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Effects of an Acute Dose of Gamma Radiation Exposure on Stem Diameter Growth, Carbon Gain, and Biomass Partitioning in *Helianthus annuus*

by

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Abstract

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by Michael E. Thiede

Chairperson of the Supervisory Committee:
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Nineteen-day-old dwarf sunflower plants (*Helianthus annuus*, variety NK894) received a variable dose (0-40 Gy) from a cobalt-60 gamma source. A very sensitive stem monitoring device, developed at Battelle's Pacific Northwest Laboratories, Richland, Washington was used to measure real-time changes in stem diameter. Exposure of plants caused a significant reduction in stem growth and root biomass. Doses as low as 5 Gy resulted in a significant increase in leaf density, suggesting that nonreversible morphological growth changes could be induced by very low doses of radiation. Carbohydrate analysis of 40-Gy irradiated plants demonstrated significantly more starch content in leaves and significantly less starch content in stems 18 days after exposure than did control plants. In contrast, the carbohydrate content in roots of 40-Gy irradiated plants were not significantly different from unirradiated plants 18 days after exposure. These results indicate that radiation either decreased phloem transport or reduced the availability of sugar reducing enzymes in irradiated plants.

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INTRODUCTION

The growth and competitiveness of native and crop plants could be affected by exposure to radioactive sources such as materials from weapons production and tests, industrial waste, nuclear power production, and nuclear accidents such as occurred at Chernobyl in Russia.

Research conducted on radiation effects on plant yield has focused on several areas, including plant lethality, growth rate, germination, photosynthesis, cellular damage, and cell-wall permeability. Studies conducted during the late 1960's and early 1970's examined the effect of acute or chronic exposures of gamma radiation on plant lethality and growth reduction. Irradiation effects varied greatly depending on the developmental stage of the plant at the time of exposure. For example, Davies (1968) noted premature death in spring sown *Triticum* spp. was caused by a dose of 20 Gy before tiller initiation was complete. In the same study, a dose of 1.3 to 7.5 Gy led to enhanced tillering. However, increased tillering has been associated with some retardation in the development of the main shoot. Davies also observed that after tillering was complete, doses of up to 20 Gy did not change the number of tillers or the number of heads. Rice, *Oryza sativa* L. was the most sensitive to radiation during the time from panicle initiation to anthesis (Siemer et al., 1971) and during the stage of meiosis (Kawai and Inoshita, 1965). Irradiation of *O. sativa* with 20 Gy at early growth stages decreased culm length, head length, and the number of spikelets per head (Kawai and Inoshita, 1965).

A number of studies have shown that irradiation of seed can result in decreased germination and reduced seedling growth. Bebawi (1984) found that the germination of *Sorghum bicolor* seeds and the subsequent root growth of seedlings were reduced by exposure to doses as low as 5 Gy. In another study, germination of *Triticum* spp. and *Sorghum* seed was reduced after parent plants were irradiated with 7 to 10 Gy at the anthesis or 1-leaf stage of development (Iqbal and Aziz, 1981). Al-Rubeai and Godward (1983) found that the biomass of the *Phaseolus vulgaris* L. was reduced by exposures of 10 Gy.

Very low radiation doses (1 to 10 Gy) can result in increased growth and yield of plants. Fowler and MacQueen (1972) found that *Triticum* spp. increased in yield when exposed to doses of 10 Gy. The study proposed that yield stimulation from low doses of radiation was due to increased seedling vigor, early modification in the pattern of auxiliary bud development, and changes in the initial rate of floral differentiation. In another study, *Lycopersicon esculentum* yield increased when exposed to doses of 5 to 10 Gy (Sidrak and Suess, 1973). Sheppard et al. (1982) also found increased fascicle needle length and total biomass in *Pinus*

sylvestris L. seedlings when they were exposed for 150 days to doses totaling 7, 26, or 220 mGy. In contrast, a high dose, 20.0 Gy reduced growth.

Cellular damage is another effect that has been observed when plants are irradiated. Meristems of *Lupinus albus* L. showed tunica-corpus damage at dosages at a threshold dose of 85 Gy (Cordero and Gunckel, 1982). In their study, vacuolation seen at 66 Gy was the earliest and most obvious radiation response. Primary tissues derived from the apical meristem showed varied radiosensitivities. The pith followed by the primary vascular tissues were the most sensitive. The epidermis and cortex showed only slight vacuolation. Morphological changes included elimination of leaves, suppression of a median leaf bundle, and leaf initiation failure. A study by Evans (1965) showed cytological changes of meristematic cells of *Vicia faba* exposed to 16 Gy, including mitotic cycle delay, chromosome aberration formation, and premature differentiation or cell death.

Gamma radiation also has been shown to modify plant membrane and cell-wall permeability. For example, radiation stimulated the leaching of reducing sugars, inorganic phosphate, and amylases from *Hordeum* spp. L. seeds (Kurobane et al., 1979). In the study, radiation reduced endosperm metabolism of reducing sugars while production of reducing sugars remained unaffected. This resulted in increased permeability, and excess reducing sugars were leached into the water in which the seeds were placed. Another study suggested that increased permeability affected the uptake and translocation of potassium ions and may influence the ability of stomata to function properly. Active accumulation of potassium ions by guard cells has been shown to be necessary for opening stomata in *V. faba* (Humble and Raschke, 1971). Roy and Clark (1970) found evidence that stomatal opening was inhibited in X-irradiated *V. faba*.

Photosynthetic processes also are influenced by plant irradiation. For example, photosynthesis of *Pinus strobus* L. decreased approximately 40% by exposure to a dose as little as 2.3 Gy (Ursino et al., 1974). Dark respiration of pine seedlings increased 23% by the same exposure. In contrast, a study by Hadley and Woodwell (1966) showed that dark respiration of seedlings *Pinus taeda* and *P. elliottii* was unaffected by doses as high as 130 Gy while photo respiration was reduced by 12.5 Gy. In an other experiment by Roy and Clark (1970), assimilation of $^{14}CO_2$ by *V. faba* was depressed by 2.5 Gy.

Chronic irradiation of old field communities (abandoned agricultural land) over several years has shown that the number of plant species progressively and continuously decreased along a radiation gradient (Woodwell and Oosting, 1965). This study found that about a 50% higher exposure was required the second year

to reduce the number of species along the radiation gradient as compared to the first year. The explanation given was that the dominant species may be inhibited by irradiation, thus permitting increased numbers of radioresistant subordinate species to be established.

Another study by Fraley and Whicker (1973) showed that the effect of radiation on a plant community was dependent upon the seasonal growth stage of the plants. For example, shortgrass prairie species diversity was reduced by 50% when chronically exposed to a dose of 450 Gy in late fall, 570 Gy during the spring, or 1,430 Gy in winter. They explained that the severity of these seasonal responses of vegetation to irradiation depended on whether the plants were in a rapid growth phase with high meristematic activity.

As Fraley and Whicker found, recovery of plant communities often did not occur until the next growing season. During this recovery, dominant plant species may shift from perennial to annual species. Recovery after late-fall irradiation was not seen until spring. Plants recovered faster from spring exposure than from summer exposure, because during the summer many shortgrass species are semidormant.

Measurements of stem diameter changes can be applied to detecting changes in plant growth from radiation stress. This is because changes in stem diameter are primarily a result of growth and stem hydration. Changes in stem growth result from increases in the number and size of stem cells and are irreversible. These growth increases can be measured as total growth in stem diameter or expressed as a growth rate. In contrast, changes in stem hydration are reversible and result from the loss or gain of water by cells. Both demand very accurate measurements to detect small changes. Recent technological improvements in the measurement of stem diameter by this laboratory should permit such a level of accuracy.

Monitoring stem diameter is not new to plant research. Crude band dendrographs used to measure diameter changes of large trees were discussed by Cooper and Herrington (1959) and were accurate to 0.76 mm. Dobbs (1969) described a band type of displacement transducer mounted on a tree stem that would measure circumference changes of about 0.05 mm. The use of linear variable differential transformers (LVDT) to measure diameter changes was discussed by Splinter (1969). Phipps and Yater (1974) compared three types of dendrographs that could measure radial change to 0.01 mm: indicator dendrographs, lever dendrographs, and gear dendrographs. Also LaPoint and Van Cleve (1971) described a portable electronic dendrograph for measuring tree growth.

Stem measuring devices have been used by researchers to observe stem diameter in relation to plant water status. Molz and Klepper (1973) found that rapid changes in stem diameter and stem hydration are caused by changes in plant water potential. They described changes in stem diameter as reversible and that this resulted from changes in the water content of living stem cells. Klepper et al. (1971) used a LVDT to examine the relationship and stem diameter of *Gossypium hirsutum* L. plant water status. This study found a diurnal hysteretic relationship between leaf water potential and stem diameter, suggesting that stem diameter reflects changes in stem tissue hydration. The study also noted immediate changes in stem diameter when plants were sprinkled with water or shaded from the sun with a large black cloth. These results indicated that the stem xylem water potential is affected by changes in evaporation. Molz and Klepper (1973) demonstrated that the mature xylem of *G. hirsutum* stem is a rigid material that shows negligible elastic deformation in the radial direction, and any measurable deformation in a water-stressed stem is due almost entirely to dehydration of the living cells found in the phloem and related tissues. Klepper et al. (1973) showed that when plants were water stressed, leaf water potential and stem diameter both decreased. Garnier and Berger (1986) demonstrated a difference in summer stem growth of water-stressed *Prunus persica* from well-watered trees. Stem diameter measurements in *Quercus alba L.* could be related to base xylem pressure potential (Hinckley and Bruckerhoff, 1975) and was related to phloem tissue hydration (Hinckley et al., 1974).

Most of the dendrographs described in the literature are large and heavy and tend to damage the stem of the plant to which they are attached. Dendrographs such as LVDT's require a reference point and, because of this, they are usually permanently fixed into the xylem by drilling into the tree, or affixed to a stem small enough in diameter so the fixed point and the LVDT are opposing. Other limitations of dendrographs include problems with calibration drift and temperature sensitivity. All the dendrographs described above are limited in their use with woody plants because the plant must support the weight of the dendrograph and attachment injures the tree.

