

A Relationship Between Photosynthesis and Translocation in  
Plants Stressed by Ionizing Radiation

James B. McCabe

Department of Biological Sciences

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Brock University

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" In science the credit goes to the man who convinces the world, not to the man to whom the idea first occurs ".

Sir William Osler

Abstract

Since previous investigations have shown that low levels of ionizing radiation can induce a reduction in the rates of apparent photosynthesis and in the magnitude of photoassimilated  $^{14}\text{C}$  exported out of a leaf, the present studies were designed and conducted to determine the relationship, if any, between the radiation effects on these two physiological processes. The experiments were particularly designed to determine if the radiation-induced reduction in export is the result of the reduction in photosynthesis and hence availability of materials for translocation or the result of a reduction in the amount of energy available for the vein loading process.

This study has shown that the radiation-induced reduction in  $^{14}\text{C}$  export out of a leaf is likely related to a loss of energy available for the vein loading process rather than a reduction in the supply of materials available for export due to reduced  $\text{CO}_2$  uptake. The process of photophosphorylation was shown to be reduced by exposure to radiation to an extent similar to the reduction in the export of  $^{14}\text{C}$  which was also observed. Both of these processes returned to their pre-irradiation rates 120 minutes following radiation exposure. The rate of photosynthetic  $\text{CO}_2$  uptake was also reduced by radiation exposure, however, this process did not return to the control level nor was the extent of reduction as large as observed for photophosphorylation and photoassimilate export. The observed relationship between the reductions of export and photophosphorylation pointed to the utilization of photosynthetically produced ATP in the vein loading process.

The radiation-induced reduction in the export of  $^{14}\text{C}$  was observed at the highest light intensity used in this study which would also imply the involvement of the photophosphorylation process as an energy source for vein loading. The lack of radiation-induced reduction in export at low light

intensities was interpreted as being due to the utilization of respiratory derived ATP, a process known to be insensitive to radiation at the levels used in this study, as the energy source for the vein loading process. Studies using plants not stressed by radiation showed that there was an increase in export of  $^{14}\text{C}$  with higher light intensities.

In summary, the data has been interpreted as showing that at high light intensities the ATP, produced by photophosphorylation, is available for use in the vein loading process. The site of ATP utilization could not be determined from the data obtained in this study but possible sites have been indicated from the work done by other physiologists and are discussed in the thesis.

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## TABLE OF CONTENTS

	<u>Page</u>
Title Page	1
Abstract	3
Acknowledgements	5
Table of Contents	6
List of Tables	8
List of Figures	9
1. Introduction	10
2. Literature Review	17
3. Materials	37
4. Methods	38
4-I. Whole Plant Irradiation	38
4-II. Measurements of Rates of Apparent Photosynthesis	39
4-III. Leaf $^{14}\text{CO}_2$ Photoassimilation and Determination of the Magnitude of Translocation	41
4-IV. Photophosphorylation Rates	43
5. Results	46
5-I. Radiation Effects on Photosynthesis	46
5-II. Radiation Effects on Rates of Photosynthetic $\text{CO}_2$ Uptake, Photophosphorylation and Photoassimilate Export	53
5-III. Magnitude of Photoassimilate Export at Different Light Intensities	58
6. Discussion	63
7. Appendices	78
1. Photosynthetic $\text{CO}_2$ uptake of soybean leaves prior to and at various times following varying acute doses of gamma radiation at a light intensity of 2,800 foot candles.	78
2. Photosynthetic $\text{CO}_2$ uptake of soybean leaves prior to and at various times following varying acute doses of gamma radiation at a light intensity of 1,600 foot candles.	81
3. Magnitude of $^{14}\text{C}$ export and photosynthetic $\text{CO}_2$ uptake of soybean leaves at 800, 1,600 and 2,800 foot candles 30 minutes following exposure to 4,100 rads of gamma radiation.	84

	<u>Page</u>
4. Photophosphorylation rate in chloroplasts isolated from soybean leaves maintained under 2,800 foot candles 15, 60 and 120 minutes post-irradiation.	87
5. Magnitude of $^{14}\text{C}$ export from soybean leaves 15 and 120 minutes following an acute exposure to 4,100 rads of gamma radiation.	89
6. Magnitude of $^{14}\text{C}$ photoassimilate export and free space $^{14}\text{C}$ content in soybean plants photoassimilating and exporting at varying light intensities.	90
8. Literature Cited	93

## LIST OF TABLES

	<u>Page</u>
Table 1. Changes in rates of apparent photosynthesis of soybean plants at various times following acute doses of gamma radiation (2,800 foot candles illumination).	47
Table 2. Changes in rates of apparent photosynthesis of soybean plants at various times following acute doses of gamma radiation (1,600 foot candles illumination).	51
Table 3. Export of assimilated $^{14}\text{C}$ and the rates of photosynthetic $\text{CO}_2$ uptake by soybean plants illuminated at 800, 1,600 or 2,800 foot candles following an acute dose of 4,100 rads of gamma radiation.	54
Table 4. Rates of photophosphorylation by chloroplasts isolated from soybean plants receiving an acute dose of 4,100 rads of gamma radiation.	56
Table 5. The magnitude of $^{14}\text{C}$ photoassimilate export in soybean plants which absorbed an acute dose of 4,100 rads of gamma radiation.	57
Table 6. The magnitude of $^{14}\text{C}$ photoassimilate export and $^{14}\text{C}$ free space content in soybean plants photoassimilating at different light intensities	59
Table 7. The magnitude of $^{14}\text{C}$ photoassimilate export and $^{14}\text{C}$ free space content in soybean plants translocating at different light intensities.	61

## LIST OF FIGURES

	<u>Page</u>
Figure 1. Diagram showing the movement of carbon through a leaf.	23
Figure 2. Diagram showing the relationship of companion cells or transfer cells to the sieve elements in the phloem tissue.	27
Figure 3. A diagrammatic representation of the Symplast and Apoplast loading theories for materials exported out of the leaf.	30
Figure 4. Schematic diagram of a possible participation of the phosphate translocator in ATP transport across the inner membrane.	35
Figure 5. Diagrammatic design of a closed system for obtaining the rate of apparent photosynthetic CO <sub>2</sub> uptake.	40
Figure 6. The effect of varying doses of gamma radiation on the rate of apparent photosynthetic CO <sub>2</sub> uptake at varying time intervals post-irradiation under 2,800 foot candles of illumination.	49
Figure 7. The effect of varying doses of gamma radiation on the rate of apparent photosynthetic CO <sub>2</sub> uptake at varying time intervals post-irradiation under 1,600 foot candles of illumination.	52

## INTRODUCTION

Studies regarding the effects of ionizing radiation in plants have been carried out using many different indicators of plant responses and damage. Growth and developmental changes (13, 43) have most frequently served as measurable responses to different radiation doses and different dose rates particularly when determined after an extended period of time following radiation exposure. Observations have also been made on the nuclear and cytoplasmic damage caused by radiation (14, 56, 92). The studies mentioned above have indicated that radiation doses above a threshold level will induce changes, normally retarding growth and development (13, 43) while the histological work has shown radiation causing aberrations and destruction to the nucleus (14, 56, 92) and to cytoplasmic organelles (92).

Physiological processes have also been shown to be affected by radiation exposure and in fact some have shown greater radiosensitivity than responses detectable at the nuclear level (8, 32, 94, 95, 100). For example the process of photosynthesis has been shown by a number of workers to be highly sensitive to ionizing radiation (8, 32, 69, 94). Boudreau and Woodwell (8) working with pitch pine (Pinus rigida) showed a reduction in photosynthetic rates following a chronic dose of only 30 rads per day. Although the reduction in rates of apparent photosynthesis was observed 210 days in plants receiving a chronic dose of 30 rads per day, it took another 30 days before there was a detectable loss of chlorophyll.

Acute doses of radiation have also been shown to reduce rates of photosynthesis (32, 69, 94). Pinus taedae and Pinus elliotti were shown to have a reduced photosynthetic rate of 8% two days following a dose of radiation as low as 1,250 rads. The extent of reduction was increased with doses of 5,950 rads or 13,200 rads and persisted for 22 days fol-

lowing radiation (32). Pinus strobus was found to be sensitive to even lower doses than those reported for Pinus taedae and P. elliotti (94). Two days following a dose of 230 rads the photosynthetic rate of Pinus strobus was reduced by 41%. A drop in photosynthetic rate 2 days following an X-ray dose of 250 rads has also been reported for Vicia faba (69). The work with white pine (94) also showed an increase of 23% in the rate of dark respiration two days following a radiation exposure to 230 rads. An increase in the dark respiration has also been observed in the field studies using chronic doses of radiation (8).

Not only do the processes involving CO<sub>2</sub> exchange in plants appear sensitive to radiation, but the processes responsible for the export and distribution of photoassimilated carbon, to all parts of the plant, have also been shown to be sensitive. The earliest study of radiation effects on translocation of photoassimilated carbon looked at the responses due to exposure of either the petiole or apical regions (100). Phaseolus vulgaris petioles, X-irradiated with 1,000 to 50,000 rads, showed no change in translocation when compared to control plants. However, apical meristems were found to be sensitive to X-ray exposures between 1 and 10 krads and gave a reduction of 97% of radioisotope moving to the apex. Addition of 5 ppm naphthaleneacetic acid was able to significantly increase the translocation to the meristematic region. More recently Roy and Clark studied the effect of whole plant irradiation on translocation to the roots of sugar beet (69). On the eighth day following a dose of 250 rads the plants were fed <sup>14</sup>C<sub>2</sub>O<sub>2</sub> in the light and allowed to translocate the <sup>14</sup>C products in the dark. The rate of translocation did not differ significantly between control and irradiated plants but the total amount exported to the root was lower in the irradiated plants.

Recently some studies have been conducted using soybean which show radiation-induced changes to the processes of translocation and photosynthesis (53, 73, 74). Soybean was selected as research material because of its importance as a food legume and also because of its extensive use in fundamental studies of physiological processes. The plant has also been shown to be sensitive to low doses of ionizing radiation (73).

In a study by Schefski (73) it was found that plants, illuminated at 1,600 foot candles (21% O<sub>2</sub>, 320 ppm CO<sub>2</sub>) and receiving doses of 3,750 or 11,250 rads of gamma radiation, exhibited a reduction in the rate of photosynthetic CO<sub>2</sub> uptake (photosynthetic rate) of 15% two hours post-irradiation. Three hours following a dose of 22,500 rads the rate of photosynthesis was reduced by 37%. Irrespective of whether the rates were measured at 21% oxygen or 1% oxygen, the extent of reductions were similar. Since low O<sub>2</sub> concentration (1%) is known to reduce photorespiration and the competition of O<sub>2</sub> with CO<sub>2</sub> for the binding site on the enzyme ribulose 5 diphosphate carboxylase (9, 12), the results at 1% O<sub>2</sub> suggested there was no increase in photorespiration which could account for reduced photosynthetic CO<sub>2</sub> uptake. The reduction in the rate of photosynthesis was shown in another study (53) to occur as early as 5 minutes following an absorbed dose of 3.5 krad and to last at least until 4 hours post-irradiation. The reduction in CO<sub>2</sub> uptake observed by Schefski (73) could not be attributed to an increase in dark respiration or to closure of stomata. Following doses of 3,750 or 11,250 rads there was no significant change in dark respiration, the number of stomata open or the aperture of the open stomata.

