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USE OF THE UNSTABLE ISOTOPE CARBON-11 FOR THE STUDY OF CARBON FIXATION TRANSPORT AND ALLOCATION IN ROOT AND TUBER CROP

Utilisation de l'Isotope instable Carbone-11 pour l'Etude de la Fixation du Transport et de Préparation du Carbone chez les Plantes à Tubercule

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SUMMARY

The short-life isotope, ^{11}C, offers two extremely important advantages in the study of root and tuber crops. It emits a form of radiation (gamma rays) which can be measured quantitavely and nondestructively in intact plants and it decay quickly to background levels allowing the experiment to be conducted repeatedly on the same plant or group of plants.

We describe both the methodology utilized with 11 C and the actual fixation, transport and allocation of photosynthetic carbon in the sweet potato, *Ipomea batatas*. We monitored net carbon dioxide exchange; absolute photosynthesis; photosyntate pool sizes, turnover and storage rates, photosynthate export rate and direction, concentration and velocity of translocates throughout the plant; and source sink relationship through time. This information was used to develop strategies for genetically enhancing the productivity of the sweet potato and other root and tuber crops.

RESUME

L'isotope ^{11}C à courte vie offre deux avantages extrêmement importants pour l'étude des cultures de tubercule. Il émet des rayons gamma mesurables sans que l'on sacrifie la plante et revient rapidement à des niveaux normaux, l'expérience pouvant ainsi être répétées sur la même plante.

La méthodologie utilisée, la fixation, le transport et la distribution effective du carbone chez la patate Ipomea batatas *sont décrits. On a suivi l'échange net de CO2; la photosynthèse absolue; la dimension, les taux de rotation et de stockage des pools photosynthétiques; la direction et le taux d'exportation des produits photosyn-*

thétisés, la concentration et la rapidité des translocations à travers la plante; et les relations source-puits au cours du temps. Cette information a été utilisée pour élaborer des stratégies de renforcement génétique de la productivité de la patate et d'autres plantes à tubercule.

Plant breeding methods have evolved through a series of stages over the past 100 years to meet our needs for increased productivity. In the past, selection decisions were made largely upon final yield and the general resistance to insects and diseases. For many crop plants, however, we have reached a point where yield increases are becoming substantially more difficult to achieve. As a consequence, it has become increasingly essential for breeders to understand the many physiological and morphological interrelationships that collectively impart high yield in their respective crops. This, however, requires expertise well beyond the scope of genetics and plant breeding. Because of this, breeding is becoming more and more a team approach where physiologists, food scientists, entomologists, and pathologists work together with the breeders and molecular geneticists to develop new, higher yielding cultivars.

Plant growth is controlled by a number of complex, integrated biophysiological and biochemical processes. From kinetic studies of enzymatic control in biochemical pathways has evolved the concept of "rate limiting steps". A rate limiting steps represents the slowest single reaction in a series of reactions and a consequence is the major factor controlling the final rate at which the entire series of reactions can proceed. Thus, if we envision an extreme hypothetical case of a plant that has a single rate limiting step; e.g. the rate of chlorophyll synthesis in a low chlorophyll mutant, then genetically eliminating this step should result in a significant yield increase. In those cases where a single step significantly limits final yield, our success in genetically circumventing the problem is generally very good. More commonly, however, there are multiple steps that limit yield and our potential for success decreases exponentionally with the number of improved steps that must be incorporated into the new line. For example, in a series of reactions where the rate is indicated by the width of the arrow (Figure 1a), increasing the rate of reaction r_1 when the différential in rate between r_1 and r_2 is very small will result in an equally small increase in yield under ideal conditions. Likewise, when there are essentially equally rates for multiple steps ($r_1' \equiv r_5' \equiv r_7' \equiv r_{12}' \equiv r_{14}'$) (i.e. colimiting steps) in a series of integrated reactions (Figure 1b), the improvement in only one step will not result in an increase in yield. If one has exceptional luck and is able to incorporate simulanious improvements in each step, a large yield increase may be realized. However, if we improve 5 of the 6 steps and use final yield as our selection criteria, the progeny is going to be discarded.

