Carbon Partitioning among Leaves, Fruits, and Seeds during Development of *Phaseolus vulgaris* L.\(^1\)

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**ABSTRACT**

Development of vegetative and floral buds was found to be a key factor in establishing the way carbon is distributed among growing leaves and fruits in *Phaseolus vulgaris* L. plants. Leaves emerged principally during a period 14 to 32 days after planting while flowers were produced during a 10- to 12-day period near the end of leaf emergence. Timing of anthesis established the sigmoidal time course for dry weight accumulated by the composite of all fruits on the plant. During the first 12 days following anthesis, fruit growth mainly consisted of elongation and dry weight accumulation by the pod wall. Thereafter, seed dry weight increased for about 1 week, decreased markedly for several days, and then increased again over the next 2 weeks. Accumulation of imported carbon in individual seeds, measured by steady-state labeling, confirmed the time course for dry weight accumulation observed during seed development. Seed respiration rate initially increased rapidly along with dry weight and then remained nearly steady until seed maturation. A number of developmental events described in the literature coincided with the different phases of diauxic growth. The results demonstrated the feasibility of relating current rates of carbon import in individual seeds measured with tracer \(^{14}\)C to the rates of conversion of imported sucrose and use of the products for specific developmental processes. The resulting data are useful for evaluating the roles of conversion and utilization of imported sucrose in regulating import by developing seeds.

Translocation and partitioning of assimilated carbon are major determinants of plant productivity and crop yield (9). Productivity is affected both by distribution of translocated carbon to new leaf growth and by accumulation of carbon in the harvestable organs. The former affects light interception for biomass production while the latter affects harvest index. As a consequence, increased plant yield requires a balanced increase in the source carbon that is partitioned to these two sites (7). In practice, higher yields of economically important crops are the result of both greater dry-matter production in leaves as well as increased carbon accumulation in harvestable organs (11).

Understanding regulation of carbon partitioning among sinks and its relation to plant growth and development is important for establishing strategies for increasing plant productivity and yield. Regulation of import and partitioning occurs by changes both in physical factors, such as conductance along the entry-path sieve tubes, and in physiological processes, such as sucrose uptake, conversion of sucrose, and use of the products in synthesis (11, 21).

A basic premise of this study is that changes in rates of accumulation of carbon imported by individual fruits often are caused by changes in rates of processes that sustain import, such as uptake, degradation, and metabolism of the imported sucrose. At the plant level, a new pattern of carbon partitioning contributed to the marked increase in overall fruit growth that was observed about 10 d AA\(^2\) in *Phaseolus vulgaris* L. (8). The growth rate of individual seeds followed a predictable pattern during its development (3, 14). These times of faster or slower import and growth provide opportunities for identifying mechanisms that sustain import into developing fruits (5, 6).

A major goal of the present study was to identify times during individual fruit development for studying how gene activation and changes in activity of certain enzymes may sustain import and regulate partitioning of carbon. Time courses for rates of carbon import, dry weight accumulation, and oxygen uptake in individual seeds showed the importance of developmental events for overall control of carbon partitioning in the plant. The sum of the import rate for a specific seed obtained from radiotracer data (8) and the respiration rate provided a measure of current carbon import rate for a specific seed. Joined with data on physiology or developmental processes, the import rate promises to provide a means for studying the molecular basis for carbon partitioning in individual organs within the context of the whole plant (5, 6).

**MATERIALS AND METHODS**

**Plant Material**

Bean plants, *Phaseolus vulgaris* L., cv Black Valentine, a determinate variety, were grown hydroponically in \(3 \times 10^3\) cm\(^3\) containers under a 14-h photoperiod as described previously (8). Irradiance was 0.75 mmol quanta m\(^{-2}\) s\(^{-1}\) at canopy level. Initial anthesis occurred approximately 28 d AP.