Ceres™, the stem measuring device designed by Battelle's Pacific Northwest Laboratories, Richland, Washington, is light, easily calibrated, and can be attached to weak-stemmed herbaceous plants (Beedlow et al., 1986). This device was designed so that it does not physically damage the plant. Thus, changes in stem diameter with time can be studied to reflect a plant's reaction to environmental stress. For example, in the study by Beedlow et al. (1986) the ability to detect

immediate changes in *H. annuus* stem growth was demonstrated when plants were exposed to automobile exhaust.

The present study uses the Ceres stem measuring device to determine real-time stem diameter growth responses to gamma radiation in *Helianthus annuus* L. and investigates the relationship between these responses and plant growth, carbon fixation, and carbohydrate synthesis. The primary objective is to show that the Ceres device is a sensitive instrument capable of measuring changes in stem diameter due to radiation damage at doses that produce very little visual growth reduction. An additional objective is to investigate the causes of this reduced stem growth, and to determine the relation between reduced stem growth, carbon partitioning, and carbon gain. The hypothesis addressed in this research is that radiation will inhibit the stem growth via effects on meristematic tissue and carbon partitioning. The basis of this hypothesis rests in following reasons: Radiation can have detrimental effects on rapidly dividing meristematic cells, hormone synthesis, and membrane permeability which can affect stem growth. Carbon partitioning is altered by increasing radiation doses due to an adverse effect of radiation on the ability of chloroplast to fix carbon. Carbon partitioning is altered by increasing radiation doses because of damage to radiosensitive cells responsible for the transport of carbohydrates in the phloem.

METHODS AND MATERIALS

IRRADIATION AND DOSIMETRY

Nineteen-day-old *Helianthus annuus* L. plants were exposed to gamma radiation supplied by an AECL Gammabeam-650™ irradiator having an initial (1987) loading of 50,000 Ci of cobalt-60. The unit has 12 vertical tubes into which the cobalt is raised and held in place pneumatically during the timed-exposure period. The 12 source tubes were adjusted to a closed position (7-cm-diameter space between the tubes). Alternate tubes were activated such that only half of the cobalt was used to provide the gamma radiation exposure. The room that contained the source was 7.3 x 7.3 m. The source was in the center of the room.

To improve the exposure symmetry, a revolving table circled the source, and the plant pots were positioned 1 m from it. The table was rotated during exposure. Pots were reversed in direction from the source at the midpoint of each exposure period. Both table rotation and pot reversal were done because the source loading was symmetric but not uniform. The dose rate of the source was calculated by placing groups of thermoluminescent dosimeters (TLD's) at four positions (front, back, bottom, and top of the plant) in the soil-filled pot of a plant receiving the largest dose. Plants were exposed at a radiation dose rate of 2.65 Gy/minute. The standard error of the TLD's readings was 13.7% of the mean TLD reading.

PLANTS AND GROWING CONDITIONS

Seeds of *Helianthus annuus* L. (variety NK894) were procured from Hyslop Farms, managed by the Crop Sciences Department of Oregon State University, Corvallis, Oregon. This plant was chosen for this research because personal involvement in several other studies concerning the effect of ozone or water stress on plant growth. This variety is a dwarf version of *H. annuus* which is more easily grown laboratory studies. Plants were grown in a Kysor-Sherer growth chamber (Model Number CEL-511-38) at a constant temperature of 24°C and a 14-hour photoperiod. Light level was set at 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and plants were grown in MALMO™ commercial potting soil, which is a blend of sterilized sandy loam, compost, and peat moss. Plants were contained in 0.5 *l* plastic pots with holes in the bottom to drain excess water. Pots were watered daily to maintain soil moisture.

STEM MEASUREMENTS

The Ceres™ device (U.S. patent number 4,549,355) was installed directly upon the plant stem portion between the first cotyledon and the soil surface (Figure 1). Each Ceres device contained four strain gauges, connected in a wheatstone bridge arrangement along the inner and outer edges of the transducer. As the plant stem diameter increased, the potential difference in voltage between the inner and outer strain gauges increased and was measured by a Campbell Scientific CR-7 micrologger. Stem measurements were made every 10 seconds, averaged over 10 minute periods, and then data were stored on cassette tapes. Data were then uploaded to a computer and analyzed.

Ceres must be calibrated to give displacement readings in millimeters from the raw Ceres mV output. Ceres were calibrated at 24°C in the same growth chamber in which the plants were grown. An inside micrometer, which had been modified to attach to the screw portion of the Ceres, was locked in position between the jaws of the Ceres with an initial jaw displacement. The jaws were then displaced in 0.10-mm increments while the Ceres output voltages were read on the micrologger. Output voltage is linearly related to jaw displacement (Figure 2). The relationship is described by a first-order linear model:

$$\text{Ceres jaw displacement} = B_0 + B_1 \text{ (output voltage)} \qquad (1)$$

The Y-intercept and slope coefficients, B_0 and B_1, were estimated through least squares linear regression. The equation is solved for displacement as a function of voltage.

GAS EXCHANGE

Whole-plant gas exchange was monitored by placing a acrylic plastic chamber (30-cm diameter, 45-cm high) over the entire plant (Figure 3). The inside walls were covered with a clear Teflon™ film to prevent adsorption of CO_2 or H_2O. A Beckman 865 CO_2 analyzer monitored CO_2 levels in the chamber. A Campbell Scientific CR-7 microprocessor recorded data pertinent to calculations of carbon gain including CO_2 levels, leaf temperature, dew point and temperature of air entering the chamber, and dew point and temperature of air leaving the chamber.

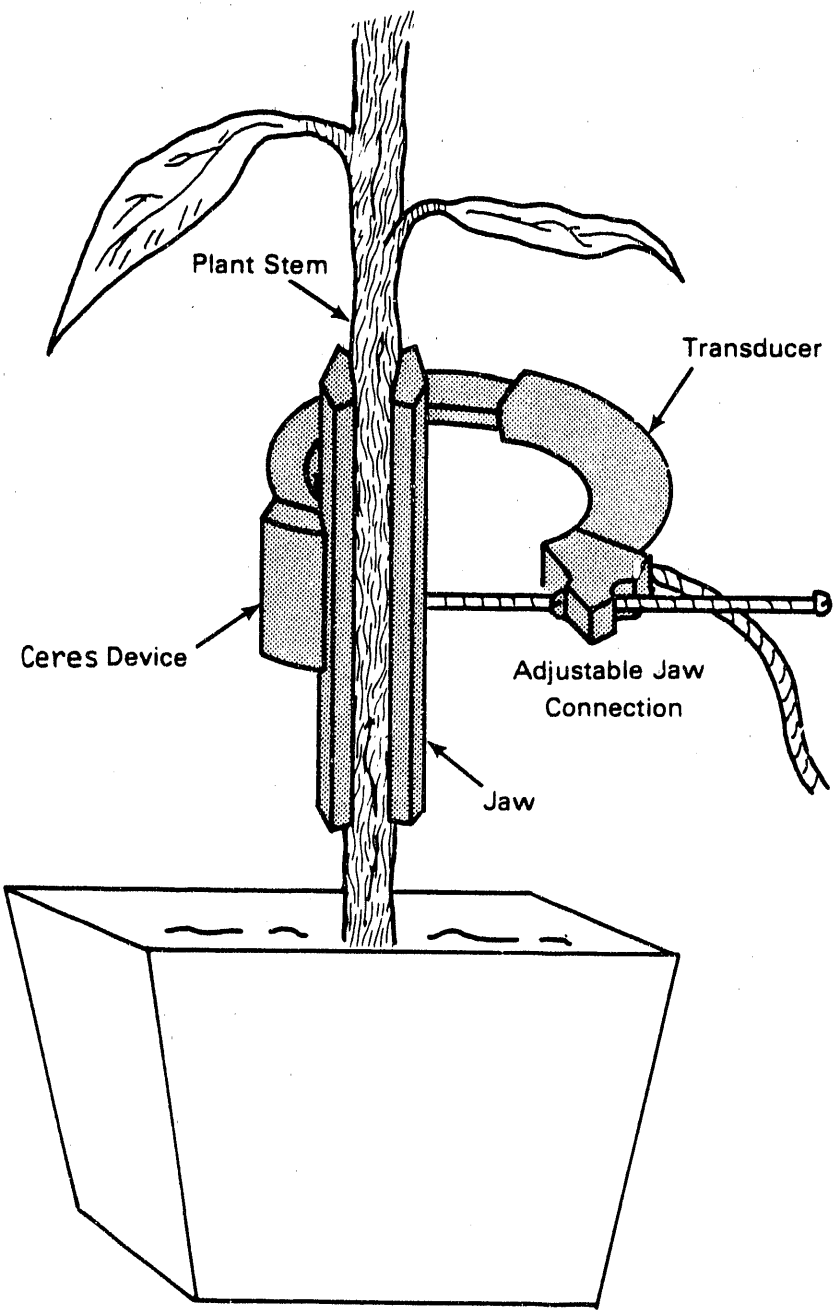

FIGURE 1. Diagram of Ceres attachment to a sunflower plant stem.

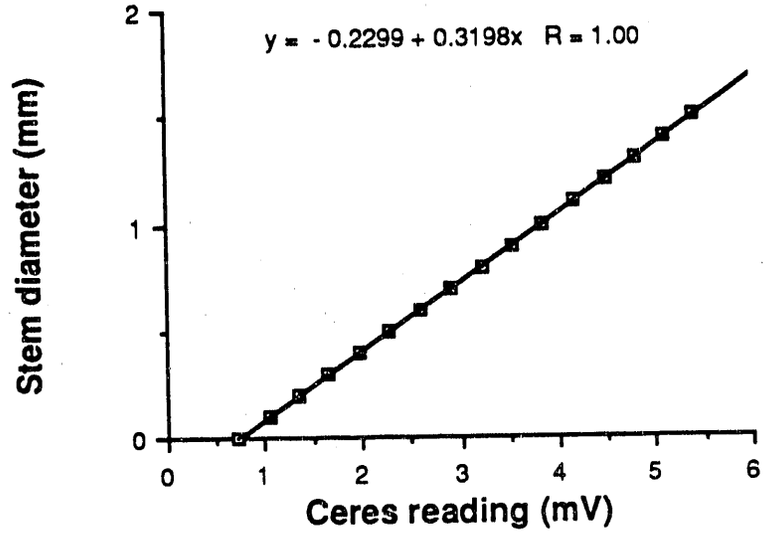

FIGURE 2. Ceres calibration curve. Each data point represents the stem diameter displacement (mm) versus the Ceres reading in mV.