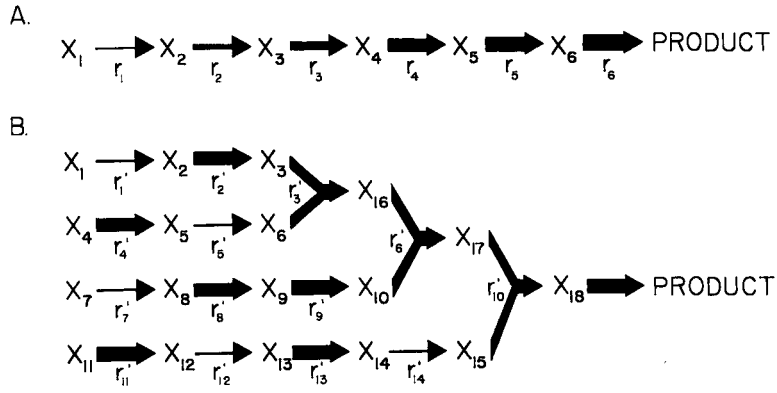


Figure 1. Schematic presentation of (A) a series of reactions and (B) an integrated series of reactions where the rate of each reaction (r) is characterized by the width of the arrow (narrow = slow, wide = rapid).

As a consequence ascertaining which step or steps are rate limiting is an essential requisite for systematically improving the species. Researchers have approached the problem by measuring general processes (e.g. photosynthate transport or a specific component of transport) and/or by trying to identify critical individual biochemical reactions. Even with identification of a target trait or traits, one must have a sufficiently precise selection criteria for monitoring improvement. This will allow the progressive incorporation of the desired traits even though their individual incorporation may not be seen in the short run as a yield increase at harvest.

Our understanding of the basic physiology of many of the tropical root and tuber crops has lagged behind that of the major temperate zone annual domesticates. There are several reasons for this. First and perhaps one of most important is funding. Most of our crops are of either very minor or of no importance in the agriculture of the northen temperate zone where much of the funding for agricultural research originates. Typically, the amount of money allocated for research on sweet potatoes, cassava, yams, or the tropical aroids is only a minute fraction of that devoted for either maize or soybeans. When assessed relative to the very limited support for research on root and tuber crops, we have, in many cases, made quite remarkable progress.

Secondly, root and tuber crops, endemic to the tropics and subtropics, display survival and reproductive strategies that are distinct from most annual domesticates of the

temperate zones. Root and tuber crops store accrued carbon in specialized fleshy organs and it is evident from the net carbon allocation patterns in these plants at harvest, that the interrelationship between the aboveground plant parts and their root system is distinctly different from other domesticates. For example, the fleshy subterranean sinks for photosynthate in root and tuber crops are generally highly plastic in total volume and length of time for deposition of carbon, in contrast to domesticates such as cereals. Hence, advances in our basic understanding of the physiology of the major temperate zone domesticates often do not translate into comparable advances in understanding of root and tuber crops.

Finally, many of the tropical root and tuber crops have envolved asexual reproduction strategies which have impeded genetic manipulation of the crop. For example, the sweet potato, *(Ipomoea batatas)*, is an autohexaploid with high levels of incompatibility and genetic instability. Likewise, several of the aroids do not flower readily, greatly decreasing the number of generations that can be produced in a given time period.

In addition to specific problems confronted in the improvement of tropical root and tuber crops, our ability to identify and study rate limiting steps in all crop plants has been impeded by lack of research and inadequate research tools. Technological advances often open the door for a rapid increase in our understanding of how plants function. One such recent advance has been the utilization of the short-lived isotope of carbon, ^{11}C especially when coupled with steady state labeling[6].

Short-lived isotopes such as ^{11}C, ^{13}N, ^{15}O, and ^{18}F, once the exclusive domain of physicists and chemists, are now being increasingly utilized to understand the complex, integrated biophysical and biochemical processes in man, animals and plants. In the past two decades short-lived isotopes have found their way into the biological sciences (including preclinical and clinical research and diagnosis in medecine). Collectively these isotopes are characterized by their short half-lives, ranging from 2.5 to 110 minutes, and their energy emission upon decay. Although they must be produced adjacent to where they will be used, they provide a number of extremely powerful advantages for research.