**Measuring Leaf and Fruit Development for an Individual Plant**

Time of flower production was calculated from the day of anthesis. Leaf production was based on leaf emergence reported as days AP when the terminal leaflet of the leaf reached a length of 3 cm. Areas of leaves and dry weight of fruits and seeds were measured nondestructively for four plants at 2- to 4-d intervals for over 2 months.

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2 Abbreviations: AA, after anthesis; AP, after planting.
Plant leaf area was determined at intervals by multiplying the area of the three circumscribing rectangles for the leaflets of each leaf by an empirically determined factor. The latter was calculated from data that were obtained by sampling a large number of leaves of different sizes from other plants growing with the four used for continuous growth measurements. The factor was computed as the ratio of the area of an individual leaf, measured by optical planimeter, and the sum of the areas of the rectangles circumscribing the three leaflets. Because leaf shape in the various plants was quite similar, the value of 0.57 was able to be used for all the conversions.

For calculating overall accumulation of dry weight by the fruits on a plant, the length, width, and thickness of individual fruits were measured and plotted against dry weight. Empirical formulas for computing fruit dry weight from fruit dimensions and final weight were based on a linear regression model established by sampling several hundred fruits of different sizes. Measured dimensions of the fruit were used to calculate estimated fruit dry weight for days 1 to 15 after anthesis. For days 16 to 35 AA, the value for the final dry weight of a fruit was included with the three dimensions to calculate estimated dry weight. Data from other plants, harvested at four times including at the start of flowering, and early and late in fruit development, were found to substantiate the accuracy of the leaf area and fruit dry weight curves. While the ranges of values for cumulative leaf area and for fruit dry weight were up to 25% of the average, the rank order of the values from the four plants was the same for each measurement time. This uniformity resulted in a family of four parallel time course curves of similar shape.

Carbon content was calculated from dry weight measurements on the assumption that 40% of the dry weight is carbon. Measurements of carbon in representative samples of fruits and leaves gave values of 36% to 42% (data not shown).

Measuring Development of Individual Fruits

The time courses for pod wall, seed, and total fruit growth in individual bean fruits were obtained by collecting fruits from plants at 10-d intervals starting near the end of the flowering period. 40 d AA, when fruits 0 to 10 d AA were present. Starting at about 10 d AA, fruits were able to be separated into seeds and pods, and fresh and dry weight were obtained for the fruit and its individual seeds. Fruits with fewer than five seeds were excluded from the time course because their fruits were smaller throughout their development.

Measuring Carbon Accumulation by Steady-State Labeling

Accumulation of recently assimilated carbon imported by parts of the plant during the 24-h experimental period was determined by measuring the tracer carbon present in these parts following steady-state labeling (8). Tracer carbon is the $^{14}$C and associated $^{12}$C that were assimilated during the 14-h labeling period. To make these measurements, the entire plant shoot was placed in an enclosure in which the atmospheric CO$_2$ was maintained at a constant concentration throughout the light period by supplying $^{14}$CO$_2$ with a specific radioactivity of approximately 20 kBq mg$^{-1}$ C. Flux of CO$_2$ into the atmosphere of the labeling apparatus was measured by a mass flow controller (model FC 260, Tylan, Carson, CA) that also regulated the level of CO$_2$ within a set range, based on data from a nondispersive infrared CO$_2$ analyzer (LIRA 3000, MSA, Pittsburgh, PA). Relative humidity in the enclosed atmosphere was regulated to maintain the desired dew point, while fans provided mixing and reduced boundary layer resistance over the leaf surfaces.

For plant growth analysis, plants that had been kept in labeled CO$_2$ through the light period were freeze-dried, weighed, and reduced to a fine powder. Weighed aliquots were oxidized to measure radioactivity and carbon content (8).

Respiration Rate of Seeds

Oxygen uptake was measured with a YSI model 53 oxygen analyzer (Yellow Springs, OH). Seeds were collected just prior to the measurement and placed in 4 mL of 200 mM sucrose solution at 25°C for the determination of oxygen uptake. Tabulated values for oxygen solubility in water at the assay temperature were used for calculating oxygen consumption rates.