Schefski (73) also studied translocation in soybean following an acute exposure to radiation. Plants were treated similarly to those used in the photosynthesis studies, except that one hour after irradiation

the plants photoassimilated  $^{14}\text{CO}_2$  for 15 minutes which was then followed by a 45 minute illumination period before the distribution of  $^{14}\text{C}$  in the plant was assayed. In this study the translocation of  $^{14}\text{C}$  was found to be reduced by 70% following exposure to 3,750 rads. As well as a reduced export of translocate there was a decrease of 29% in the relative amount going to the apex and an increase of 8% directed below the node of the leaf which assimilated the  $^{14}\text{C}$ . If the radiation dose was increased to 11,250 rads the only observable change was a further reduction in the per cent of translocate moving to the apex.

It was also shown (73) that changes caused by gamma irradiation to the distribution pattern could be restored to normal with application of a 20 ppm IAA solution to the stem apex. The addition of IAA, however, was not able to restore the magnitude of  $^{14}\text{C}$  exported to the values obtained with non-irradiated controls. From this data it was concluded that the radiation induced changes in translocation were likely the result of damage to both the source leaf ( $^{14}\text{CO}_2$  photoassimilating leaf) and the stem apex.

It was proposed that changes in the distribution of translocate were due to damage at the apex, possibly in the IAA synthesizing mechanism. This view was supported by the fact that the addition of IAA to the cut stem apex of irradiated plants returned the distribution pattern to normal, as well as observations that radiation resulted in a reduced rate of stem elongation and an extension in the time required for new leaf emergence (73). The reduction in export was attributed to damage incurred by the source leaf possibly related to the reduction in the rate of photosynthetic  $\text{CO}_2$  uptake which was also observed following radiation.

To possibly account for the radiation-reduced export of photoassimilates Shelp (74) studied the effect of radiation on the availability of

translocatable materials and the metabolism of the photoassimilating leaf. Translocation was measured following radiation and the 80% ethanol soluble extract of the leaf was chromatographed to look at the kinds and relative amounts of  $^{14}\text{C}$ -metabolites. When the magnitude of translocation was reduced by radiation there was no significant change in the distribution of  $^{14}\text{C}$  in translocatable compounds or in the leaf metabolites. Furthermore, the distribution of  $^{14}\text{C}$  between the ethanol-soluble and -insoluble fractions did not change. Approximately half of the photoassimilate was present in each of the fractions. It was concluded therefore that the reduction in export was not due to a change in leaf metabolism causing a shortage in materials for translocation.

Shelp (74) further examined the radiation-induced changes in export and distribution pattern of translocation by studying the extent of the responses as a function of the absorbed dose of radiation. Distribution pattern was shown to be altered at a dose as low as 490 rads with no further changes in the radiation effect occurring over a dose range of 990 rads to 4,930 rads. The extent of export was not affected until the plants received a dose of 2,960 rads. The different radiosensitivities of these two translocation parameters indicated that the site of radiation damage is not the same for the magnitude of export as it is for the distribution pattern, thus supporting the conclusions of Schefski (73).

The previous studies of Schefski (73), Shelp (74) and McCabe (53) have identified and characterized the responses of the translocation and photosynthetic processes in soybean to low levels of ionizing radiation. Their efforts however have not identified the cause of reduced export or reduced photosynthesis in the source leaf although such possibilities as increased stomatal resistance (73), increased photorespiration (53, 73), increased dark respiration (73) and shifts in relative magnitudes of

biosynthetic pathways (74) were investigated. In each instance however no changes were evident in irradiated plants.

Shelp (74) has suggested that the reduction of photosynthetic rate and the radiation-induced reduction in export of photoassimilated carbon may be related. In fact he has suggested that the radiation sensitive site could be the process of photophosphorylation which not only provides energy for the dark reactions of photosynthesis but which might possibly provide necessary energy for the process of vein loading (74) and hence export.

On the basis of the studies of Schefski (73) and Shelp (74) the following radiation-induced changes to the process of photosynthesis and translocation have been identified:

(a) photosynthesis

(1) photosynthetic CO<sub>2</sub> uptake in soybean is reduced by ionizing radiation at absorbed doses as low as 3,750 rads,

(2) the reduced CO<sub>2</sub> uptake is not due to increased photorespiration or dark respiration,

(3) there is no increase in stomatal resistance within 3 hours to account for the reduced uptake of CO<sub>2</sub>,

(4) photosynthetic CO<sub>2</sub> uptake measured 2 hours after radiation exposure is not significantly different between 3,750 rads to 11,250 rads.

(b) translocation

(1) the magnitude of export is sensitive to an absorbed dose of 2,960 rads of ionizing radiation and it has been suggested that the initial site of radiation damage resides in the leaf,

(2) the distribution pattern of translocate is sensitive to doses as low as 490 rads and it has been suggested that the stem apex may be the site of radiation damage,

(3) both responses, that is decrease in export and change in distri-

bution pattern, show a maximum response following threshold and do not change at least up to an absorbed dose of 4,930 rads.

To extend some of the previous investigations reported, this thesis study was undertaken. The initial experiments were designed primarily to investigate the relationship between the responses of photosynthesis and translocation to gamma radiation. The subsequent experiments were planned to increase our understanding of the interrelationships existing between these two fundamental physiological processes. To achieve these goals, experiments were conducted to measure the extent of radiation-induced reductions in rates of apparent photosynthesis and to measure the effect of light intensity and leaf photosynthesis on the magnitude of photoassimilate export.

LITERATURE REVIEW

The work reviewed in this section is addressed to the relationship between the physiological processes of photosynthesis and translocation, particularly as the rate of CO<sub>2</sub> fixation and fate of photoassimilated carbon influence the availability of substrates for export from a source leaf and as the rate of carbon exported from a leaf affects the carbohydrate levels in the leaf and hence the rates of photosynthesis. In dealing with the export of photoassimilated carbon (e.g. amino acids, sucrose) from a source leaf, it becomes necessary to review evidence that vein loading, the process whereby translocatable materials in the source leaf enter the translocating system, is an energy-requiring process. The factors which control the photosynthetic rate of plants or the movement of organic compounds out of a photosynthesizing leaf may be expected to have reciprocating effects as they are functionally related, one producing the compounds which are substrates for the latter.

As early as 1868 Boussingault (60) suggested that accumulation of photosynthetic products in a leaf could cause a reduction in the rate at which carbon dioxide is taken up by that leaf. To account for a reduced photosynthetic rate it was suggested that increased levels of photosynthetic products may reduce the availability of carbon dioxide to the chloroplasts, or subject the biochemical reactions of carbon dioxide reduction to a negative feedback system or lastly, to reduce the illumination available for the light reactions. The hypothesis that there is a reduction in photosynthesis due to a build up of photoassimilation products remains a controversial one to this day.

Upmeyer and Koller (93) found that the photosynthetic rate of soybean although relatively stable 4 to 10 hours following the beginning of illumination gradually fell to 85% of the optimum rate near

the end of a 16 hour photoperiod. Starch levels were observed to increase steadily throughout the photoperiod until a limit was reached, at which time the soluble carbohydrate production increased. The increasing soluble carbohydrate concentration was accompanied by a reduction in photosynthetic capacity, an increase in stomatal diffusion resistance (stomatal closure) and an increase in residual mesophyll resistance to  $\text{CO}_2$ . Their data was interpreted to show a correlation between decreasing photosynthetic rate and increasing levels of soluble carbohydrates. A correlation between apparent photosynthetic rate and carbohydrate levels was also shown by Nafzigir and Koller (58) but they observed a more direct response to starch concentration rather than to soluble carbohydrate level. Three levels of starch were obtained in different soybean leaves by exposing them to different  $\text{CO}_2$  concentrations. Leaves with the highest starch levels had photosynthetic rates lower than the other plants while the leaves containing the least amount of starch had the highest activity when determined at 300 ppm  $\text{CO}_2$ .

Evidence for high levels of starch concentration causing a reduction in sugar beet photosynthesis was also shown from experiments in which rates of photosynthesis were measured under high irradiances following either a dark or light pretreatment (57). Dark pretreated plants contained less carbohydrate than light pretreated ones and showed higher photosynthetic rates until the carbohydrate levels in the leaves were equal to those in the leaves of plants pretreated with light. Sucrose, glucose and fructose levels in the leaves under any treatment remained relatively constant while starch was found to accumulate under high light. Also using sugar beet Habeshaw (31) found that photosynthetic rates were highly correlated with the concentration of carbohydrates and he observed that photosynthesis was reduced to the greatest degree when sugar levels were high in the cytoplasm surrounding chloro-

plasts. Starch levels in the sugar beets used by Habeshaw (31) were 10 times less than those of Milford and Pearman (57) and this may account for some of the observed difference.

The work of Lui et al (49) and Hofstra and Nelson (40) has suggested that the extent of export of photoassimilated carbon out of the leaf, and therefore the levels of carbohydrate in the leaf, is correlated with photosynthetic efficiency. The photosynthetic rates of two varieties of Phaseolus vulgaris were found to be significantly different by about 30% (49). The variety which loaded photoassimilate into the phloem tissue at a faster rate was the variety which also photosynthesized more efficiently. Studying a variety of plants such as sorghum, millet, tomato, tobacco and soybean Hofstra and Nelson (40) found that those plants with high photosynthetic rates had generally higher export rates and moved a greater total of the photoassimilated  $^{14}\text{C}$  out of the leaf. The authors did not however suggest explicitly which process resulted in the other process occurring at a high rate.

In any evaluation of carbon movement out a leaf and its affect on photosynthetic rate the influence on the source area (leaves) by the sinks (sites of utilization of translocated materials) must be considered. For example removal of some leaf tissue might be expected to alter the photosynthetic rate of remaining leaves due to a change in the ratio of source tissue to tissues of utilization. Indeed this was demonstrated in experiments by Sweet and Wareing (82) who removed all or one third of the fully elongated needles of Pinus radiata six days prior to measuring photosynthetic rates. Experimental plants in which needle removal occurred six days before photosynthetic measurements showed increases in photosynthetic ability. The increased photosynthetic rates were attributed to increased demand by the sinks on the remaining leaves. If removal of the stem apex, an important sink, occurred at the same time

as the defoliation there was no increase in the photosynthetic CO<sub>2</sub> uptake of the remaining needles. Similarly, Wareing et al (98) partially defoliated Phaseolus vulgaris, Zea mays, Salix viminalis and S. acutifolia and found an increase in the photosynthetic ability of the remaining leaves three days later. Removal of the root, a sink region, following the partial defoliation showed that the photosynthetic increase was due to sink demand because an increase in photosynthesis did not occur.

Thorne and Koller (86) using soybean altered the sink to source ratio by shading 7 of the 8 existing leaves and followed the changes in the source leaf over an 8 day period. The photosynthetic rate was found to increase by the second day, the rate increasing to more than 125% of control by the eighth day. The level of carbohydrates in the experimental source leaf also changed with a small increase in sucrose from 1% (0 day) to 3% (8 day). However a very large decrease in starch from 23% to 2% was observed. The authors suggested that reduced starch may have caused an increase in the photosynthetic rate and that the response was ultimately due to sink-source hormonal interaction. The sink-source hormonal interaction was suggested due to observed increases in Pi levels, ribulose diphosphate carboxylase concentration and the time needed for the photosynthetic rate increases to occur.