^{11}C, like the other short-lived isotopes has two principal advantages : 1) it emits a form of radiation which can be measured quantitively and nondestructively in live, intacts plants, animals or humans; and 2) it decays quickly to background levels allowing the experiment to be conducted repeatedly on the same subject and as a consequence does not pose significant health of disposal problems. ^{11}C has a tremendous advantage over the more stable isotope of carbon, ^{14}C, in that it decays by positron-emission, followed by a nuclear annihilation reaction, emitting two 0.5 million volt V-rays which leave the decay site at 180° orientation

to another. This unique property has allowed the development and utilization of two very powerful research tools: 1) coincidence counting; and 2) positron-emission tomographic imaging. These two techniques afford the researcher the ultimate goal in any biological study, simultaneous collection of qualitative and quantitative information on the dynamics of a physiological process in a living system.

To illustrate the potential for the use of ^{11}C in the study of root and tuber crops, we describe recent work[5] by our research group characterizing the fixation, transport, and allocation of carbon in the sweet potato.

METHODS AND MATERIALS

The research was conducted at the Duke University Phytotron due to the availability of a 4 MeV Van de Graaff accelerator in the nearby (~100m) in the Physics Department Nuclear Laboratory. Although the research we describe is on the sweet potato, it could be just as readily conducted on cassava, yams, or any of the many other root and tuber crops grown around the world.

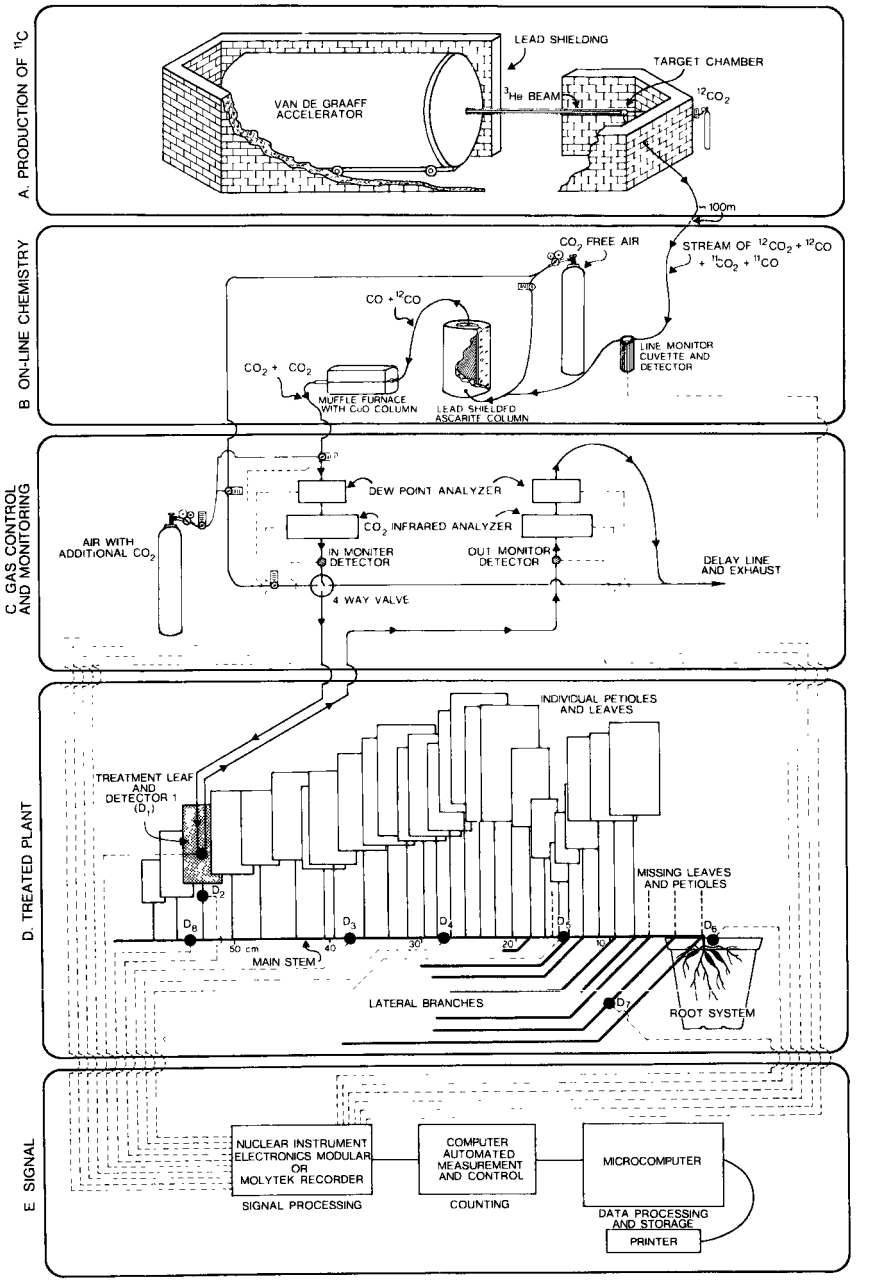
Plants for the study were grown under controlled environment conditions in the Phytotron. The precise geometry of the plant (node number from the base and node length; petiole length and position; and leaf blade length and width) was ascertained several days before each run. This allowed precise positioning of detectors and knowledge of their exact locaton from the source leaf.