RESULTS

Plant Leaf Growth and Photosynthesis

Cumulative leaf area (Fig. 1B) and area growth rate (Fig. 1C) increased during the first 6 weeks of vegetative growth as a result of continuing leaf initiation (Figs. 1A and 2). Emergence of individual leaves followed a similar pattern in each of the plants. Primary leaves emerged about 4 d AP and trifoliate leaves emerged principally between 16 and 36 d AP, with the highest frequency in the middle of this period (Fig. 1A). The enlargement rates of successive trifoliate leaves were similar for the majority of leaves on a plant (Fig. 2), but the rates slowed progressively for the last several leaves. There was some variation in the total number of leaves and the time of their emergence, and this contributed to the variation in total leaf area among the four plants. From 12 to 19 trifoliate leaves were present on a mature plant (Fig. 1A), corresponding to the low and the high range of leaf area values in Figure 1B. Leaf area increased until about 45 d AP and then decreased as leaves began to senesce (Fig. 1B).

Photosynthesis rate per unit leaf area, measured for the entire canopy, was highest during the first 3 weeks AP and gradually decreased as leaves aged, and mutual shading increased with increasing leaf area index (data not shown).

Plant Reproductive Growth

Anthesis started on day 26 to 30 in the four plants for which data are presented (Fig. 1A). The plants produced from 21 to 32 flowers, mostly over a 10- to 12-d period and had an abortion rate of 20% to 30% (data not shown).

Total dry weight accumulation in the fruits began to increase markedly near the middle of the main flowering period and reached its highest rate about 5 d later, that is, 2 weeks after first anthesis (Fig. 1, B and C). The rate held nearly steady for about 10 d and thereafter decreased gradually over the next 3 weeks.
CARBON PARTITIONING DURING DEVELOPMENT

Figure 1. Times of leaf and flower initiation and growth in overall leaf area and leaf and fruit dry weight in bean plants. A, Time of leaf emergence (●) and anthesis (●) in days after planting for four plants. B, Dry weight accumulation in fruits (●, left ordinate) and growth in overall leaf area (▲, right ordinate). C, Rates of dry weight accumulation (left ordinate) for leaves (solid line) and for fruits (dashed line) obtained from the smoothed curves in B. Fruit dry weight and leaf area were calculated from measurements of leaf and fruit dimensions at the times indicated by data points. Averages and ranges are shown for data obtained from four plants. The decrease in area after 45 d was determined from the area lost when leaves senesced. Leaf dry weight values were derived from leaf area values by means of specific leaf area measurements taken at the sampling times. Carbon accumulation rates (right ordinate) were calculated from leaf dry weight.

Growth of Individual Fruits and Seeds

Accumulation of dry weight by individual bean fruits, their pod walls, and enclosed seeds is shown in Figure 3. Fruits began to grow in length soon after anthesis (Fig. 3B), and appreciable dry weight began to accumulate several days thereafter. Fruits with fewer than five seeds grew more slowly, had a shorter final length (Fig. 3B), and accumulated less dry weight. Nearly all fruits on the plants had five to seven seeds, and these showed no clear relationship between seed number and final length or dry weight accumulation. For this reason, analysis of growth of individual fruits, pods, and seeds generally was limited to data from fruits with five or more seeds.

Although seed volume increased as fruits grew in length, appreciable dry weight accumulation by individual seeds occurred only after the fruits stopped elongating, about 12 d AA (Fig. 3, B and C). From about 19 to 22 d AA, there was a temporary decrease in growth in dry weight accumulation by the seeds, followed by an increase in the rate of dry weight accumulation by fruits. Both pod walls and seeds lost dry
weight during the final period of maturation starting about 30 d AA. The timing of developmental events in individual fruits is summarized in Figure 4.

**Dry Weight Accumulation by Individual Seeds**

Accumulation of tracer carbon by individual seeds (Fig. 5) confirmed the pattern for the rate of dry weight accumulation in seeds during their growth (Fig. 3C). Variation in import rate among seeds of a given age, as measured by tracer carbon accumulation, became quite large during the phase of cladode development (Fig. 5).

