *Activities of Enzymes Extracted from Leaves of Fruiting and Vegetative Cucumber Plants*

Leaf tissue was composited from leaves at the fruiting node and three additional nodes above the fruiting node. Each datum is the average of four plants. Fruit weight at the time of leaf harvest was about 300 g. The experiment was repeated on an additional set of plants with similar results. Samples for both experiments were taken near noon.

Plant Condition	Galactinol Synthase	Sucrose-P- Synthase	Stachyose Synthase
		units/g fresh wt[a]	
Vegetative	45.0 ± 20	5.5 ± 1.2	1.1 ± 0.1
Fruiting	46.8 ± 20	5.8 ± 0.9	2.0 ± 0.4

[a]A unit is the amount of enzyme which produces 1 μmol product h^{-1}.

DISCUSSION

Accelerated carbon metabolism was evident in cucumber leaves in response to the strong sink demand imposed upon the plant by a growing fruit. This was evident from higher rates of photosynthesis, starch accumulation, and assimilate export by cucumber leaves on fruiting plants. In sucrose translocating plants, more rapid export may be associated with increased photosynthetic rate as described earlier, or increased export may be achieved without alteration in photosynthetic rate by altered partitioning of fixed carbon between leaf starch and sucrose in favor of sucrose for export (22). In cucumber leaves in fruiting plants, more rapid assimilate export was associated with approximately proportional increases in leaf photosynthesis and starch accumulation rate (Fig. 4). Thus, there was little indication of altered carbon partitioning between leaf starch and assimilates for export.

Evidence from these experiments suggest that cucumber leaves respond to increased sink demand by increased photosynthetic rate associated with more rapid stachyose biosynthesis. In addition to higher activities of St Syn assayed in extracts from leaves of fruiting plants, the lower galactinol pool in fruiting leaves constitutes additional evidence for an activation of stachyose biosynthesis *in situ*. Because galactinol is the galactosyl donor for stachyose biosynthesis it might be expected to exist at lower steady-state concentration during rapid stachyose synthesis. The activity of St Syn detected in leaves of fruiting plants (Table I) was sufficient to account for the synthesis of 3.2 mg of stachyose/ $dm^2 \cdot h^{-1}$, which is about 50% of the export rate observed from cucumber leaves (Fig. 4). It is not certain that St Syn as assayed here represents the maximum catalytic potential for this enzyme, as only limited information is available concerning its regulation (6). Despite this uncertainty, the present results provide an indication that the biochemical strategy for providing higher translocate synthesis in response to sink demand by fruit may be different in a stachyose translocator than in sucrose translocators (3, 11).

Finally, it is of interest to comment on the photosynthetic capacity of cucumber leaves in relation to fruit growth. The highest rate of fixation observed in fruiting leaves on the average was about 30 mg $CO_2/dm^2 \cdot h^{-1}$ which constitutes a primary production rate of about 225 mg of assimilate/dm^2 during an 11-h photoperiod. Fruit in the range of 200 to 300 g fresh weight were frequently observed to increase in fresh weight by as much as 60 g in a 24-h day-night period. The dry weight percentage of a cucumber fruit is known to remain constant throughout its development at about 5 to 6% of its fresh weight. Thus, the demand for assimilate import by an actively growing cucumber fruit of this size can be conservatively estimated to be about 3300 mg within a 24-h cycle. This is a minimum estimate because a significant proportion of the incoming assimilate will be respired. This implies the daily photosynthetic participation of a minimum of 14 dm^2 of leaf canopy which corresponded to about 40% of the source leaf canopy of fruiting plants (estimated from weight to area conversion from data in Fig. 2). These observations may explain the strong tendency of cucumber plants to grow limited numbers of fruit simultaneously (17), and they are also consistent with the strong interplant competition between fruit growth and vegetative growth (Fig. 2).

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