Isotope Production and On-line Chemistry: A continuous flow of ^{11}C was produced by bombarding a stream of $^{12}CO_2$ in a specially constructed target chamber with a ^{3}He beam[6] (Figure 1a). The nuclear reaction[1] was ^{12}C (^{3}He, ^{4}He) ^{11}C with the ^{3}He beam produced using a 4 MeV Van de Graaff accelerator. This provided a continuous stream of $^{12}CO_2$, $^{11}CO_2$, ^{11}CO and ^{12}CO which flowed through a Teflon capillary line from the accelerator to the Phytotron where the labeling experiments were conducted.

To increase and control the specific activity of the carbon dioxide, it was essential to remove the $^{12}CO_2$ and $^{11}CO_2$ present in the stream of gases. This was accomplished by passing the gases through an Ascarite column which removed CO_2 but not CO. The CO_2 free air was then passed through a heated column of CuO (600°C), which oxidized the ^{11}CO and $^{12}CO_2$ to $^{11}CO_2$ and $^{12}CO_2$ (Figure 1b). This resulted in approximately a 1000 fold increase in specific activity over the original gas mixture from the target chamber. The gases were then passed through a dew point analyzer to determine the moisture content, an infra-red CO_2 analyzer to determine total CO_2 concentration and a curvette equipped with a -ray detector to determine the specific activity of the gas mixture (Figure 1c). The flow rate and CO_2 concentration were adjusted with CO_2 free air or air with supplemental CO_2[20].

Application of $^{11}CO_2$ to the plant. The $^{11}CO_2$ containing air and CO_2 appropriate concentration (375 cm^3m^{-3}) (tested by bypassing the leaf chamber to the infrared CO_2 analyzer), before passing through a curvette-detector arrangement to monitor the specific activity of the gas ("In Monitor"). The air with $^{11}CO_2$ then moved into a specially constructed clear plastic Lexan^R leaf chamber with a water jacket on the top and bottom of the leaf (Figures 1d and 2). The chamber was constructed of a 2-mm thick closed-pore neoprene gasket on each water jacket. The gas was passed over the upper surface of the leaf (20 cm^2), exiting the opposite end, then entered the lower chamber passing over the lower surface of the leaf. The exiting gas then moved through a second curvette-detector ("Out Monitor") where the remaining $^{11}CO_2$ was measured. The $^{11}CO_2$ could then be determined by subtracting the radioactivity of the "Out Monitor" from the "In Monitor" and correcting for decay and detector sensivity. The gas subsequently flowed through a second infra-red CO_2 analyzer and dew point analyzer before passing on to a delay line and vent (Figure 1c).