Tracer carbon accumulation among seeds in an individual fruit varied with seed dry weight (Fig. 6). The degree of dependence, slope of the regression line, was similar for fruits of the same age but changed with age. The correlation coefficients for the regression lines were in the range of 0.74 to 0.84.

**Respiration of Individual Seeds during Development**

Respiration per unit of dry weight, measured as oxygen consumption, was highest in young seeds and decreased rapidly as their dry weight increased (Fig. 7). As a result, total oxygen uptake per seed increased rapidly with initial dry weight accumulation up to about 10% of final weight (Fig. 5). For the remainder of seed development, respiration in individual seeds remained relatively steady. Respiratory carbon loss was calculated from oxygen consumption rate on the assumption that the respiratory quotient was 1, in the absence of measured values. It is unlikely that all of the carbon respired was from the newly imported carbon and, for this reason, the calculated rates of import likely are overestimate. There was no marked change in respiration rate that corresponded to the transient decline in dry weight accumulation midway in seed development.

**DISCUSSION**

**Partitioning of Carbon between Vegetative and Reproductive Growth**

The relative frequency of development of vegetative and floral buds during plant growth (Fig. 1A) was consistent with the pattern of partitioning of carbon between leaves and fruits expected for a determinate variety of bean. Leaf initiation and subsequent growth formed a positive feedback loop with total leaf photosynthesis and resulted in a 6-week period of rapid leaf growth (Fig. 1C) and increasing canopy photosynthesis. The timing of anthesis of individual flowers (Fig. 1A), along with the relatively uniform time course for dry weight accumulation in individual fruits (Fig. 3), produced the sigmoid time course for overall fruit dry weight accumulation (Fig. 1B). As a result, there was a marked increase in the proportion of newly assimilated carbon partitioned to reproductive growth, beginning about 1 week after the start of anthesis (8). The diversion of carbon to development of young fruits (Fig. 1B) likely was a dominant factor in the cessation of flowering and increasing abortion of flowers, which were observed late in the anthesis period in this determinate variety (data not shown). It appears that growth of the individual developing fruits was not altered to a noticeable extent by the overall determinate development pattern of the plant.

**Growth and Development of Individual Fruits and Seeds**

Three periods of fruit growth were observed during the development of individual fruits. During the first 12 d following anthesis, fruit grew rapidly, primarily by elongation and by dry weight accumulation in the pod wall (Fig. 3, B and C). Thereafter, fruit growth consisted mainly of the two phases of diauxic seed growth (Fig. 3, A and C; ref. 3). During the first period, seed growth appears to result mostly from growth and dry weight accumulation by the seed coat (10, 12, 13, and refs. in Fig. 4). Although the volume of a seed increases rapidly soon after anthesis (12, 13, 22), the enclosed ovule remains small during this time (17). The second phase of rapid seed growth appears to occur mainly from accumulation of dry weight in the cotyledons as starch and seed proteins (17). The cause for the period of slow growth that separated the two periods of rapid growth is not known, although there are a number of possibilities, such as transition from a period of rapid cell division to a period of synthesis of compounds that are stored in the developing cotyledons (4, 22, and refs. in Fig. 4). It is likely that termination of the expression of certain genes also is involved (15).

**Import of Carbon by Seeds**

The rate of tracer carbon gain by individual seeds over a 24-h period (Fig. 5) generally increased with seed age until maturation and showed a time course similar to that for gravimetrically measured dry weight accumulation in the seeds contained in a single fruit (Fig. 3C). Import during the same period is the sum of net carbon accumulated by a seed plus its respiratory loss of carbon imported during the period.
The latter is not measured readily, but was estimated from the rates of oxygen uptake (Fig. 7), which was relatively constant throughout most of seed development. As a consequence of the latter, the ratio of carbon lost by respiration:imported carbon accumulated decreased with age. For seeds 8 d AA, the ratio was about 2:3, and this value decreased to less than 1:9 by 30 d AA. The pause in dry weight accumulation midway in seed development appeared to result from a marked slowing of import rather than a high rate of carbon loss by respiration at that time (Fig. 7).