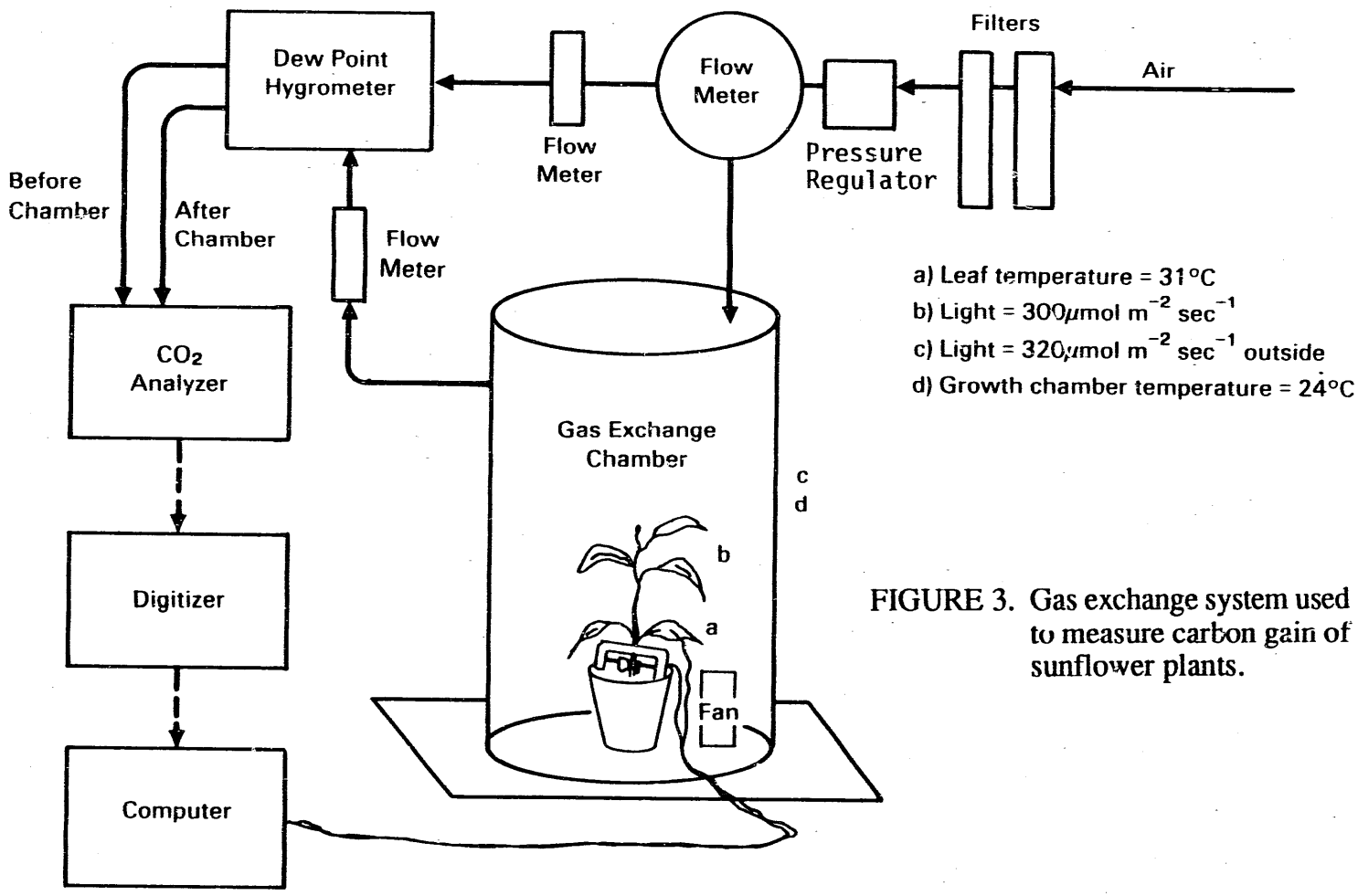

FIGURE 3. Gas exchange system used to measure carbon gain of sunflower plants.

Chamber flow rate and sample air flow rate and chamber back pressure were determined with flow meters and pressure gauges, respectively. Chamber air turnover rate was 3.3 minutes with an air volume of 10 l/min. All air-line components were constructed of stainless steel piping, Teflon or Bev-e-lin™ tubing to prevent absorption of CO_2. The effect of soil respiration from the pot was determined by comparing the ppm CO_2 with both the soil-filled pot and plant in the chamber with the soil-filled pot only. Soil respiration and was then factored out of the gas exchange data. Building air drawn from a roof duct and passed through a pressure regulator and filters provided a constant supply of air for the chamber flows. The isolated aspect of the laboratory from roads, vehicles, and industry provided an air source sufficiently constant in CO_2 ppm. Building air was calibrated before and after each gas exchange test period for ppm CO_2 using a two-way calibration against two standard gases. Gas exchange data were collected on days 1, 5, 8, and 12 after exposure for three control plants and three plants exposed to 40 Gy. Carbon gain was calculated from gas exchange information.

STEM MEASUREMENT CALCULATIONS

Stem diameter measurements were made using Ceres devices and were monitored continuously at 10-second intervals with a microprocessor; resulting 10-minute averages were automatically recorded on tape. (Stem diameter refers to the size of the cambial layer.) To determine stem diameter changes, the initial stem diameter measured was subtracted from subsequent observations by:

$$d^* = d_i - d_0 \tag{2}$$

where d^* is the difference between the initial stem diameter (d_0) and stem diameter (d_i) is at subsequent times (i).

Stem diameter growth rates were obtained by regressing a two hour change in stem diameter (2400 - 0200 hr) (d^*) against day (t).

$$d^* = b_0 + b_i t, \tag{3}$$

where d^* is defined in eq. (2), t is time in days, and b_i are linear regression parameters. The growth rate was then obtained as:

$$G = \Delta d^*/dt = b_i \tag{4}$$

This period of stem growth was selected during a 2-hour period near the end of the each day's dark photoperiod (Figure 4). During this period, growth was nearly linear. For the purpose of comparison, the rest of the daily cycle was eliminated because the daily stem growth was cyclic in nature. The beginning of the light photoperiod showed a rapid decrease in stem diameter because of open stomata and water loss. Stem diameter remained at a low value, and subsequent growth was erratic in nature. Any small amount of stem growth during this period was usually hidden by the changes in the water balance of the plant. A rapid increase in stem diameter occurred at the beginning of the dark photoperiod because of the closing of the stomata and reduced water loss. A fairly constant growth period showing an increase in stem growth was seen from near the beginning to the end of the dark photoperiod. Small fluctuations in growth were seen during the first portion of the growth period, which stabilized as the dark cycle progressed. Thus, the most consistent growth period enabling comparison between treatments was near the end of the dark photoperiod.

BIOMASS MEASUREMENTS

Each plant was separated into leaves, stems and petioles, and roots. The plant parts were dried at 55°C for 48 hours in a Precision™ mechanical convection oven (GCA Corporation) and weighed with a Mettler™ balance (type H6), which is accurate to four decimal places. Portions of each plant were weighed separately to see if there was a change in dry matter accumulation within the whole plant. Soil was washed from the roots of each plant. The roots were then dried and weighed.

LEAF AREA MEASUREMENTS

Leaf area as a function of leaf length and width was modeled to provide estimates of leaf area for the experimental plants during the experiment. The following regression relationship was developed:

$$LA = b_0 + b_1L + b_2W + b_3L*W \qquad (5)$$

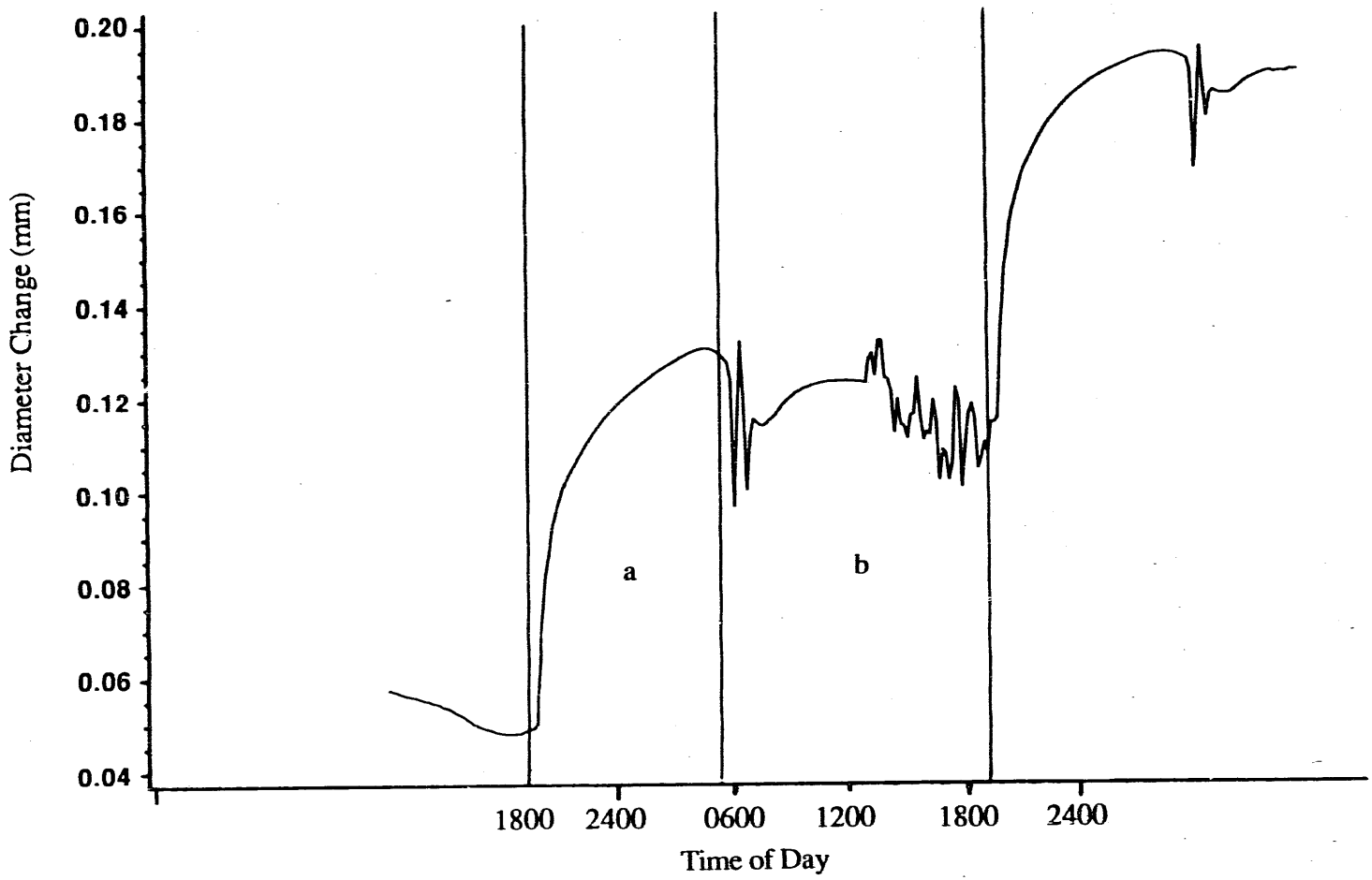

FIGURE 4. The diametrical change of the stem with time. a = dark photoperiod and b = light photoperiod.

where leaf area (LA) is the leaf area (cm^2), L is the greatest length (cm), W is the greatest width (cm), and b_i are linear regression parameters. Leaf area was determined by measuring width and length for all leaves on each plant at 1, 4, 6, 9, 13, and 18 days after exposure. At the end of 18 days, plants were harvested. Leaves from each plant were measured for length and width, and then the corresponding leaf area was measured with a LiCor (Model LI-3100) leaf area meter. Estimated leaf areas from the final day of harvest were tested for accuracy by comparing the leaf area of plants passed through the leaf area meter.

CARBOHYDRATE MEASUREMENTS

Leaves, stems, and roots of three control plants and three plants exposed to 40 Gy were analyzed for carbohydrates to determine the amounts of hexose, disaccharide, and starch in the plants. A carbohydrate analysis procedure described by Dickson (1979) was modified to determine hexose, disaccharide, and starch. Harvested plants were separated into subsamples consisting of leaves, stems, and roots and then oven dried at 55°C. Subsamples from each of three 40-Gy-irradiated plants and three control plants were used in the following procedure:

1) 50.0 mg of dried tissue was homogenized with 5.0-ml hot (>70°C) 80% Ethanol (EtOH) in a Tenbrock™ tissue homogenizer. The sample was transferred to a centrifuge tube and rinsed with an additional 2-3 ml EtOH. The solution was then vortexed in a hot water bath at 85°C. The centrifuge tubes were spun at 5000 g for 5 minutes. The supernate was decanted into a scintillation vial and processed according to step 2. The remaining pellet was further processed according to step 3.
2) To the supernate was added 2-ml carbon tetrachloride ($CHCl_4$) and 1-ml distilled H_2O. The sample was vortexed and spun in the centrifuge at 5000 g to form two phases. The chloroform layer separated to the bottom and was decanted and discarded. The water/alcohol solution was freeze-dried overnight and constituted to a 5-ml volume with distilled H_2O. A 10-µl sample was taken for analyzing total hexose. A 1.0-ml sample was combined with 1 ml of 0.1% (weight/volume) invertase in 20-mM 2[Morpholino]ethanesulfonic acid (MES) at pH 4.5. This was incubated for 1 hour at 45°C and then placed in a boiling water bath for 5 minutes and cooled; then a 10-µl sample was taken and analyzed for glucose.
3) The pellet was treated by placing the centrifuge tube in a boiling water bath for 10 minutes, cooling it, then adding 5 ml of a 0.5% (weight/volume)

Amyloglucosidase solution in 20-mM MES pH 4.5 to the tube. The tubes were then vortexed and incubated overnight in a water bath at 45°C. The next day, the starch pellet was centrifuged, and a 10-µl aliquot taken from the supernate. This sample was analyzed for glucose.

4) A total of 54 test tube samples were then determined for glucose content, plus a standard curve of 6 tubes (0, 5, 10, 25, 50, 100 µg), 1 tube of a sucrose control, 1 tube of invertase, and 1 tube of starch enzyme = 63 assays.

STATISTICAL ANALYSIS

Several types of statistical analyses were used in this study, depending on the type of data compared. To compare variation between the treatment groups, the analysis of variance (ANOVA) model was used when cell sizes were equal, and the general linear models (GLM) were used when cell sizes were unequal. The Duncan test was used to determine the significance of multiple comparisons. The Student t-test was used when only two populations were compared. Hypotheses were tested at the alpha level of 0.05. Stem growth was assessed every 3 to 4 days during the experiment to determine significant differences between the treatments. Growth rates were sorted by treatment, and the treatment averages were compared daily for differences using Duncan's multiple range test. The 95% confidence interval for multiple mean comparison when shown was calculated as:

$$\text{mean}(x) \pm (t, n_2, 0.95) * \sqrt{(MSE/n_1)}. \qquad (6)$$

where x is the mean for a sample group, n_1 is the number of individuals in that sample described by that mean, and t is the t-test value where n_2 is the degrees of freedom for error in the one-way GLM, and MSE is the mean square error for all the groups combined. The confidence interval for comparison of means for the t-test was as follows:

$$\text{mean}(x) \pm t(0.95, n-1) * sd/\sqrt{n} = t(0.95, n-1) * SE \qquad (7)$$

RESULTS

Figure 5 shows the stem growth of seedlings as a function of time after exposure. These results show a dose dependent decrease in stem growth. As a result, eighteen days after exposure to either 5, 10, 20, or 40 Gy, the stem diameter was 9, 40, 48, and 44% smaller, respectively, than the stem diameter of the unirradiated plants. The mean stem diameter of plants exposed to 10, 20, or 40 Gy were significantly smaller than the stem diameter of control plants as early as day 5. Plants exposed 5 Gy were not significantly different in stem diameter from control plants at all time points.

To look at stem growth recovery the daily growth rate was computed for each plant. Daily growth for plants exposed to either 10, 20, or 40 Gy showed a departure from that of the control as early as the day 1, as shown in Figure 6. Plants exposed to 10, 20, or 40 Gy of gamma radiation had significantly less daily growth than control plants as early as 8, 9, or 4 days after radiation, respectively. Once a significant difference was found between irradiated and control plants, this difference continued to the eighteenth day post exposure. The lowest treatment group (5 Gy) was never significantly different in stem growth rate from the control plants.

To see if factors that influence stem growth also affect biomass, the total plant dry weight (i.e., leaves, stems, and roots) were measured 18 days after irradiation. The resulting radiation dose response curve is shown in Figure 7. Comparison of the dose groups using Duncan's multiple-range test showed no significant differences in total biomass measured at 18 days at all dose levels, even though there is a trend for the average biomass to decrease with increasing dose of radiation.

To see if plants express morphologic plasticity after irradiation, the leaf, stem, and root biomass was measured 18 days after irradiation. The results in Figure 8 show a significant increase in leaf (27-45%) biomass with increasing doses of radiation above 5 Gy. In contrast there was a significant decreases in stem (27-34%) and root biomass (26-53%) with increasing doses of radiation above 5 Gy. The Duncan's multiple-range test controls the type I comparison error rate, and significant differences between dose groups were found even though confidence intervals overlapped.

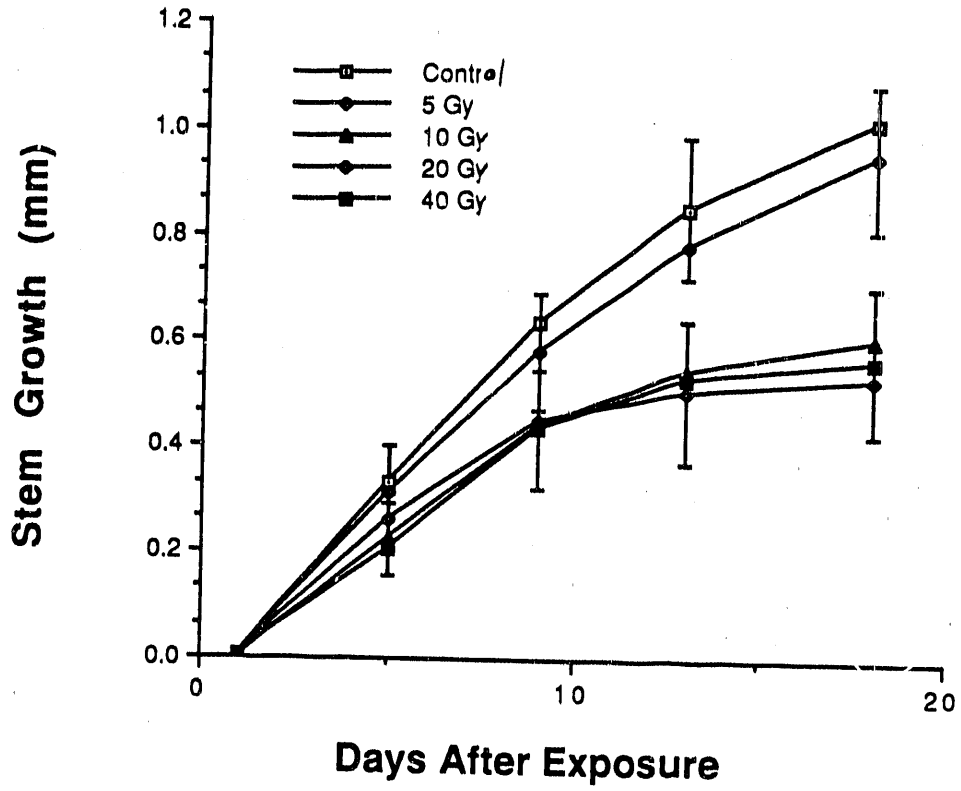

FIGURE 5. Effects of irradiation on total stem growth. Each point represents the mean total stem diameter growth of 5 plants for each dose at the indicated time after exposure. The error bar represents the 95% confidence interval.