Figure 2. Schematic presentation of the system used for steady-state production of ^{11}C and measurement of its introduction into and transport within a sweet potato plant. (A) ^{11}C is produced by bombarding a stream of $^{12}CO_2$ in a specially construted target chamber with a beam of ^3He from a Van de Graaff accelerator (4 Mev.) (B) The carbon products of the reaction, $^{11}CO_2$, ^{11}CO, $^{12}CO_2$, ^{12}CO, are transported to an on-line chemistry station within the Phytotron. Carbon dioxide is removed from the air stream using a shielded ascarite column. The remaining carbon monoxide (^{11}CO and ^{12}CO) is oxidized by passing the gas through a CuO column at 600°C. (C) The air containing $^{11}CO_2$ is monitored for flow rate, moisture, CO_2 concentration and specific activity (In Monitor). Air with additional CO_2 or without CO_2 is added at a 4-way valve to maintain the CO_2 concentration at 375 cm^3 m^{-3}. (D) The air containing $^{11}CO_2$ enters a leaf chamber (see Figure 2), passing over the upper surface of the leaf then over the lower surface. It then moves to an "Out Monitor" (C) to determine the change in radioactivity, and a CO_2 analyzer and dew point meter before passing into a delay line and vent. The plants (D) had detectors (denoted as D_{1-8}) at various positions on, above, and below the treated leaf. Illustrated is the precise position of each leaf and lateral branch from the base of a representative plant, and the length of the individual petioles and leaf blades. (E) Electrical signals from the various detectors and other monitoring equipement were fed into a nuclear instrument electronic module for signal processing, then to a computer automated measurement and control unit for quantification and finally to a HP microcomputer for data processing and storage (after Kays et al. [5].)


A. PRODUCTION OF ^{11}C
LEAD SHIELDING
TARGET CHAMBER
VAN DE GRAAFF ACCELERATOR
^{3}He BEAM
$^{12}CO_2$
100m
B ON-LINE CHEMISTRY
CO_2 FREE AIR
STREAM OF $^{12}CO_2 + ^{12}CO + ^{11}CO_2 + ^{11}CO$
$CO + ^{12}CO$
$CO_2 + CO_2$
LINE MONITOR CUVETTE AND DETECTOR
MUFFLE FURNACE WITH CuO COLUMN
LEAD SHIELDED ASCARITE COLUMN
C. GAS CONTROL AND MONITORING
DEW POINT ANALYZER
CO_2 INFRARED ANALYZER
AIR WITH ADDITIONAL CO_2
IN MONITER DETECTOR
OUT MONITOR DETECTOR
DELAY LINE AND EXHAUST
4 WAY VALVE
D. TREATED PLANT
INDIVIDUAL PETIOLES AND LEAVES
TREATMENT LEAF AND DETECTOR 1 (D_1)
MISSING LEAVES AND PETIOLES
D_2
D_8
D_3
D_4
D_5
D_6
50 cm
40
30
20
10
MAIN STEM
LATERAL BRANCHES
D_7
ROOT SYSTEM
E. SIGNAL
NUCLEAR INSTRUMENT ELECTRONICS MODULAR OR MOLYTEK RECORDER
SIGNAL PROCESSING
COMPUTER AUTOMATED MEASUREMENT AND CONTROL
COUNTING
MICROCOMPUTER
DATA PROCESSING AND STORAGE
PRINTER

Positioned below the leaf chamber was a single γ-ray detector to monitor the build-up of activity within the labelled leaf (Figure 2). The "Line", "In Monitor", "Out Monitor" and "Leaf" detectors were lead shielded to prevent excitation by other sources of radiation.