The removal of tubers or the major sink in potato plants (63) was observed to cause a reduction in photosynthesis which took a couple of days to become apparent. The photosynthetic rate of wheat leaves has been shown to be affected by sink demand (ear) by inhibition of ear photosynthesis or removal of the ear and this effect was attributed to a direct sink demand for assimilates controlling carbohydrate levels in the leaves (44). Manipulation of sink demand by means other than removal of the sink has also been shown to affect photosynthetic ability. The larger roots on sugar beet plants which were germinated and grown in a

growth chamber under controlled conditions were correlated to higher photosynthetic abilities in these plants. Sugar beets grown in the field which had smaller roots also had lower rates of photosynthesis (41). Cooling of the roots of sugar beet plants, thus lowering sink demand, has been attributed to lowering export and causing a reduction in the photosynthetic rate (31). The sugar beet leaves exhibiting lower photosynthetic rates also had greater levels of starch and reducing sugars than the controls (31).

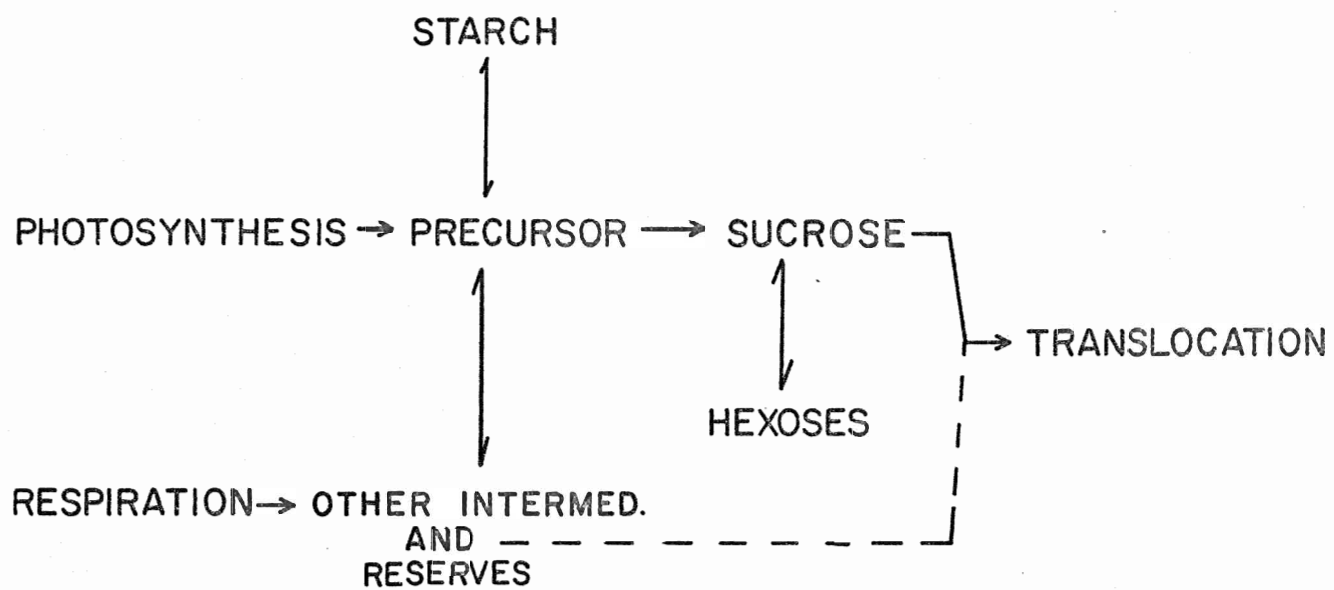
The review presented thus far has been selected from studies that support the idea of a control of photosynthetic rates by a build up of products in the leaf or a change in demand upon a source region by the sink regions. There is however data to suggest that these factors may not be controlling mechanisms of photosynthetic rates. Correlations between carbohydrate levels, extent of export and photosynthesis rates have not been demonstrated in studies involving diurnal analysis (65), seasonal analysis (48), cooling of the translocation pathway and sites of utilization (18, 33, 99) or defoliation and sink removal (3, 18, 50, 51, 59). Whether or not there is a definite control mechanism between the level of photoassimilate export, leaf carbohydrate level and the leaf's ability to photosynthesize remains uncertain. If the sink is not controlling the movement of photoassimilate from the leaf and indirectly the photosynthetic rate then an alternative explanation is that export is controlled by the production and availability of leaf assimilates for translocation or by the availability of the energy for the entry of assimilates into the translocation pathway. The control of leaf translocatable metabolites and energy may be controlled by the light intensity or the potential energy available to be trapped and converted into usable forms by the leaf.

Evidence exists that the amount of organic compounds exported from a leaf can be affected by light intensity (30, 38, 72, 87). For instance Throver (87) showed that a soybean plant illuminated at 1,500 foot candles translocated 14% more  $^{14}\text{C}$  than a plant maintained at 600 foot candles for the same two hour period and sugar beet translocation was observed to increase two and a half times with an increase in light intensity (30). Although the export was increased two and a half times,  $\text{CO}_2$  fixation rate increased by only 50%. A relationship between the photosynthetic rate as affected by light and the amount of carbon translocated has, in fact, recently been observed by Ho (38). The proportional relationship of fixation and translocation occurred above a light intensity of  $25 \text{ Wm}^{-2}$  when the carbon fixed was  $2 \text{ mg C dm}^{-2} \text{ hr}^{-1}$  or greater. Export of 60-66% of the fixed carbon ( $> 2 \text{ mg C dm}^{-2} \text{ hr}^{-1}$ ) was maintained with increasing fixation rates. When the level of export was proportional to the rate of  $\text{CO}_2$  fixation the sucrose level was maintained at 1% of the total organic carbon and starch was 17%. When photosynthesis became limiting, that is when the  $\text{CO}_2$  fixation rate was less than  $1 \text{ mg C dm}^{-2} \text{ hr}^{-1}$ , the translocation rate was still maintained at  $1 \text{ mg C dm}^{-2} \text{ hr}^{-1}$  principally at the expense of starch which decreased to 8% of the total organic carbon while the sucrose level was only halved to 0.5%. The changes in sucrose and starch levels were attributed to control mechanisms dependent upon light intensity which partition fixed carbon between two carbohydrates. The partitioning mechanisms control export by regulating mobile sucrose with starch being a potential source of sucrose at low light intensities, when fixation is lower than translocation. To show the relationship between newly formed photosynthate for translocation and the reserve, starch, Charles-Edwards and Ho (11) presented a model (see Fig. 1).

Identified in Figure 1 is sucrose the major translocatable carbo-

Figure 1. Diagram showing the movement of carbon through a leaf.

Sucrose is the primary photosynthetic product and is readily available for export from the leaf.



hydrate, whose concentration controls the export rate; starch whose synthesis is dependent on a precursor pool which also serves as a source for sucrose synthesis; other intermediates may be transported as they exist or possibly converted into sucrose and exported; sucrose and hexoses are in a simple equilibrium with little exchange between the two pools.

Studying the rate of  $^{14}\text{C}$  export under varying light,  $\text{O}_2$  and  $\text{CO}_2$  concentrations Servaites and Geiger (72) found the increase in fixation of  $\text{CO}_2$ , under these varying conditions, was reflected in the export rate out of the leaf. They increased photosynthetic rate up to 4 times the rate at 'normal' conditions and did not saturate the ability of the leaf to translocate the excessively produced translocatable materials. They suggested therefore that the mass transfer rate, that is, the movement of  $\text{CO}_2$  assimilated compounds for export, is directly related to the production of carbohydrates by photosynthesis. They did not however measure the concentrations of the carbohydrates in the leaf under the varying conditions.

In contrast to the interpretation of Servaites and Geiger (72), Nelson (61) found that a soybean plant photoassimilating at a constant light intensity but receiving varied  $\text{CO}_2$  concentrations (0.03% - 0.3%) showed no increase in the amount of material translocated even though the  $^{14}\text{C}$  fixed in the leaf increased by a factor of 3 between the low and high  $\text{CO}_2$  concentrations. It is unfortunate that the concentrations of organic compounds produced under the varying  $\text{CO}_2$  were not determined to see if the extra carbohydrate was potentially available for export.

The ability of a leaf to translocate photoassimilate may depend not only on the availability of translocatable materials for export, but also, on the ability of these materials to enter the translocate pathway. The loading of translocatable materials into the vascular tissue is an

energy requiring process (25, 77, 91) and therefore energy availability could potentially become limiting to the export process. The site of loading of the vascular system is not satisfactorily acknowledged by all scientists even though description of phloem tissue is well advanced.

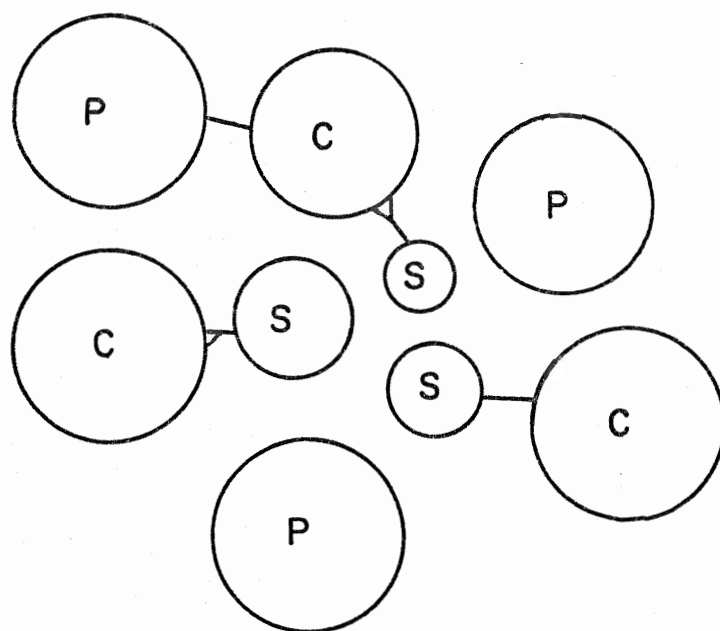
The phloem tissue which is principally responsible for the movement of organic molecules throughout the plant is part of the complex vascular conducting tissue which forms a netted pattern in the leaf. Such a network of venation allows maximum proximity of mesophyll cells where photosynthate is produced with the phloem transport cells, the sieve elements. The strings of sieve elements or veins are classified according to their size, the largest or main vein in the leaf being the first order vein, while the veins in closest contact to mesophyll cells are classified as fifth order veins (15, 88). The smaller or minor veins of the leaf are unique from other veins in that they have associated with them 'companion cells' which are equal in size or larger than the associated sieve element cells (15, 16, 24). The companion cells, believed to have arisen from the same mother cell as the sieve element, are physically joined to the sieve element cell, though the two cells are very different morphologically when in their functional states (47).

The sieve element is generally longer than it is wide and contains a fluid-filled central region called the 'lumen', through which translocated materials move. A thin layer of cytoplasm is oppressed to the cell wall and contains a few small mitochondria, stacks of smooth endoplasmic reticulum and a unique plastid. Each sieve element is joined to the next by a sieve plate, an area where end walls of two sieve elements join and develop a pore system through which flow of translocate may occur. The pore system is lined with a proteinaceous material called P-protein and when injury occurs flow is stopped by deposition of callose between the sieve elements (15, 16, 47, 79, 88).