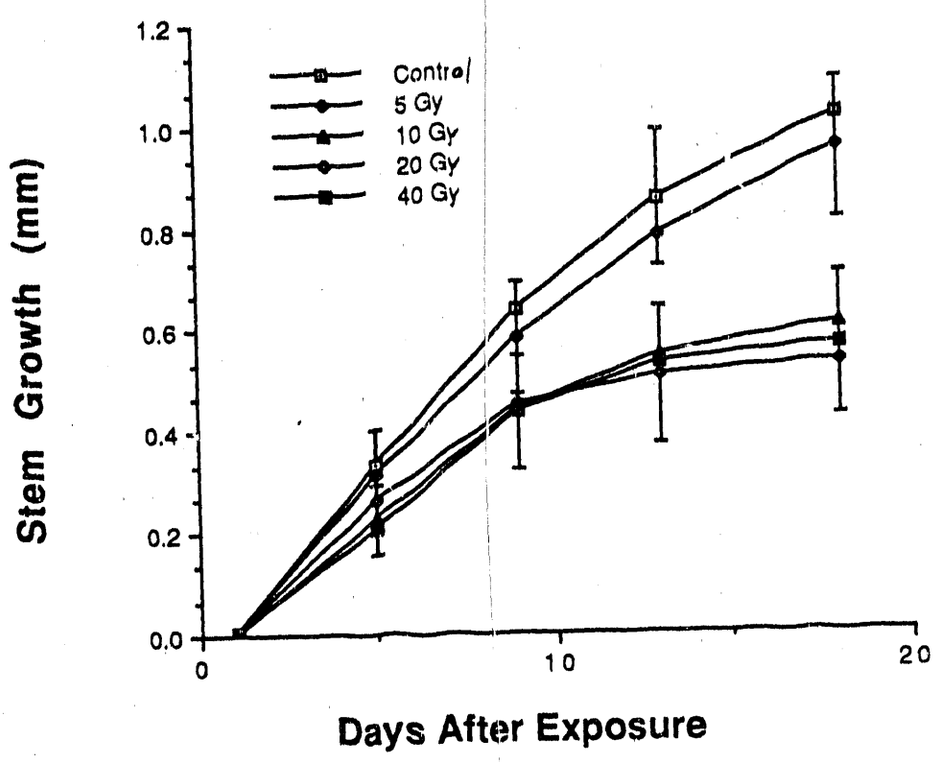

FIGURE 5. Effects of irradiation on total stem growth. Each point represents the mean total stem diameter growth of 5 plants for each dose at the indicated time after exposure. The error bar represents the 95% confidence interval.

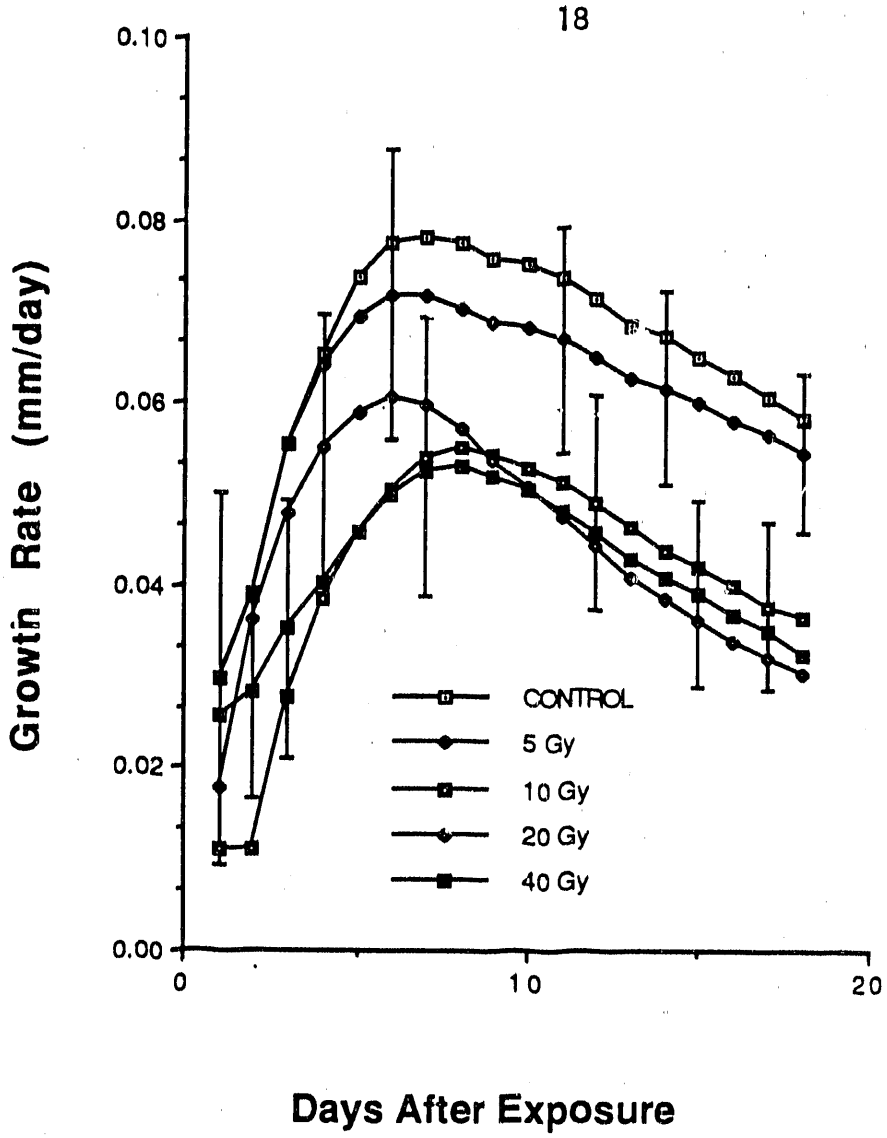

FIGURE 6. Effect of irradiation on the daily growth rate. The growth rate was determined during a 2 hour period near the end of the dark cycle. Each point represents a mean of five plants. The error bar represents the 95% confidence interval.

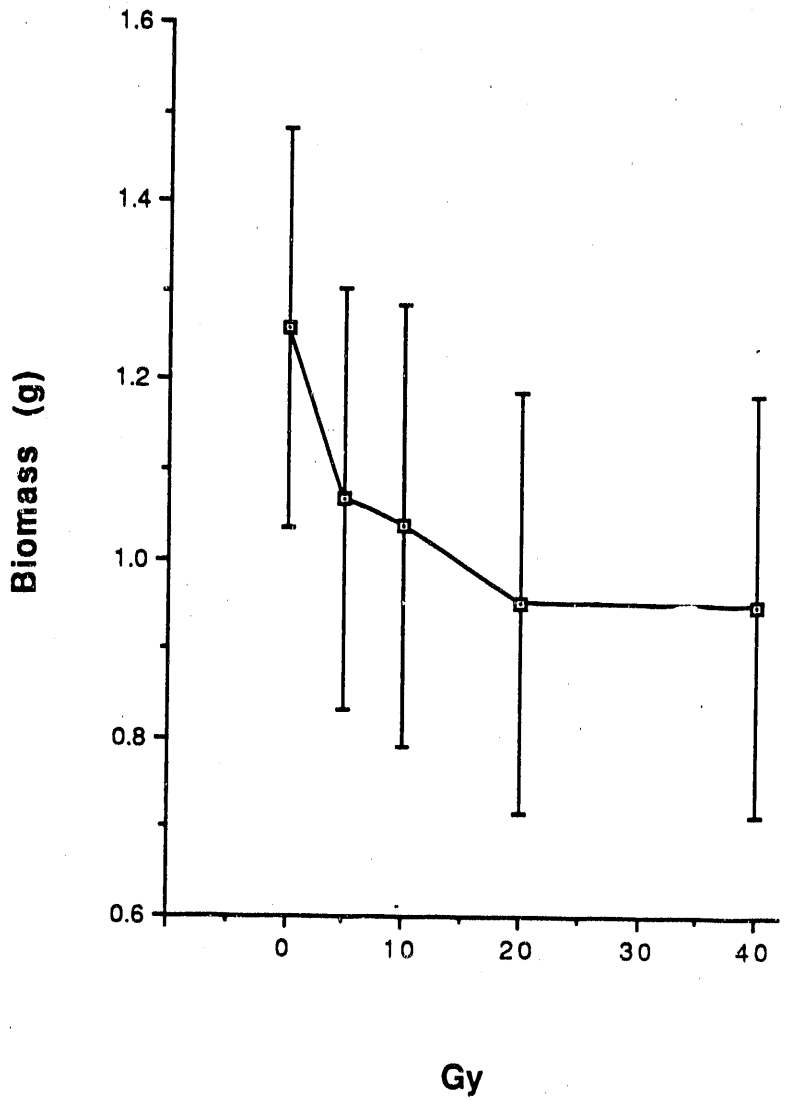

FIGURE 7. Dose-response curve for total plant biomass measured 18 days after exposure. Each point represents the mean of 10 plants. The error bar represents the 95% confidence interval.