Additional detectors were positioned on the plant's main stem at various intervals from the treated leaf to monitor the movement of labelled photosynthates throughout the plant (Figure 1d). These detectors were positioned as pairs on each side of the main stem to allow coincidence counting. Because the energy level of the emitted γ-rays was sufficiently high to excite any unshielded detectors in the room, interference by peripheral radiation was eliminated by time-coincidence counting. Since 2 γ-rays are emitted from a single positron at an 180° orientation from one another, the paired detectors were electronically required to "see" both γ-rays simultaneously. This allowed precise localization of the source (decaying ^{11}C) and minimization of undesired background. The geometry of the paired detectors and the stem was therefore critical. This was standardized using specially constructed styra-foam fittings. Generally, 5 pairs of detectors in addition to the single detectors previously mentioned were used; one on the petiole of the treated leaf, one on the main stem above the treated leaf and three on the main stem below the treated leaf (Figure 1d). One additional shielded detector was placed on the media surface over the developing storage roots.

The detectors consisted of either NE 102 (singles detectors) or CsF (coincidence detectors) optically coupled to photomultiplier tubes.[20] Each detector or set of paired detectors were calibrated prior to and after each run using a specially configured γ-ray source (^{22}Na).

Signal Processing and Counting. Signals from the detectors were processed using a standard nuclear instrumentation electronics moduler (NIM) and counted with a computer automated measurement and control system (CAMAC) (Figure 1e). Modules in the CAMAC also received signals from the CO_2 infra-red analyzers, dew point analyzers and flow meters. The CAMAC was interfaced with a Hewlett Packer 9845B (option 250) microcomputer with 186K byte memory. This allowed CRT graphic display of the experimental data in real time as the experiment progressed and direct screen copies using a thermal printer. Raw data inputs were displayed every 60 sec on the CRT during the course of the experiment. The specialized software for data handling and manipulation was developed by one of the authors (C.E.M.). Additional details on signal processing and counting have been published previously[6].

Data Manipulation and Parameters Measured. Due to the high energy of emitted V-rays and the short half-life

of ^{11}C (20.4 min), a number of computations must be made during the processing of raw data. These include corrections for background, induced background, calibration sensitivity of each detector or pair of detectors, geometry of the detectors, relative specific activity ($^{11}CO_2/^{12}CO_2$) and decay of ^{11}C during transit. In addition, data inputs were made for plant geometry, detector location on the plant, leaf chamber area and flow rate which were utilized in several specific computations. All raw data were procesed using a smoothing routine to minimize random data spikes[7].

We measured the following characteristics of the carbon aquisition, transport and allocation system within the sweet potato.

a. Leaf Parameters	Units of Measurement
1. net rate of ^{11}C assimilation	nCi $cm^{-2}sec^{-1}$
2. rate of export of ^{11}C photosynthate from the leaf	nCi $cm^{-2}sec^{-1}$
3. rate of storage of ^{11}C photosynthate in the leaf	nCi $cm^{-2}sec^{-1}$
4. turnover time of the export pool	min
5. percent export	%
6. steady-state ^{11}C activity of the export pool Y/	nCi cm^{-2}

b. Transport Parameters	Units of Measurement
1. speed of photosynthate transport throut the plant	cm min^{-1}
2. mean transit time to various sites in the plant	min
3. average steady-state activity at the various sites	nCi cm^{-1}(stem)cm^{-2}

In order to obtain both time dependent and steady-state information, we used an extended square wave of radioactivity which gave a leading edge, a steady-state portion, and a tailing edge once labeling was discontinued. Carbon allocation within the leaf was treated as a 2 compartment system where activity from the storage compartment is lost only by isotope decay and activity in the export department is lost both by decay and export Z/ (i.e. replaced by ^{12}C in a steady-state turnover of carbon). Parameters such as carbon exchange rates, relative pool sizes, turnover rate, and export rate were calculated through the analysis of the leaf radioactivity loss curve after cessation of steady-state labeling. For a detailed description of the theoretical assumptions and mathematics used to analyze steady-state and time dependent curves of the isotope within the leaf, see Fares, et al.[2]

The net storage rate was determined from the amount of ^{11}C remaining in the leaf after cessation of labeling (corrected for half-life, background and induced background). The export component was calculated from the slope of the washout curve which was an exponential function.[2] The total rate of assimilation was the composite of the storage rate and the export rate. Export turnover time was determined from the steady-state activity level in the export pool and the rate of export of label from the export pool.