The associated companion cells and phloem parenchyma cells have a more dense cytoplasm than the sieve elements. Higher metabolic activity is associated with these cells because they have an abundance of ribosomes and numerous large well developed mitochondria. The companion cell is physically associated with a sieve element by plasmodesmata. The plasmodesmatal canal is wider and unbroken at the sieve element surface and may branch out on the companion cell side joining to the surface at different but spatially close points. Companion cells can be associated with phloem parenchyma cells by plasmodesmata and parenchyma cells bordering on the mesophyll cell layer are also commonly joined to these cells by plasmodesmata (22, 23). Gunning (29) has reported the existence of specialized modified companion cells in Pisum arvense which he called 'transfer cells'. The cells are unique in that they have numerous cell wall invaginations thus increasing the cell surface:volume ratio (Fig. 2).

Numerous workers have suggested that the phloem minor vein tissue actually accumulates solutes for translocation, that is they maintain a higher concentration of organic solutes than other leaf tissue. The term used to describe the accumulation of solutes into the minor veins is vein loading or phloem loading, a process whereby materials are actively and selectively accumulated into the phloem minor vein tissue (19, 20). Gunning (29) observed that 'transfer' and sieve element cells would accumulate labelled photosynthate following photoassimilation of  $^{14}\text{CO}_2$  and he proposed that the transfer cells actively accumulated sugars making these sugars available to the sieve elements. Using autoradiography it has also been shown that minor veins will accumulate photoassimilated  $^{14}\text{C}$  over and above that found in the mesophyll (17, 25, 39). Studies using incipient plasmolysis (23) indicated that the solute concentration, basically with sucrose as the solute, was equal in the sieve element and companion cell and was 3 to 4 times higher than other leaf

Figure 2. Diagram showing the relationship of companion cells or transfer cells to the sieve elements in the phloem tissue.



—  $\triangleright$  plasmodesmata.

P phloem parenchyma cell.

C companion cell ; if membrane invaginated "transfer cell".

S sieve element.

tissues. Sovonick et al (77) calculated that of the total sucrose in a sugar beet leaf 80% was in the minor veins at a given time.

Two explanations have been advanced to account for the route that translocatable materials travel before active accumulation into the minor veins occurs. The first explanation states that the organic solutes produced in the photosynthetic mesophyll symplast moves through the symplast via plasmodesmata to the phloem parenchyma and companion cells and ultimately to the sieve elements (10, 90). Symplast is defined as the region of bulk cytoplasm which includes cytoplasm filled plasmodesmata. The previously discussed morphology would allow for such a movement because of the existence of plasmodesmatal connections between mesophyll and phloem parenchyma, phloem parenchyma and companion cells and finally, companion cells and sieve elements. In this pathway, the site of active accumulation is suggested as being the branched plasmodesmata between adjacent walls of phloem parenchyma, companion cells and sieve tubes (10). Tyree (90) has determined that plasmodesmata are common among a wide range of plants and that plasmodesmata would thermodynamically constitute the pathway of least resistance. Cataldo (10) enzymatically separated the mesophyll cells and the minor vein bundles from tobacco and subjected the separate fractions to endogenous sucrose. The sucrose uptake data of minor veins and mesophyll cells, when compared to tobacco leaf disks, indicated that mesophyll cells took up sucrose at a rate comparable to the disks. Since the isolated minor veins accumulated sucrose at a much reduced rate he concluded that mesophyll cells normally produce sucrose and transport it to the sieve elements symplastically. A diagram illustrating the pathway is shown in Figure 3.

The second hypothesis regarding the pathway of transport to the sieve elements suggests a movement of sucrose from the photosynthesizing cells through the symplasm to associated phloem parenchyma of mesophyll cells.

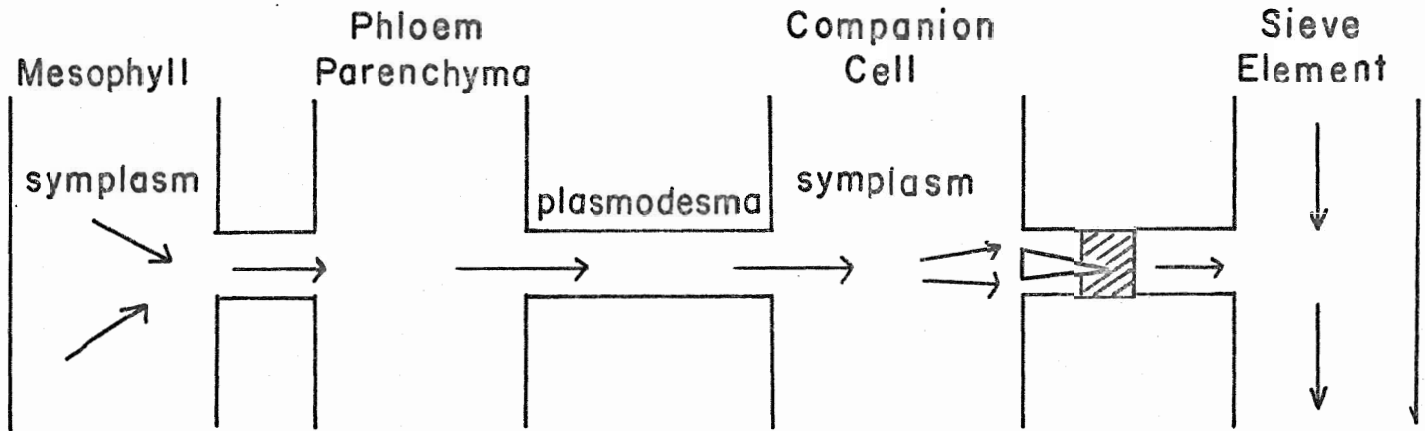
However, after reaching the parenchyma the sucrose enters the free space (apoplast) by crossing the parenchyma plasmalemma either by diffusion or an active process. Once in the apoplast (extracytoplasmic region) the sucrose and other organic solutes are actively accumulated into the companion cell-sieve element complex (19, 20, 25, 28). The proponents of the 'apoplast loading' theory have amassed a great deal of data in its support and it will be dealt with in this review to a greater extent than the symplastic theory. The pathway for apoplast transport is shown in Figure 3.

In support of the 'apoplast hypothesis' for phloem loading Geiger has shown a close physiological relationship between the companion cell and sieve element (23). The two cells plasmolyzed at the same osmotic pressure indicating that the solute concentrations were similar in the cells. Furthermore, the energy requiring process of accumulation was implied to be located at the site of the companion cells because of their large number of mitochondria and ribosomes, an indication of high metabolic activity (15, 24, 29). Analysis of sugar beet leaf morphology indicated that a 33  $\mu\text{m}$  length of minor vein was no farther away than 65 to 100  $\mu\text{m}$  from 30 mesophyll cells. Although the sieve element-companion cell complex was found to account for only 0.6% of blade volume (22), this complex has a surface of 0.9  $\text{cm}^2$  in a leaf tissue area of 1  $\text{cm}^2$  (77), a situation therefore, where the exposed area for uptake is large, as is the amount of solute produced in close proximity, with only a short distance for solutes to travel.

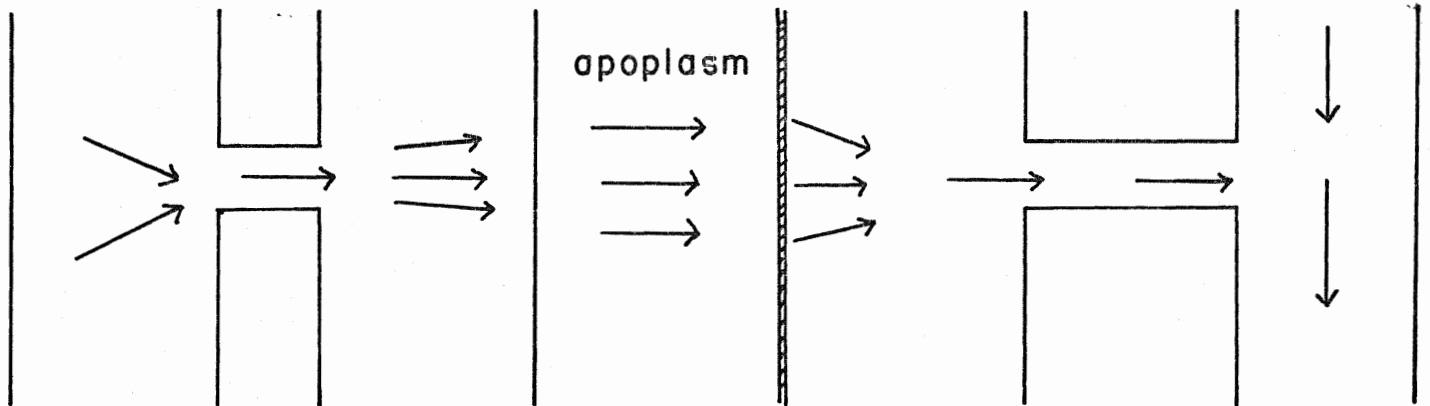
Support for the accumulation of sucrose from the apoplast also comes from experiments in which exogenously supplied  $^{14}\text{C}$  sucrose was actively accumulated by the minor veins from the apoplast. A sucrose concentration of 20 mM can be loaded from the apoplast into the minor veins




Figure 3. A diagrammatic representation of the Symplast and Apoplast loading theories for materials exported out of a leaf.

### VEIN LOADING VIA SYMPLASM



### VEIN LOADING VIA APOPLASM



-  indicates movement of translocation material.
-  site of active accumulation.
-  branched plasmodesmata.

at a rate similar to the rate of photosynthetically fixed  $^{14}\text{CO}_2$  by leaf disks (25). Furthermore in whole plants the export rate was maintained by the addition of 20 mM sucrose at the same value as the rate of export of photosynthetically produced  $^{14}\text{C}$  sucrose (25, 77). When mesophyll cells were plasmolyzed in an effort to destroy symplastic connections (not totally successful) the addition of 20 mM sucrose brought the mass transfer rate back to or slightly below the rate produced by photosynthesis before plasmolysis (25). More recent work (28) has shown that accumulation of exogenously supplied sucrose or  $^{14}\text{CO}_2$ -derived assimilates into the minor veins of leaf disks is inhibited by the addition of p-chloromercuribenzenesulfonic acid (PCMBS) and that the inhibition by PCMBS, did not affect the metabolic functions of photosynthesis or respiration. Also the inhibition was completely reversible using dithiethriitol indicating no permanent damage due to PCMBS treatment. The inhibition of active uptake of sucrose by PCMBS, a sulfhydryl blocking reagent, indicates that a sulfhydryl carrier system on the membrane may be active in the movement of sucrose to the minor veins. The data did not allow determination of whether the carrier system was associated with the phloem parenchyma or the companion cell-sieve element complex or possibly both.

Evidence for the movement of sucrose into the apoplast around the minor vein before loading was demonstrated by use of a isotope trapping methodology (25). In this study the upper abraded surface of the leaf was exposed to a circulating buffer solution containing 20 mM unlabelled sucrose while the lower surface was exposed to  $^{14}\text{CO}_2$ . The circulating buffer with the sucrose exchanges non-radioactive sucrose with photosynthetically produced radioactive sucrose. Using such a procedure Geiger has argued that sugars do enter the apoplast prior to final loading into the phloem tissue.

The requirement for energy in the accumulation of translocatable

material in the minor veins (vein loading) has been shown by a number of workers (25, 77, 46, 91). Ulrich (91) observed that the addition of ATP to the leaf veins of Pelargonium zonale together with fluorescein dye would increase the intensity of the dye transport. If the dye and ATP were applied at separate points on the vein there was no affect. Kuranov and Brovchenko (46) showed that ATP stimulated outflow of organic solutes from a sugar beet leaf and they suggested that the increased translocation was due to an initial activation of hexoses into their phosphoric esters thus increasing transport to the phloem and also increased accumulation into the phloem of hexose phosphates by the addition of ATP.