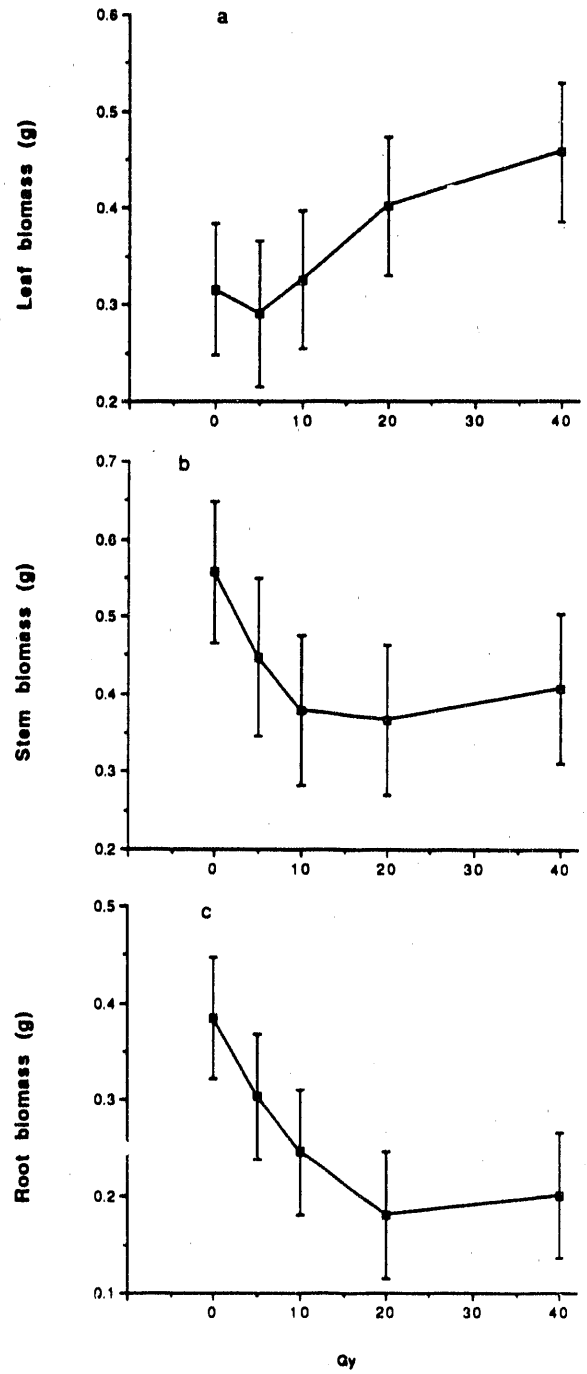

FIGURE 8. Radiation dose response curve for leaf biomass (a), stem biomass (b), and root biomass (c) measured 18 days after exposure. Each point represents the mean of 10 plants. The error bar represents the 95% confidence interval.

Survival of a plant in the environment may depend on the ability of a plant to assimilate nutrients and water through its root system and support the aboveground biomass. The ratio of the biomass of a plant's roots to its aboveground biomass (root/shoot ratio) is commonly used indicator of plant survival under environmental stress such as water stress. Figure 9 shows a dose-response curve of the root/shoot ratio measured 18 days after exposure. The results show that with doses between 0 and 20 Gy there is a dose dependent decrease (0-46%) in root/shoot ratio. At radiation dose levels above 20 Gy, there is no further decrease in root/shoot ratio.

A reduction in the root/shoot ratio could be due to increased leaf size or increased leaf density. To see if this was the case, leaf size and leaf density were measured 18 days after graded doses of radiation (Figure 10). The results show no statistical difference in leaf area for all dose levels. However, the leaf density increases with increasing radiation dose up to 20 Gy, with no further increase with larger doses. The maximum increase (46%) in leaf density was observed for the 20-Gy plants. This increased in leaf density corresponds to a similar decrease in the root/shoot ratio at each dose level (see Figure 9).

Measurements of photosynthesis can indicate whether increased carbon production is one of the mechanisms causing increased leaf density. Figure 11 shows that net photosynthetic rates for control plants and plants exposed to 40 Gy on days 1, 5, 8, and 12 after irradiation. At all time points after irradiation, the mean photosynthetic rates of irradiated plants were lower than control plants. Photosynthetic rates (10-20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in unirradiated plants in this study were generally less than 20% of those in the published literature. Lower chamber light levels (300 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) and above optimum chamber temperature (31°C) in the present experiment probably responsible for this reduced photosynthetic rates (Figure 3). The slope of the regression of carbon gain for plants exposed to 40 Gy during the 12-days post exposure was negative and significantly different from zero ($\alpha <$ than 0.05). In contrast, the slope of carbon production for control plants during the same 12-day period was not significantly different from zero.

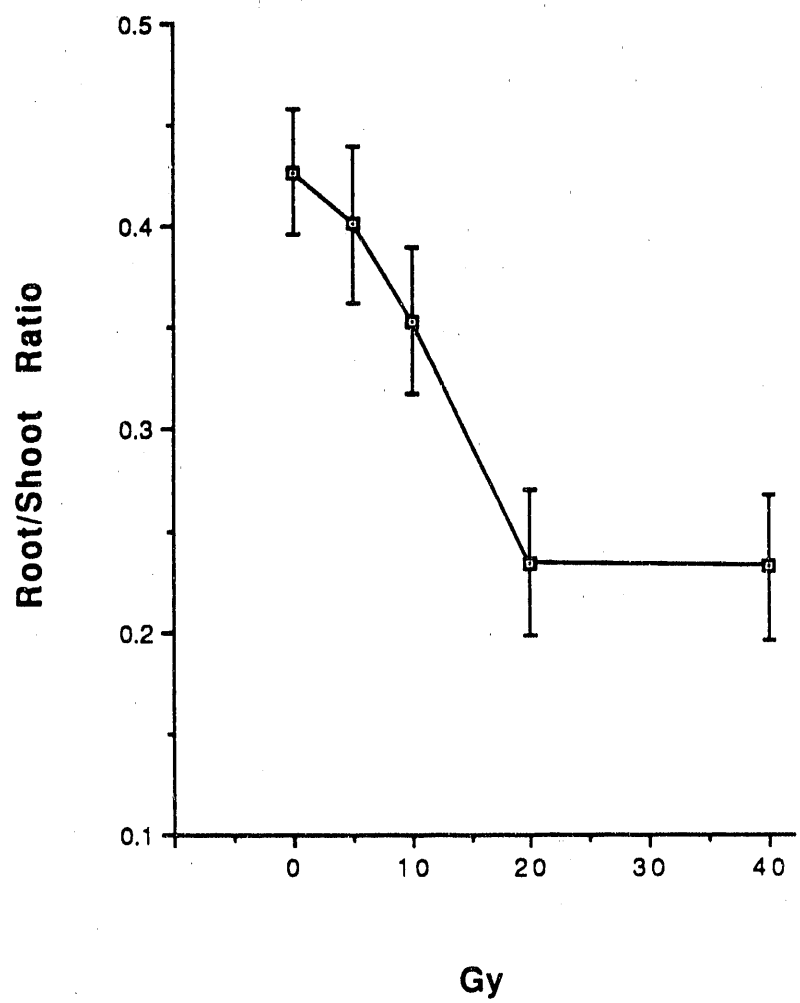

FIGURE 9. Radiation dose response curve for the root/shoot ratio measured 18 days after plants were exposed. Each point represents the mean of 10 plants. The error bar represents the 95% confidence interval.

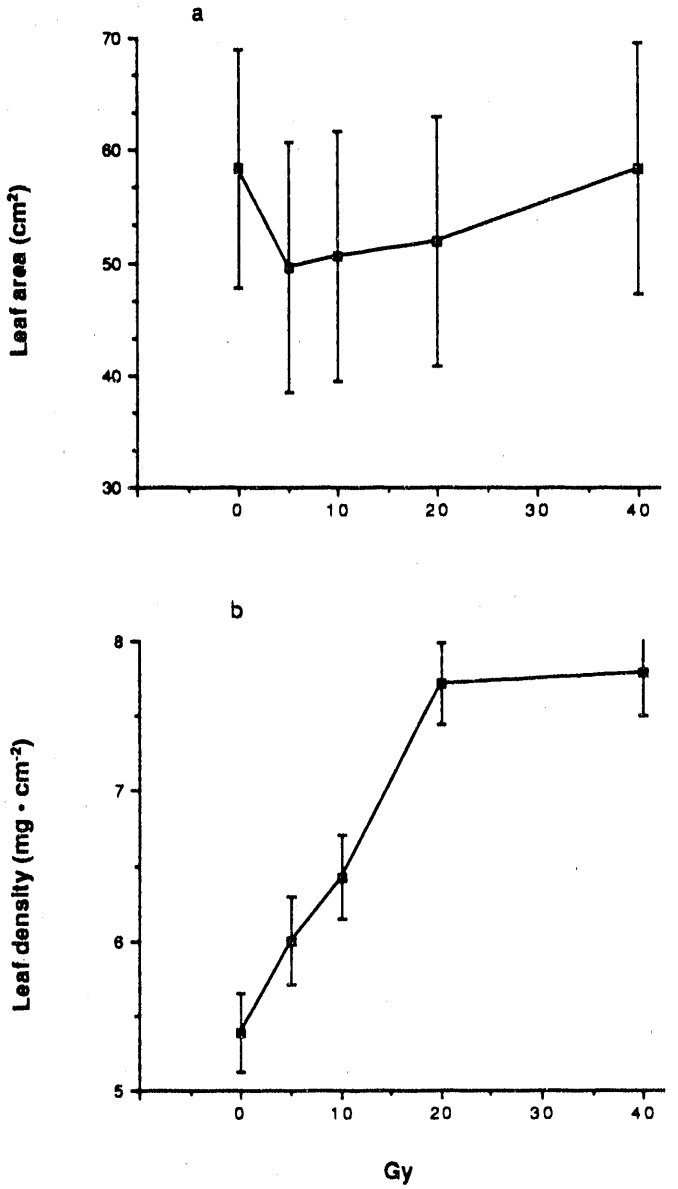

FIGURE 10. Radiation dose response curve for leaf area (a) and density (b) measured 18 days after exposure. Each point represents the 95% confidence interval. Each point represents a mean of 10 plants. The error bar represents the 95% confidence interval.