The rate of import into individual seeds in the same pod varied, and these differences appeared to be a function of their dry weight (Fig. 6). For reasons not known, the degree of this dependence changed at different stages of seed development (Fig. 6). As seeds filled, specific import, the ratio of carbon imported per day to the standing dry weight, decreased from 0.075 per day at 8 d AA to 0.0045 per day near end of seed growth (Fig. 5).

**Studying Potential Mechanisms That Regulate Carbon Import during Seed Growth**

The contribution of a sink to keeping itself supplied with organic solutes, sometimes termed potential sink strength (11), includes physical factors and physiological processes that are involved in import of assimilated carbon in the phloem and its subsequent unloading from the sieve elements (5). During at least part of bean seed development, solutes that are imported through sieve elements are thought to enter the reticulate phloem of the seed coat and exit symplastically into
the parenchyma of the seed coat (21). Solute and water exit through the membranes of the seed coat parenchyma and form the apoplast solution that bathes the developing embryos (16, 21). Under these circumstances, increases in membrane permeability and in the rates of metabolic processes that prevent solute build-up in developing cells would promote import of solutes.

In many plants, sucrose is a major component of the imported solutes and a common starting point of sink carbon metabolism (1). In view of this, it has been proposed that, in plants, glycolysis should be more correctly termed sucrolysis (20). Activity levels of the enzymes of sucrolysis, which convert and metabolize sucrose for respiration and synthesis (2, 19, 20), likely are important in sustaining the supply of carbon.

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Figure 4. Timing of developmental events in the growth of bean fruits under controlled conditions. Seed dry weight is for a single seed. Time shown is days after anthesis.

Figure 5. Rates of tracer carbon accumulation and respiratory loss by individual seeds over 24-h periods throughout the course of fruit development. The sum (○) of tracer carbon accumulation rate (●) and the loss rate for respired carbon derived from Figure 7 likely gives an overestimate of import rate. The standard deviation bars for carbon accumulation data are not shown if they fall within the symbol. The data are from 145 seeds from 32 fruits. Time shown is days after anthesis.

Figure 6. Relationship between seed dry weight and rate of tracer carbon accumulation for seeds of a given age. Scales for the bottom abscissa and left ordinate refer to data from days 16, 23, and 29 after anthesis, and the top abscissa and right ordinate refer to the data for 12 d after anthesis only. Dashed lines are for the overall regression data for seeds from three fruits. Regression lines (solid lines) for seeds from individual fruit and for individual fruit are shown for each set of seeds for three fruits (●, ○, ▲) analyzed at 12 and 29 d after anthesis. Two regression lines coincide for data from individual fruits for day 12. To avoid crowding, data and regression lines for individual fruits are not shown for values from 16 and 23 d. Correlation coefficients for regression lines fell between 0.77 and 0.84.
to sink tissues. Consequently, it is likely that regulation of the enzymes responsible for sucrolysis (2, 19, 20) and for the various processes that utilize imported sucrose (Fig. 4) play an important part in the changes of import into seeds observed in the present study.

Times during seed development when there are changes in rates of carbon accumulation or shifts from one dominant carbon-consuming process to the another are times when the processes that sustain import and regulate partitioning among sinks likely are most in evidence. Knowing the time course for dry weight accumulation in individual fruits and seeds is helpful for scheduling sampling for studying these processes. Because of variability among seeds and fruits measurements of rates and current import by steady state labeling prior to sampling allows us to evaluate the roles of molecular events related to sucrolysis and synthesis in regulating import into developing seeds and fruits. Currently, we are studying the involvement of gene activation and the activity of key enzymes of sucrolysis and molecular synthesis in regulation of carbon import by individual seeds and carbon partitioning among seeds and fruits.

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LITERATURE CITED