The more recent data of Geiger et al. (25) and Sovonich et al. (77) indicates the response to ATP to be more specifically at the minor vein surface possibly increasing uptake of sucrose. The effect of 4 mM DNP (dinitrophenol) added to the free space was to lower the total ATP level of the source leaf to 40% of the control, to increase CO<sub>2</sub> production by 210% and to reduce translocation rate of exogenously supplied sucrose to the sink leaf by 80%. If 4 mM ATP was added following DNP-induced reduction of translocation the level of export returned to the level of that prior to the inhibition (77). Using isotope trapping Geiger and coworkers (25) were able to show that the addition of 4 mM ATP increased both the sucrose entering the free space and the translocation from the leaf. Sovonick et al. (77) found that uptake and translocation of sucrose into the minor veins could be increased simply by increasing the concentration of exogenously supplied sucrose. This may indicate the controls on the vein loading process are complex and deal with energy supply as well as organic solute availability for export.

After reviewing the literature, Geiger (19) has suggested three possible ways that ATP may affect accumulation into the minor veins:

(1) phosphorylation of hexoses for increased exit from mesophyll and entry into the phloem or minor vein tissue; (2) increase membrane permeability to translocatable organic solutes and; (3) promotion of phloem loading and possibly promotion of leakage in adjacent parenchyma cells.

The studies referred to previously have shown the possible ways in which translocatable photoassimilates produced in the chloroplast-rich palisade and mesophyll cells may enter into a leaf's phloem system for export. The question still remains as to what controls the rate of movement of sucrose into the minor veins under steady state sink demand. As previously discussed increases in translocation have been observed when light intensity is increased (27, 38, 72) and certainly light-mediated control of the amount of photosynthate available or more specifically control of levels of translocatable materials such as sucrose, would be one way in which light could control export. A second way in which light may increase export is via photophosphorylation and hence the availability of energy for the vein loading process.

The suggestion that light-mediated ATP production can influence translocation by increasing energy available through photophosphorylation has been made by Plaut and Reinhold (67) who showed that DEMU (3,3,4 dichlorophenyl) 1,1 dimethyl urea) an inhibitor of photosystem II phosphorylation, could reduce the translocation of exogenously supplied sucrose by more than 50%. Hartt (34) also implicated light produced energy in translocation when she observed increased movement of sucrose at increasing light intensities below the compensation level of photosynthesis. Since the cyclic production of ATP is not immediately necessary to the maintenance of normal photosynthetic rates (83) it has been suggested that this extra ATP could be funnelled into the cytoplasm for use by other physiological processes, possibly vein loading. Indeed, Raven (68) has shown a dependence of such ATP-requiring processes as  $\text{Na}^+$ ,  $\text{H}_2\text{PO}_4^-$  or  $\text{K}^+$  influx and

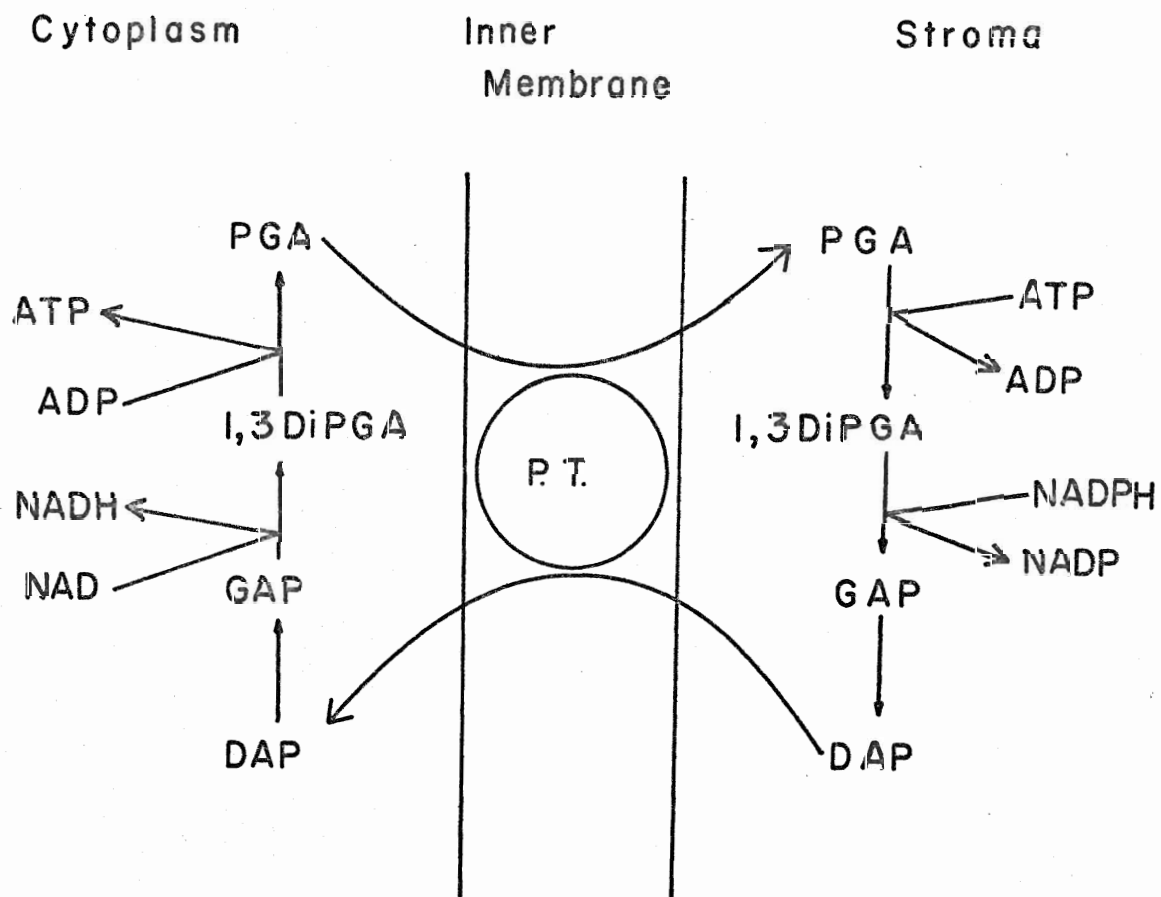
glucose uptake and metabolism on the ATP from cyclic photophosphorylation.

To account for the cytoplasmic utilization of ATP, initially produced in the chloroplasts, mechanisms of ATP transfer, either directly or indirectly, have been sought. In isolated spinach chloroplasts it has been observed that the early products of photosynthesis PGA (phosphoglyceric acid) and DHAP (dihydroxyacetone phosphate) are found in the suspending medium following a short period after the onset of photosynthesis (4, 36). The exchange of ATP from the chloroplast to the cytoplasm has been suggested by Heldt and Rapley (37), as an indirect transfer, proceeding by a DHAP-PGA shuttle. The exchange of DHAP and PGA occurs by means of their proposed 'phosphate translocator' with photosynthetically produced DHAP being transferred across the chloroplast inner membrane to the cytoplasm. In the cytoplasm the DHAP is isomerized to glyceraldehyde phosphate (GAP) which in turn is oxidized to 1,3-diphosphoglyceric acid (1,3-DiPGA) and finally dephosphorylated to PGA. The enzymes involved in the production of PGA from DHAP in the cytoplasm are proposed to be the same as those responsible for the reverse reactions in the chloroplast. The important aspect of this shuttle is the production of ATP during the dephosphorylation in the cytoplasm. A diagram showing the participation of the phosphate translocator in ATP transport across the inner membrane of the chloroplast can be seen in Figure 4.

Mangat et al. (52) have demonstrated that when leaves are exposed to light there is a large increase in the leaf ATP levels which was depleted within 10 minutes. This depletion of ATP occurs minutes after photosynthesis is at a steady state suggesting that the ATP is used in processes in the cytoplasm. Indeed experiments showed that ATP levels in the cytoplasm rose by 115% compared to chloroplast levels in the same 10 minute period suggesting ATP movement from the chloroplast to the cytoplasm.

There is evidence to suggest therefore that the process of photophos-

Figure 4. Schematic diagram of a possible participation of the phosphate translocator in ATP transport across the inner membrane.



P.T. phosphate translocator.

phorylation may supply energy to processes other than for the reduction of  $\text{CO}_2$  in the chloroplast and that photosynthetically produced ATP transported outside of the chloroplast should be considered available for the loading of translocatable materials into the minor veins. The work described in this thesis will be directed at the problem of energy availability for the process of vein loading and the relationship of this process to the process of photosynthesis.

### MATERIALS

The soybean plants (Glycine max L. cultivar Haro'soy 63') used in this study were obtained by germinating seeds supplied by the Agriculture Research Station, Harrow, Ontario.

Seeds were planted, three to a 12 cm - diameter pot, at a depth of approximately 1.5 cm in 'Terra-Lite' horticultural grade vermiculite. The plants were watered daily and kept in the greenhouse on a 14 hr light: 10 hr dark photoperiod. Sunlight was supplemented by a row of Sylvania 60 watt fluorescent bulbs which supplied 700 foot candles of illumination at the top of the pots. The day and night temperatures were approximately 30°C and 23°C, respectively.

On the seventh day following planting the pots were moved to an environmentally controlled growth chamber (Colmat Environmental System Model 255-6). The photoperiod in the chamber was 16 hr light (6 a.m. - 10 p.m.) and 8 hr dark with the respective temperatures at 25°C and 18°C. Illumination was provided by eight 60 watt fluorescent bulbs supplemented by two rough surface 60 watt incandescent bulbs. Light intensity measured at the level of the first trifoliolate leaf was 700 foot candles.

The plants in the growth chamber were watered daily and supplied with nutrients in a solution of 20-20-20 All Purpose Feed (Plant Products Co. Ltd.; 3.5 grams per litre of H<sub>2</sub>O) twice weekly.

Plants were selected for use in experiments when they were 18-22 days old and the length of the developing second trifoliolate leaf was approximately 0.5 to 0.6 the length of the first trifoliolate leaf. The length of trifoliolates was measured from the node to the tip of the central leaflet.

## METHODS

The experimental procedures used in this study were initially designed to study radiation-induced changes in photosynthesis and in the magnitude of translocation following exposure to radiation. Later experiments were conducted to study the effect of light on the process of translocation.

The procedures used consisted of:

- (i)  $^{60}\text{Co}$  gamma-irradiation of whole soybean plants.
- (ii) measurement of the apparent photosynthetic rates before and following exposure to radiation.
- (iii) photoassimilation of  $^{14}\text{CO}_2$  by a soybean trifoliolate leaf and the extraction of ethanol-soluble compounds in the plant as well as in the free space.
- (iv) measurement of  $^{32}\text{P}$  incorporation into ATP by isolated chloroplasts as a measure of leaf photophosphorylation rate.

### I. WHOLE PLANT IRRADIATION

The radiation source for this study was a  $^{60}\text{Co}$  Gammacell 220 unit, manufactured by Atomic Energy of Canada Ltd. in December 1960. The original source contained 2,430 curies of  $^{60}\text{Co}$  radioactivity and produced 3,617 rads/minute. The average dose rate over the period of this study was 458 rads/minute.

The basic unit consists of the annular shaped  $^{60}\text{Co}$  source, a lead shield surrounding the source and a drawer which moves vertically between the 'loading' and 'irradiating' positions. Exposure to radiation is the same at each point in the drawer (irradiating position) due to the 'squirrel cage' source which is 8 inches high and 8 inches in diameter. The drawer moves between the loading and irradiating positions in a time of 7 seconds.