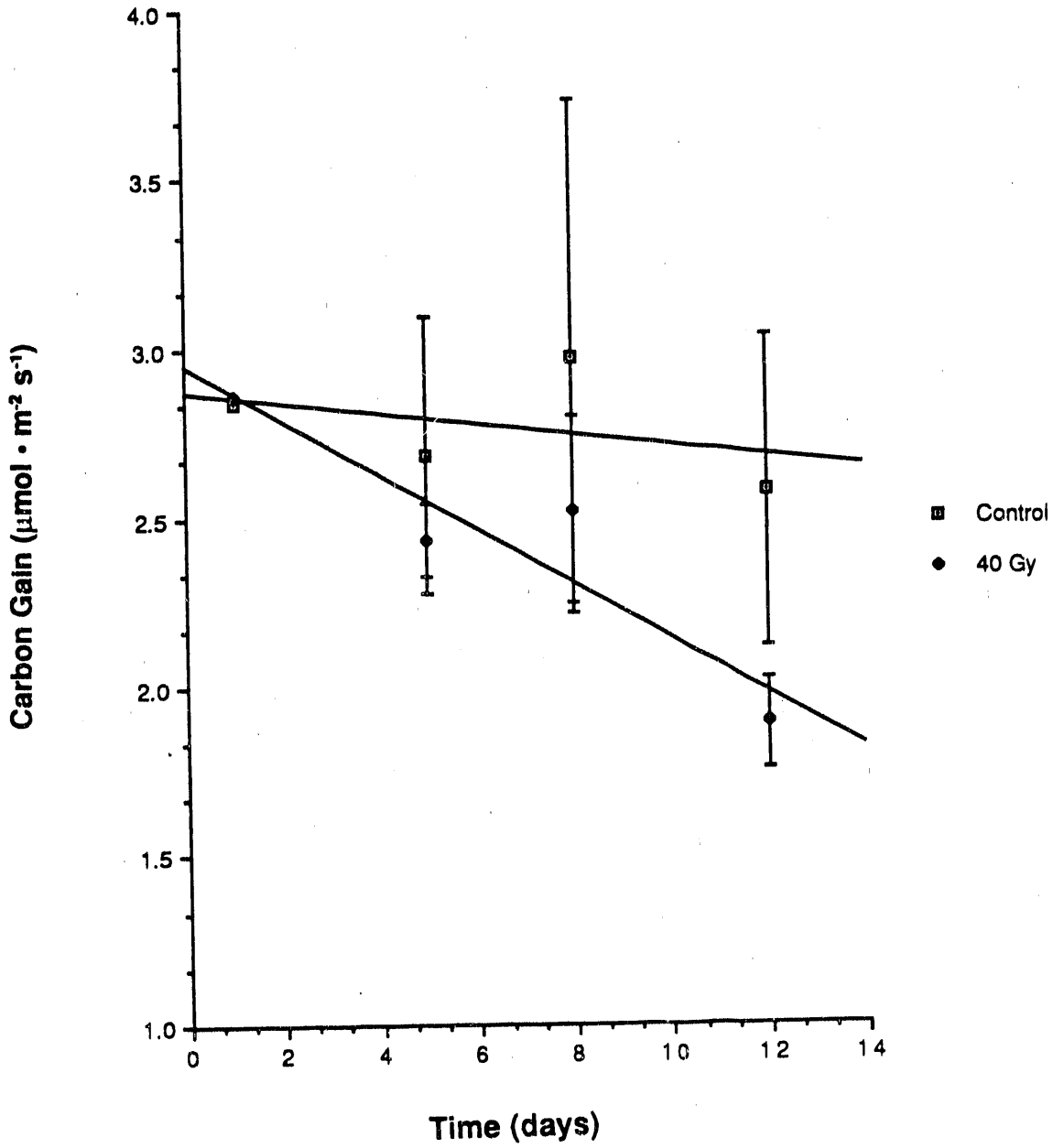

FIGURE 11. Effect of irradiation on carbon gain during light photosynthesis. At day 1 the value is based on one plant. At days 5, 8, or 12 the value represent the mean of 3 plants. The line represent the slope of the regression for carbon gain during the time period. The error bar represents the standard error of the mean.

Small decreases in carbon gain should lead to decreased leaf density. However, increased leaf density was observed in this study (see Figure 10b). In an attempt to clarify this apparent contradiction, the carbohydrate content of control and 40-Gy irradiated plants leaves, stem, and roots were analyzed 18 days after exposure. The results presented in Figure 12 show that leaves of irradiated plants contained 22% more starch than control plants. This difference was significant when the two groups were compared using a Student's t-test. Hexose levels of irradiated plant leaves were slightly (6%) but not significantly higher than control plants. Sucrose levels in leaves of irradiated plants were not significantly different from control plants. In contrast the starch levels in irradiated stems were 94% lower than unirradiated stem, whereas, sucrose and hexose levels were not significantly affected by irradiation. Irradiation also had no effect on starch, sucrose, hexose levels in roots.

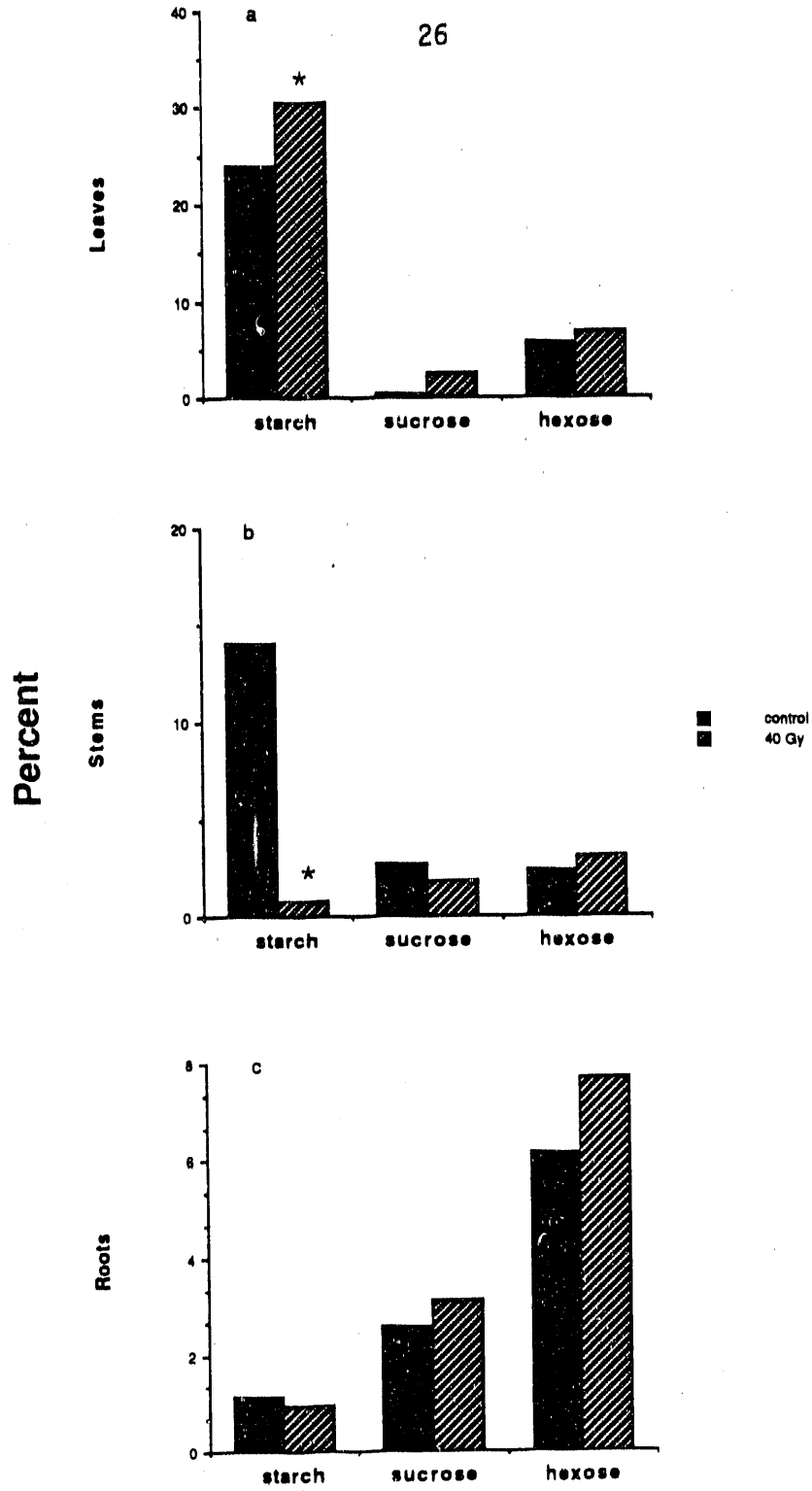

FIGURE 12. The effect of irradiation on carbohydrate content of leaf, stem, and root portions of irradiated and unirradiated plants. "*" indicates that the values are significant different.

DISCUSSION

All the observations described in this experiment (changes in stem, root, leaf biomass, leaf density, and carbohydrate allocation) suggest possible hypotheses that could help explain why stem diameter was reduced in *H. annuus* while leaf biomass was stimulated, including the following: Phloem transport may be disrupted and cause a buildup of sugars or starches in the leaf tissues, or enzymes responsible for reducing sugars to transportable forms may be damaged.

Other studies have noted that changes in stem diameter occur when a plant is subjected to a stressful environment. For example, Keitel and Arndt (1983) detected stem diameter changes when *Nicotiana tabacum* was exposed to ozone. Beedlow et al. (1986) observed reduced stem growth when *H. annuus* plants were exposed to automobile exhaust. Garnier and Berger (1986) recorded reduced growth of water-stressed *Prunus persica*. This study confirms that measurements of stem diameter are useful in determining the effect of radiation on plants (Figures 5 and 6). Significant reduction in total stem growth between control plants and plants exposed to 5, 10, 20, or 40 Gy 4 days after exposure showed threshold effects between doses of 5 and 10 Gy. A study by Sparrow et al. (1971) predicted a 50% yield reduction for *Helianthus* spp. at exposures of 40 to 60 Gy using chromosome volumes as a comparison. They determined the growth reduction of a few plants and then regressed yield reduction against the interphase chromosome volume. In contrast, this study showed a 40% reduction of stem diameter growth for plants exposed to as little as 10 Gy. Although a reduction in stem diameter does not necessarily mean that there is a corresponding reduction in plant biomass, it does indicate that radiation causes permanent changes in processes that influence stem diameter. Sparrow et al. (1965) found that the xylem increments of *Pinus rigida*, which were measured after several years' exposure to less than 40 mGy/day, were reduced in width. After 8 years of exposure, 50% of these pine trees had been killed by a cumulative exposure of 58 Gy (31 mGy/day).