Transport speeds between various positions on the plant were calculated from the arrival time of the ^{11}C front at each pair of detectors minus the arrival time at the nearest upstream detectors and the distance (cm) between the respective pairs of detectors on the stem. Arrival time was determined by using an expanded arithmetic plot of the counts min^{-1} in paired detectors along the transport path (corrected for background, induced background). Arrival of the ^{11}C front at each position was indicated when counts in the coincidence detectors increased sharpely (i.e. $>$ 1% of the final level). A line was then fitted from the first data point above 10 CPM through the next 15 points upward (60 sec intervals). The time where this fitted line extrapolated through zero CPM was used as the arrival time. Distances between individual detectors were automatically calculated from X-Y coordinates entered at the begining of the experiment and the mean transit speed (cm min^{-1}) determined between each set of coincidence detectors.

RESULTS AND DISCUSSION

Representative data characterizing mid-day photosynthate fixation, allocation and export patterns in a fully expanded apical leaf of sweet potato is presented in Table 1. This includes information on the rate of ^{11}C assimilation, the size of the pool of photosynthate that is to be exported out of the leaf, the rate at which ^{11}C-photosynthate is exported from the leaf storage pool of carbon (presumably for export during the night), the length of time required for one complete turnover of carbon in the export pool and the percent of the photosynthate that is exported from the leaf.

The rates of transport of photosynthate between the petiole of the labeled leaf (D_2) and various positions down the mainstem toward the storage roots are presented in Figure 3. Basipetal transport velocity was not constant throughout the plants and changed significantly depending upon the time of the day it was assessed. During the morning and mid-day there typically was an increase in transport speed toward the base of the plant. This appeared to decline somewhat upon entry into the root system.

Table 1. Representative data characterizing the photosynthate fixation, allocation and export system within a fully expanded apical sweet potato (cv. Jewel) measured at mid-day. The plants had 140 to 160 leaves, a LA of 4000 to 4500 cm^2 and small storage roots.

Net rate of ^{11}C assimilation	1.54 nCi $cm^{-2}sec^{-1}$
Size of the leaf export pool	946 nCi cm^{-2}
Rate of export of ^{11}C photosynthate from the leaf	.491 nCi $cm^{-2}sec^{-1}$
Rate of storage of ^{11}C photosynthate within the leaf	.608 nCi $cm^{-2}sec^{-1}$
Turnover time of the export pool	22.3 min
% export of photosynthate	44.7 %

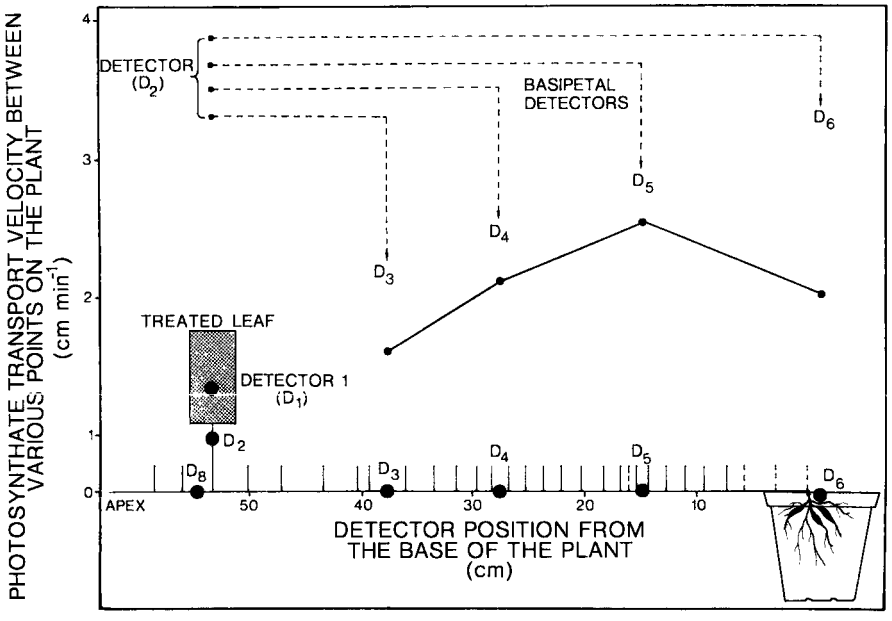


Figure 3. Changes in the mean transport velocity of photosynthates between the petiole of the treated leaf (detector 2) and various basipetal positions on the mainstem from the treated leaf.