A plant was irradiated by placing it in the chamber (loading position)

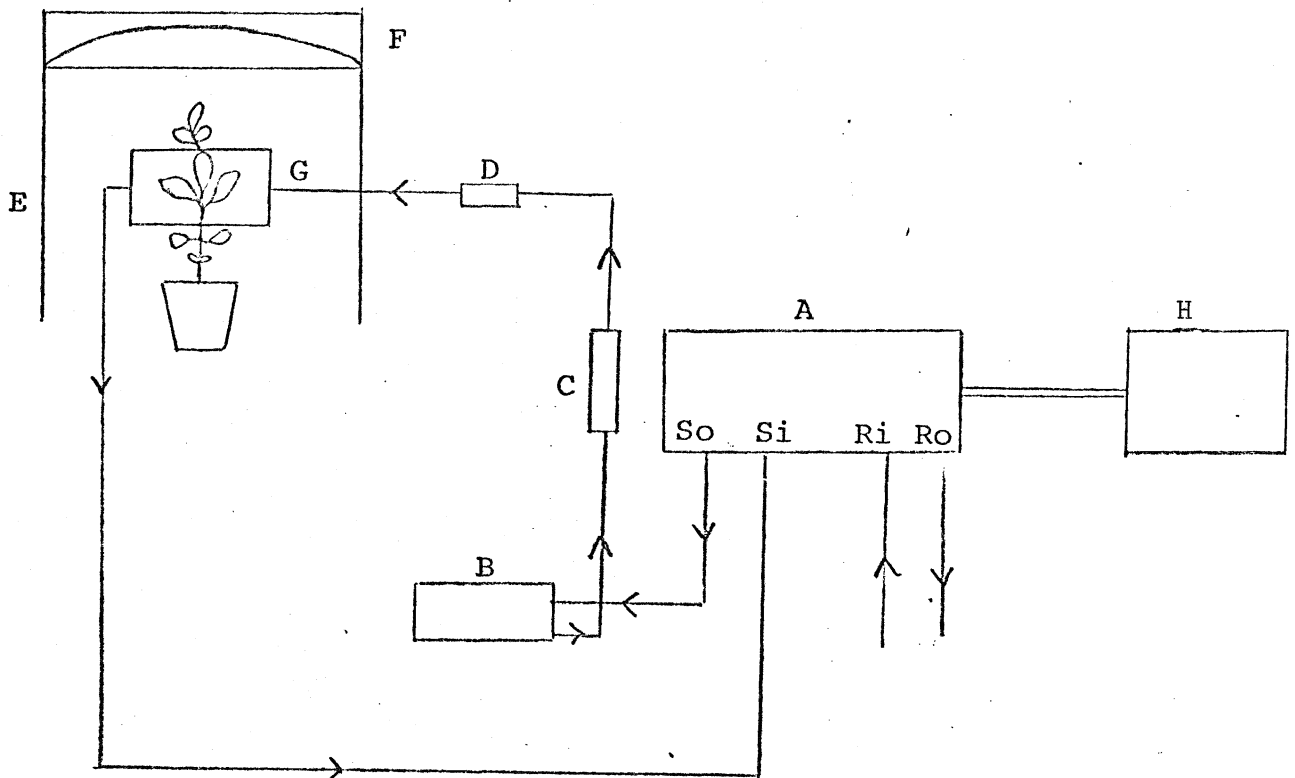
and lowering the chamber into the 'irradiating' position. Timing of the exposure period began when the chamber reached its final point of descent. The chamber remained in this position for the required time after which, the chamber was brought to the 'loading' position and the plant removed. During the irradiation period a flow of fresh air was maintained by a pump circulating the air at a rate of 7.5 litre/min. Non-irradiated plants were handled similarly except that the chamber remained in the 'loading' position for the same time period.

## II. MEASUREMENTS OF RATES OF APPARENT PHOTOSYNTHESIS

Gas exchange measurements were made using a closed system which consisted of (1) a transparent rectangular plexiglass leaf chamber; (2) infrared gas analyzer; (3) electric pump and (4) a gas flowmeter. The components were joined together using 'Tygon' tubing fitted with ground glass joints. The glass joints were sealed using Dow-Corning stopcock grease and secured by use of elastic bands. The total volume of the system was 260 ml and the gases were circulated through the system at a rate of 2.8 litres/minute. Changes in CO<sub>2</sub> concentration in the closed system were recorded by a Perkin Elmer recorder as measured and monitored by the IRGA. Light was supplied by an illumination chamber with a light source of twelve 20 watt Cool White fluorescent and twelve 150 watt Reflector Flood incandescent bulbs. The light was filtered through a flowing water shield of 5 cm in depth which kept the temperature in the illumination chamber at approximately 25°C. A diagrammatic representation of the closed system and light source can be seen in Figure 5.

Thirty minutes prior to measuring the pre-irradiation photosynthetic rate the plant was placed in the illumination chamber under illumination of 2,800 or 1,600 foot candles measured at the level of the mature trifoliolate leaf. The three leaflets and a portion of the petiole of the trifoliolate were enclosed in the plexiglass leaf chamber 15 minutes before

Figure 5. Diagrammatic design of a closed system for obtaining the rate of apparent photosynthetic CO<sub>2</sub> uptake. Reaction vessel (D) used only during <sup>14</sup>CO<sub>2</sub> generation.



- A - Beckman 215 Carbon dioxide Infrared Gas Analyzer
- B - Pump - Universal Electric Co. (Model No. AA1E122)
- C - Flowmeter - Grilmont (Model No. L95)
- D - Vessel for the production of radioactive carbon dioxide ( $^{14}\text{CO}_2$ )
- E - Illumination Chamber
- F - Light Source
- G - Leaf Chamber
- So - Sample out
- Si - Sample in
- Ro - Reference out
- Ri - Reference in
- - Tygon Tubing - showing direction of gas flow
- H - Chart Recorder

the rate was to be taken. Prior to use the IRGA was calibrated with gases of known CO<sub>2</sub> concentration purchased from and analyzed by the Linde Corporation. Rates of apparent photosynthesis were taken at an average CO<sub>2</sub> concentration of 300 ppm using laboratory air as a source of CO<sub>2</sub>. The plant was then taken to the gammacell where it absorbed a total radiation dose of between 60 and 4,930 rads.

After removal from the gammacell the plant was returned to the illumination chamber and the same trifoliolate isolated in the plexiglass chamber. Photosynthetic rates were again taken under the same conditions used for the pre-radiation measurements. Rates of apparent photosynthesis were determined 15, 20, 25, 30, 45, 60 and 75 minutes following isolation. The procedure followed was the same for irradiated and non-irradiated plants.

Following the final measurement of photosynthetic rates the portion of the leaf inside the plexiglass chamber was separated from the plant and weighed to yield a fresh weight of leaf. Photosynthetic rates were expressed as mg CO<sub>2</sub> taken up per gram fresh weight on leaf tissue.

### III. LEAF <sup>14</sup>CO<sub>2</sub> PHOTOASSIMILATION AND DETERMINATION OF THE MAGNITUDE OF TRANSLOCATION

The <sup>14</sup>C was introduced into the plant by feeding <sup>14</sup>CO<sub>2</sub> into the closed system for a period of 5 or 15 minutes. A glass reservoir reaction vessel was introduced into the closed system between the flowmeter and the plexiglass leaf chamber. The <sup>14</sup>CO<sub>2</sub> was generated by adding 1 ml of 8 M lactic acid, by syringe, into the reservoir containing 10 μCi of sodium <sup>14</sup>C carbonate (specific activity 60 mC/mmmole).

In the first experiments the plants were illuminated at 800, 1,600 or 2,800 foot candles for 30 minutes before a rate of apparent photo-

synthesis was taken.  $^{14}\text{CO}_2$  was then circulated in the system for 5 minutes and followed by a 10 minute translocation period after which the plant was removed to the gammacell and irradiated with a dose of 4.1 krads. The plant was then returned to the closed system and illuminated as before for a 30 minute period during which time laboratory air continuously flowed (2.8 litre/min) over the leaf. After the 30 minutes an apparent photosynthetic rate was taken.

The amount of photosynthate that was translocated was determined by comparing the  $^{14}\text{C}$  exported out of the leaf (leaflets and portion of petiole) to the total  $^{14}\text{C}$  extracted from the plant. The plant was divided into (1) fed leaf, which includes the petiole enclosed inside the plexi-glass chamber and (2) the sink or areas into which  $^{14}\text{C}$  compounds are translocated. The two sections were extracted twice in 80% aqueous alcohol (V/V) with the two extracts from each section being combined. The volume of the combined extracts was measured and the  $^{14}\text{C}$  radioactivity determined by counting duplicate 100  $\mu\text{l}$  aliquots contained in vials having 15 ml ACS (Aqueous Counting Scintillant purchased from Amersham/Searle Ltd.). The  $^{14}\text{C}$  content in the vials was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer (model 3310).

For some experiments translocation rates and free space  $^{14}\text{C}$  content were measured to observe the effects of light intensity on these parameters. In these experiments the plants were illuminated for 1 hour before the 15 minute photoassimilation period of  $^{14}\text{CO}_2$  at the same light intensity. This was followed by a 45 minute translocation period with the plant under illumination. Details regarding the intensity of light during the translocation period are given for each experiment in the Results section. After the translocation period the plant was divided into fed leaf (leaflets), petiole (inside plexiglass chamber) and sink. The petiole and sink were extracted in boiling 80% aqueous alcohol (V/V),

while the surface of the fed leaf was abraded using Carborundum 600 and extracted for 30 minutes in 100 mls of distilled water. The water extraction was followed by an extraction in boiling 80% alcohol. Samples of the alcohol extracts were counted by taking 100  $\mu$ l duplicate aliquots while the water extract was reduced in volume by evaporation before the  $^{14}\text{C}$  content was determined. Translocation was again determined by calculating the  $^{14}\text{C}$  outside the fed leaf (which included petiole inside chamber) as per cent of the total  $^{14}\text{C}$  recovered from the plant. Free space  $^{14}\text{C}$  was determined as a per cent of the total amount of  $^{14}\text{C}$  found in the fed leaflets, that is water and ethanol soluble.

#### IV. PHOTOPHOSPHORYLATION RATES

The rates of photophosphorylation were measured by the incorporation of  $^{32}\text{P}$  into ATP by isolated chloroplasts. Plants were illuminated at 2,800 foot candles for 1 hour after which they were exposed to 4.1 krads of radiation. The plant was then returned to the illumination chamber (2,800 ft.c.) for 15, 60 or 120 minutes. After the post-radiation illumination period leaves were removed and homogenized in 50 mls of cold STN buffer (0.4 M sucrose, 0.05 M tricine, 0.01 M NaCl) using a Virtis Homogenizer (Super 30 Type). The homogenate was filtered through a double layer of nylon cloth to remove large debris and the resulting filtrate was spun down using a desk top centrifuge (setting 7) for 10 minutes. The supernatant from this centrifugation was poured off and the pellet was resuspended in 1 to 2 mls of cold STN buffer. The resulting solution served as the source of chloroplasts used in the  $^{32}\text{P}$  studies.