A threshold response is suggested because plant stem diameter growth was reduced from 40-48% when exposed to 10-40 Gy (Figure 5). Treatment plants exposed to 10 Gy or higher were not significantly different in stem diameter growth, but were significantly different than either control plants or plants exposed to 5 Gy. This suggests that the factors that damage plant growth and metabolism affect stem diameter growth irreversibly above 10 Gy, while below 10 Gy the damage is not significant or is reversible.

The results of measuring stem diameter of plants exposed to radiation levels used in this experiment indicate that plant damage expressed as reduced stem

diameter growth is not proportional to dose. That is, the response is either expressed as significant reductions in stem diameter or is not expressed at all. Once initial differences in stem diameter were observed, they remain present during 18 days post exposure. This would indicate that the cells or processes responsible for stem diameter growth are permanently damaged at the time of radiation.

Evidence of repair was not supported by stem growth rates. However, repair was suggested, but not supported statistically during the first 2 days after exposure to radiation. The average growth rate of plants exposed to 10-40 Gy was reduced by 14-63% 1 day and 7-17% 2 days after exposure (Figure 6). This suggests the partial recovery of irradiated plants within 48 hours after exposure.

The pattern of growth appears to be similar between the control plants and the plants exposed to radiation; the growth rate of plants increased for approximately 6 days and then decreased the next 12 days (Figure 6). However, this pattern was delayed a couple of days for the plants exposed to 10 or 40 Gy. Plants exposed to 20 Gy were not delayed in growth when compared to that of control plants. A delay of plant growth was described by Cordero and Gunckel (1982) for leaves in a study of the plastochron index (the relationship of leaf length with age) of *Lupinus albus* L. The plastochron index was described as the developmental age of a particular plant by correlating leaf length with age. Repair mechanisms are suggested because the plastochron index for plants measured immediately after irradiation was increased, but was indistinguishable from the controls 11 days after exposure.

Different plant tissues may respond differently to changes in environmental influences. For example, biomass partitioning was affected by exposure to radiation. Leaf biomass significantly increased by exposure to as little as 20 Gy while stem or root biomass decreased significantly by exposure to doses as low as 10 Gy (Figure 8). Bradshaw (1965) discussed the change in the growth development of species such as *H. annuus*, which tend to respond to plant density by changes in the size of the parts. This is because for a crop growing with stored soil moisture, the plasticity of leaf size provides an important mechanism for conserving water during vegetative growth. *Helianthus* plants have shown decreased leaf size because of water stress (Boyer, 1970; Rawson and Turner, 1982; Shinichi et al., 1981). Water deficits can reduce shoot dry matter and seed yield in *H. annuus* (Sobrado and Turner, 1983). After relief of a severe water stress, a higher proportion of dry matter was allocated to leaf production and a smaller proportion to stems (Sobrado and Turner, 1986). In contrast, this study shows that radiation stress did not affect leaf size (Figure 10).

The root/shoot ratio was decreased by exposure to radiation (Figure 9). Survival of a plant in the environment may depend on the ability of a plant to assimilate nutrients and water through its root system and support the aboveground biomass. The ratio of the biomass of a plant's roots to its aboveground biomass (root/shoot ratio) is an indicator of plant survival under environmental stress such as water stress. A larger portion of aboveground biomass supported by a smaller portion of roots could tax the ability of the roots to provide enough water during drought. The overall root/shoot ratio was unaffected by water stress in studies by Sobrado and Turner (1986). They found that water stress decreased the proportion of dry matter allocated to the stem and increased the proportion allocated to reproductive parts. This would enable the plant to still produce at least some seed. Although plants in this experiment were not allowed to reach maturity, and thus flower, the opposite relationship seemed to occur. Both the stem and roots decreased in biomass when exposed to radiation, while leaf biomass increased.

This increase in leaf biomass was due to increases in leaf density. The leaf density seemed to be the most sensitive response to radiation of all the parameters measured (stem diameter, biomass portions, leaf area), as it was significantly different from control plants at exposures as low as 5 Gy (Figure 10).

Results of this experiment indicate that photosynthesis was reduced only slightly in plants exposed to 5-40 Gy (Figure 11). A decreasing trend in the average total biomass with increasing doses suggest that there was a radiation effect on carbon gain. Even though a decreasing trend was observed, the large scatter of the data precluded significance. Reduced carbon gain was also supported by Ursino et al. (1974) who found that photosynthesis was reduced by half in *Pinus strobus* exposed to 10 Gy. Hadley and Woodwell (1966) found that seedlings of *Pinus taeda* and *Pinus elliottii* were absorbing CO_2 at between 60 and 90% of pre-irradiation rates 21 days after exposure to 12.5 Gy. Dosages used in these studies are considered to be lethal, however. The present experiment shows that the dosages were not lethal to the plants, and they caused very little visual damage to the plants. Absence of significant differences between total biomass would not suggest a change in the rate of fixed carbon. However, since the sample size was small (only 3 plants) and light levels were low, this experiment's data should only be used to indicate trends and not necessarily considered statistical support. These data indicate a decreasing photosynthetic trend over 13 days post-exposure for plants exposed to 40 Gy. The negative slope of the photosynthetic data for plants exposed to 40 Gy was significant from zero and tends to support a

decreasing trend of photosynthetic rates for irradiated plants. The rate of carbon gain for control plants was not statistically different from zero.

Dark respiration, measured 1 day after exposure, was not reduced in *H. annuus*. Ursino et al. (1974) also found that respiration of *Pinus strobus* L. did not change 1 day after exposure to 75 Gy, but was significantly higher 2 days following radiation. Dark respiration was not affected in pines by 12.5-13.2 Gy for as long as 3 weeks after exposure (Hadley and Woodwell, 1966).

This study found that radiation did not reduce leaf area, while showing a slight negative relationship to photosynthesis and a strong positive relationship to leaf density. Photosynthesis has also been used to study dry matter accumulation and distribution in relation to water deficits in *Helianthus* spp. (Rawson et al., 1980; Shinichi et al., 1981; Sobrado and Turner, 1986). Unlike radiation stress, these studies suggested that water deficits reduced leaf area production and net photosynthesis. These findings suggest that other sites such as those responsible for carbon transport or metabolism are possible sites affected by irradiation.

The transport of sucrose down the phloem to the stem is one possible site of radiation damage. This possibility is supported by evidence of significant increases in the levels of starch were found in leaves of plants receiving 40 Gy when compared with control plants (Figure 12). The stem is a reserve tissue for starch storage, and excess accumulation of sugars could end up as storage in stem parts. However, starch was reduced 94% in stems of irradiated plants. Less root biomass in irradiated plants could indicate lack of sucrose movement through the stem tissues or possible depletions of stored starch by the roots rather than direct radiation damage to root meristem cells, or the use of carbohydrates for repair of leaf and stem tissues.

Starch is the only product of photosynthesis that is retained by the chloroplast, and Ursino et al. (1974) suggested that the chloroplast is a radiosensitive organelle. Starch reserves of the chloroplast are used by the plant during darkness and/or when there is limited photosynthesis. Starch usually accumulates in the light period and is depleted in the dark, but when the rate of triose phosphate conversion to sucrose is less than carbon fixation, starch synthesis in the chloroplast is increased.

There were no significant increases in levels of sucrose in the leaf, stem, or root. Greater amounts of sucrose found in leaves of irradiated plants could suggest that enzyme activity has been inhibited. However, sucrose levels were not found to be higher in the leaves of irradiated plants. Sucrose is a highly soluble sugar that can accumulate to considerable concentrations without an apparent inhibitory effect on most of the biochemical reaction of plant metabolism (Foyer,

1984). The sucrose molecule is electrically neutral, readily transports across biological membranes, and is a useful component of the mechanisms that regulate osmotic pressure and water relationships between the cellular compartments in the plant (Foyer, 1984). Although sucrose moves easily across cell membranes, it has a limited use in the plant. Sucrose is hydrolyzed by the enzyme invertase to forms used in cell metabolism, such as glucose and fructose.

The reduced root and stem growth suggested that radiation reduced phloem transport. This phenomena may have a result similar to that of farmers girdling the base of grape plants to increase the size of the fruit. The procedure simply restricts the movement of carbohydrates assimilated in leaves during photosynthesis from moving down the phloem to the stem and roots, so that there is a buildup of carbohydrates in the upper plant tissues. Similar results with more complex causes could be responsible for observations of other researchers: Sidrak and Suess (1973) found increased size of *Lycopersicon esculentum* when exposed to 5-10 Gy, and Sheppard et al. (1982) noted stimulation of both fascicle needle length and total biomass in *Pinus sylvestris*.

Some researchers have suggested that small amounts of radiation can be beneficial to the organism (Luckey, 1980), a concept called radiation hormesis. If increased leaf biomass caused by gamma irradiation in this study were examined, it would suggest that this study supports the idea of radiation hormesis. However, examining just leaf biomass could lead to a false conclusion because only one growth attribute was used to predict radiation effects on the plant. For example, this experiment showed that leaf biomass increased with radiation exposure. However, the total biomass, including leaves, stems, and roots could not be related to dose. Radiation has been shown to damage mechanisms within plants so that carbohydrates remain in the leaf. More information is needed before low dose radiation can be labeled as causing a stimulative response because this experiment showed that carbon allocation measured 18 days after exposure was affected for all plant parts (Figure 8) at doses used in the present experiment.

CONCLUSIONS

These results suggest that exposure to gamma radiation at relatively low levels causes complex biochemical changes in plants that are ultimately expressed as nonreversible morphological growth changes. These results show that stem diameter is a sensitive indicator of radiation stress. Radiation caused reduced stem and root growth while leaf density increased. Carbon fixation was reduced only slightly while starch levels in leaves increased. These findings suggest that radiation may damage phloem transport or enzymes responsible for reducing sugars to transportable forms.

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