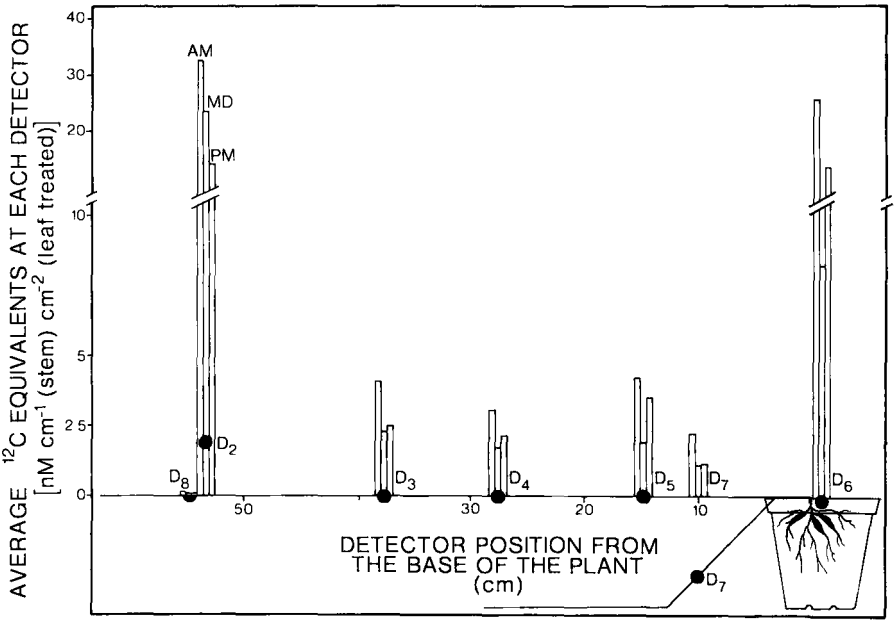


Figure 4. The average ^{12}C equivalents of photosynthate from the treated leaf at each detector and changes in the concentration at three times during the day AM, mid-day (MD) and PM (after KAYS et al. [5].

The average ^{12}C equivalents at each detector position on the plant provides a measure of how the photosynthate is being allocated within the plant (Figure 4). Maximum allocation was into the root zone where there were developing storage roots. Essentially no photosynthate was translocated acropetally from the treated leaf, suggesting that the growing tip of the vine is dependent upon carbon from other sources and/or carbon which is recycled from the storage pool of photosynthate within the leaf during the dark period. A small amount of photosynthate was translocated basipetally and then acropetally in lateral branches during the light period.

From this data it can be seen that ^{11}C applied as an extended square wave provides an extremely powerful tool for understanding the basic physiology of root and tuber crops and locating and identifying rate limiting steps or processes in the carbon aquisition, allocation and storage system. This information can be used to assess potential parential genotypes and progeny from breeding programs for traits needed for progressive improvements in yield. For example, high rates of carbon assimilation in some species have been associated with a high percent export of carbon from the leaf during the light period, a trait readily measured using ^{11}C.

Initial research should be directed toward characterizing the basic physiology of each root and tuber crop species. For example, it is necessary to know how the carbon aquisition, transport and storage system changes during the day, and with leaf position on the plant, age of the plant, and sink strength and position. This will allow a more accurate determination of critical traits that differ between high and low yielding lines. Once limiting traits have been identified, a cross section of the genepool of the species is assessed for genotypes that can be used for parental lines. This, in turn, is followed by screening of the progeny from crosses in individual breeding programs.

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