Since the amount of  $^{32}\text{P}$  incorporated by photophosphorylation is calculated on a chlorophyll weight basis, it was necessary to determine the concentration of chlorophyll added to the reaction vessel. The chlorophyll concentration was calculated using the method of Avron. An aliquot of 50  $\mu$ l of the prepared chloroplast suspension was added to 10 ml of 80%

aqueous acetone (V/V) and spun down at setting 7 on a desk top centrifuge for 2 minutes. The optical density of the resulting supernatant was determined at 645 and 663 m $\mu$  on a Spectronic 20 spectrophotometer and the chlorophyll concentration calculated using the following equation:

$$\text{Chl } \mu\text{g/ml} = (\text{OD}_{645} \times 202) + (\text{OD}_{663} \times 80.2) \times 20$$

The ability of chloroplasts to incorporate  $^{32}\text{P}$  into ATP was tested in a 15 ml test tube containing 45  $\mu\text{moles}$  tricine, 12  $\mu\text{moles}$   $\text{MgCl}_2$ , 60  $\mu\text{moles}$   $\text{NaCl}$ , 6  $\mu\text{moles}$   $\text{KH}_2\text{PO}_4$ , 4  $\mu\text{moles}$  ADP, 0.015  $\mu\text{moles}$  phenazine methosulfate (PMS) and between  $5.0 \times 10^4$  and  $2.5 \times 10^5$  dpm of  $^{32}\text{P}$  (purchased from Amersham/Searle as orthophosphate in dilute HCl, pH 2-3) making a total volume of 3 mls. The reaction was started when 50  $\mu\text{ls}$  of the chloroplast suspension was added to two test tubes. One test tube was kept in the dark and the other was exposed to 16,000 foot candles of light. The reactions were stopped after 2 minutes by adding 0.4 ml of 30% trichloroacetic acid (TCA) and the test tubes were put on ice for 10 minutes.

The TCA denaturated mixture was spun down using a clinical centrifuge for 10 minutes and the resulting supernatant was assayed for  $\text{AT}^{32}\text{P}$ . The volumes of the supernatants were determined and two 100  $\mu\text{l}$  aliquots were counted to determine the total  $^{32}\text{P}$  present. One ml of the supernatant was added to a 50 ml test tube followed by 1.5 ml of  $\text{H}_2\text{O}$  saturated with 1:1 isobutanol-benzene, 1.2 ml of acetone and 0.8 ml of molybdate reagent (5%  $\text{NH}_4$ -molybdate in 4N HCl). This mixture was incubated for 2 minutes, then 7.0 ml of 1:1 isobutanol-benzene saturated with  $\text{H}_2\text{O}$  was added and mixed for 30 seconds using a Vari-Whirl mixer. The resulting lower organic phase was discarded (separatory funnel) and to the upper aqueous phase containing the  $\text{AT}^{32}\text{P}$  was added 0.2 ml of 0.06 M  $\text{KH}_2\text{PO}_4$ . This solution was separated again by the addition of 1:1 isobutanol:benzene. The volume of the aqueous phase was recorded and duplicate 100  $\mu\text{l}$  aliquots from the dark and light reactions were

placed in 15 ml of scintillation fluid and the radioactivity determined using the Packard Tricarb-3310 scintillation spectrometer.

The counts per minute obtained from the scintillation counting were corrected for efficiency and volumes, giving values for total counts ( $^{32}\text{P}$  available), light counts ( $^{32}\text{P}$  fixed into ATP) and dark counts ( $^{32}\text{P}$  fixed into ATP). The amount of  $\text{PO}_4$  (6  $\mu\text{moles}$ ) and time of reaction (2 minutes) were constant so the  $\text{AT}^{32}\text{P}$  produced by photophosphorylation was determined from the following calculation:

$$\frac{\text{Light counts} - \text{Dark counts}}{\text{Total counts}} \times \frac{6 \mu\text{moles}}{\text{of } \text{PO}_4} \times \frac{60 \text{ min/hr}}{2 \text{ min}} \times \frac{1000 \mu\text{g/mg}}{[\text{Chl}] \text{ in re x}}$$

=  $\mu\text{moles of ATP/hr/mg chlorophyll}$ .

#### STATISTICAL ANALYSIS

The statistical test used to identify significant differences between the data, expressed as per cents, was the t-test. The parametric test determines whether or not two samples have the same population mean. Comparison of pre-irradiated photosynthetic rates with the rates 15 through 75 minutes post-irradiation were performed for the null hypothesis:

$$\mu_1 = \mu_2: \text{ where sample 1 and 2 are paired variates;}$$

$$\mu_1 = \text{population mean of sample 1.}$$

other samples were compared using the assumption:

$$\mu_1 = \mu_2: \sigma_1 \neq \sigma_2 \text{ where } \sigma_1 = \text{standard deviation for sample 1}$$

on the Wang 2200 system. Data identified as significant in this thesis represents significance at the 5% level.

The parameteric test (Wang 2200 system) to determine significant differences is a method used for large sample sizes which were not available in this study. A non-parameteric test the Mann-Whitney U test showed that the differences were significant as determined by the parameteric test.

## RESULTS

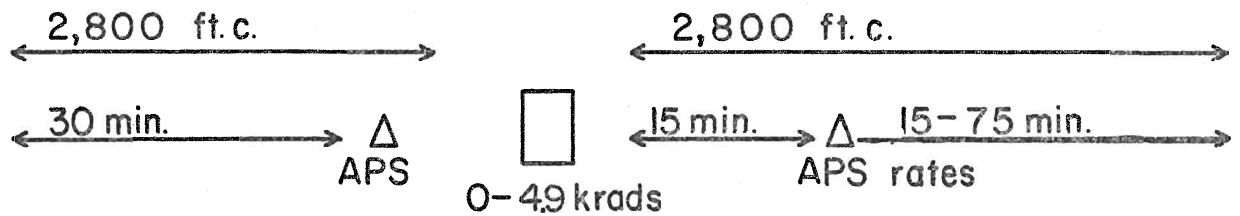
The initial group of results presented in this section have come from experiments designed to investigate radiation-induced changes to the processes of photosynthetic CO<sub>2</sub> uptake, photophosphorylation and photoassimilate export. The second group of results are from experiments directed primarily towards photoassimilate export, particularly as this process is affected by light of different intensities. In fact all of these experiments are directed to a hypothesis which considers the availability of light-mediated ATP production as an energy source for vein-loading, and the relationship between these experiments will become more evident in the Discussion.

### I. RADIATION EFFECTS ON PHOTOSYNTHESIS

The first set of data was obtained from experiments in which the rates of CO<sub>2</sub> uptake (21% O<sub>2</sub>, 300 ppm CO<sub>2</sub>) were determined prior to gamma irradiation and at times from 15 to 75 minutes post-irradiation. The net photosynthetic or CO<sub>2</sub> uptake rates were determined at a light intensity of 2,800 foot candles and the plants exposed to gamma radiation, absorbed doses of, from a minimum of 60 rads to a maximum of 4.9 krads.

The primary data for these results is contained in Appendix 1, a summary is presented in Table 1. It can be observed from Table 1 that plants illuminated at 2,800 foot candles and receiving either no radiation or only 60 rads showed no significant change in photosynthetic rates during the 75 minute post-irradiation period. Those plants receiving 120 rads however, showed a significant reduction of 7.9% at 60 minutes and a 10.5% reduction by 75 minutes post-irradiation. The same response was evident following an absorbed dose of 250 rads. At a dose of 490 rads however, the initial reduction was apparent at 45 minutes post-irradiation and at a dose of 1.97 krads the initial reduction was first evident in

Diagrammatic illustration of experimental procedure used to obtain data shown in Table 1.



ft.c. foot candles.

APS apparent photosynthetic CO<sub>2</sub> uptake rate taken.

Unless otherwise stated plants were exposed to lab air as a CO<sub>2</sub> source.

TABLE 1. Changes in rates of apparent photosynthesis of soybean plants at various times following acute doses of gamma radiation (2,800 foot candles illumination).

Absorbed dose (rads)	Significant Changes* (as % of pre-irradiation rate)						
	Time Post-irradiation (min)						
	15	20	25	30	45	60	75
0 + <sub>n</sub> = 6	-	-	-	-	-	-	-
60 n = 4	-	-	-	-	-	-	-
120 n = 4	-	-	-	-	-	-7.9	-10.5
250 n = 4	-	-	-	-	-	-5.6	-7.9
490 n = 4	-	-	-	-	-7.7	-7.7	-11.5
990 n = 4	-	-	-	-	-3.0	-7.8	-14.6
1970 n = 4	-3.5	-2.0	-	-	-1.4	-6.2	-11.6
2960 n = 4	-11.4	-9.2	-4.2	-2.1	-9.0	-14.1	-21.9
3940 n = 4	-9.8	-7.1	-4.3	-1.5	-4.6	-13.2	-17.6
4930 n = 4	-13.6	-11.3	-9.4	-5.9	-7.6	-12.9	-23.1

+<sub>n</sub> = sample number

\* significance level is 5%

Rates taken at average [CO<sub>2</sub>] = 300 ppm; O<sub>2</sub> = 21%

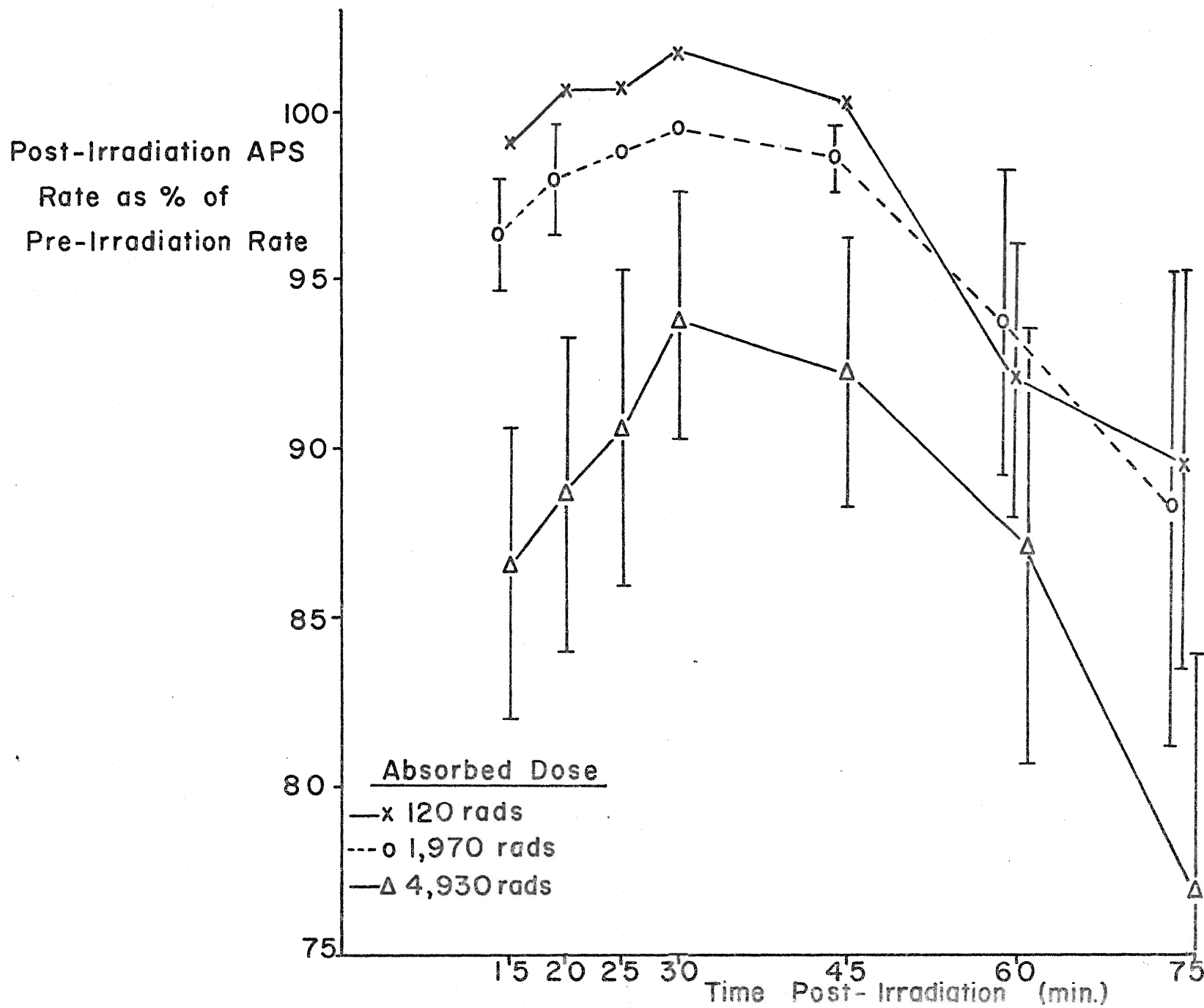
15 minutes following radiation exposure. Table 1 shows that 15 minutes after plants absorbed a radiation dose of 1.97 krads the rate of photosynthetic CO<sub>2</sub> uptake was reduced by 3.5%, a small, but statistically significant amount. This initial period of reduced rate of photosynthesis was then followed by a period in which the rate of photosynthetic uptake of CO<sub>2</sub> returned to normal (25 and 30 minutes post-irradiation) which was followed by a reduction in the rates of apparent photosynthesis. At 75 minutes post-irradiation a reduction of 11.6% was observed. For plants receiving doses of 2.96 krads, 3.94 krads and 4.93 krads a similar response was shown, namely an initial reduction in CO<sub>2</sub> uptake followed by an increase in photosynthetic rate 25 and 30 minutes post-irradiation and hence a smaller extent of reduction followed by a large reduction in the apparent photosynthetic rates at 45 minutes. Furthermore at doses above 1.97 krads a greater initial reduction was observed and the rate did not recover to the level of the pre-irradiation photosynthetic rate by 25 to 30 minutes following radiation exposure.

To more clearly show the patterns of responses evident in Table 1 the responses of plants absorbing radiation doses at three different levels are shown in Figure 6. The curve for 120 rads shows the reduction of CO<sub>2</sub> uptake 60 minutes following irradiation, while the curve for 1.97 krads illustrates the early response at 15 and 20 minutes followed by a return to the pre-irradiated photosynthetic rate which is then followed by a further reduction observable at 45 through 75 minutes. The curve for the absorbed dose of 4.93 krads shows essentially the same trends as 1.97 krads except in magnitude the reductions are greater and the CO<sub>2</sub> uptake does not return to the pre-irradiated rate.

A similar experiment to the one just presented was performed except that the light intensity used in this second study was 1,600 foot candles. The primary data of Appendix 2 is summarized in Table 2 which

Figure 6. The effect of varying doses of gamma radiation on the rate of apparent photosynthetic CO<sub>2</sub> uptake at varying time intervals post-irradiation under 2,800 foot candles of illumination.

Standard deviations are shown only for those values which were significantly (5%) different from the pre-irradiated rate.



shows significant responses of photosynthetic CO<sub>2</sub> uptake at 1,600 foot candles of light following varied radiation exposures. The responses were measured 15 through 75 minutes following absorbed doses from 60 rads to 4.93 krads. From Table 2 it can be seen that the first evidence of a significant reduction in the rate of photosynthetic CO<sub>2</sub> uptake is at 60 minutes after a dose of 120 rads with reduction still apparent at 75 minutes post-irradiation. Plants absorbing radiation doses of 250, 490, 990, 1970 and 2,960 rads all show a reduction in the photosynthetic rate but the time post-irradiation at which the reduction initially appears fluctuates between 60 and 75 minutes. An early response (15 minutes) to radiation exposure was exhibited in plants only after an absorption of 3.94 krads which caused a reduction in CO<sub>2</sub> uptake of 6.4%. The reduced uptake of CO<sub>2</sub> following 3.94 krads is prevalent throughout the time period that photosynthetic rates were determined following radiation exposure. The observations from plants absorbing a dose of 4.93 krads seem to be consistent with those for 3.94 krads, that is, a significant reduction in the rates of photosynthetic CO<sub>2</sub> uptake at all times post-irradiation.

The results shown on Figure 7 are three representative responses with time, of those found on Table 2. The curve for plants absorbing a dose of 120 rads shows the delayed reduction in rates of CO<sub>2</sub> uptake while the curves for the plants absorbing the 3.94 krads and 4.93 krads show the initially rapid reduction and the lack of total recovery to the pre-irradiated value.

Table 1 and 2 show that rates of apparent photosynthesis measured at 2,800 and 1,600 foot candles respectively are reduced when soybean plants are exposed to radiation above a threshold dose. Following absorption of 120 rads the reduction was first evident after 60 minutes at both light intensities (1,600 and 2,800 ft.c.). There was an initial response 15 minutes following radiation exposure occurring a 1.97 krads when plants

Diagrammatic illustration of experimental procedure used to obtain data shown in Table 2.

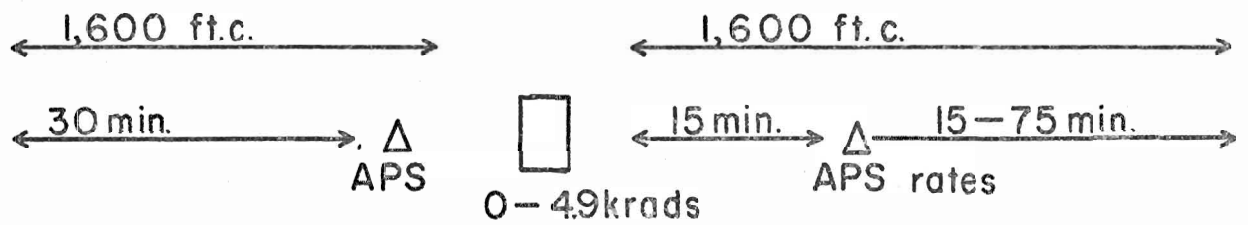


TABLE 2. Changes in rates of apparent photosynthesis of soybean plants at various times following acute doses of gamma radiation (1,600 foot candles illumination).

Absorbed dose (rads)	Significant Changes* (as % of pre-irradiation rate)						
	Time Post-irradiation (min)						
	15	20	25	30	45	60	75
0 +n = 4	-	-	-	-	-	-	-
60 n = 4	-	-	+2.3	+2.8	-	-	-
120 n = 4	-	-	-	-	-	-3.8	-3.8
250 n = 4	-	-	-	-	-	-2.6	-3.9
490 n = 4	-	-	-	-	-	-	-6.3
990 n = 4	-	-	-	-	-	-	-5.7
1970 n = 4	-	-	-	-	-	-	-2.1
2960 n = 4	-	-	-	-	-	-8.0	-9.4
3940 n = 4	-6.4	-6.9	-7.0	-3.9	-9.7	-8.3	-13.2
4930 n = 4	-8.7	-9.0	-9.1	-6.1	-6.4	-8.9	-11.0

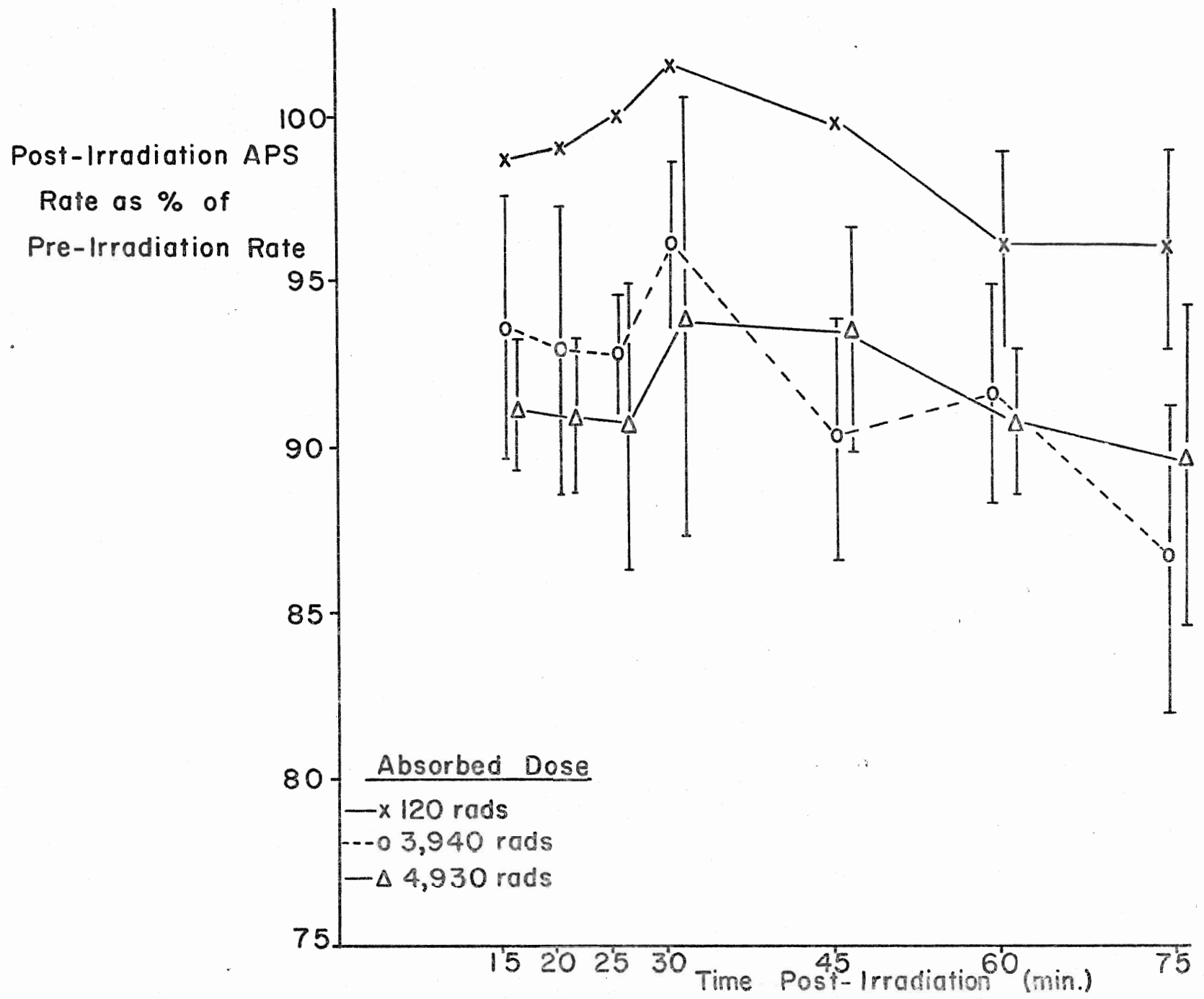
+<sub>n</sub> = sample number

\* significance level is 5%

Rates taken at average [CO<sub>2</sub>] = 300 ppm; O<sub>2</sub> = 21%

Figure 7. The effect of varying doses of gamma radiation on the rate of apparent photosynthetic CO<sub>2</sub> uptake at varying time intervals post-irradiation under 1,600 foot candles of illumination.

Standard deviations are shown for only those values which were significantly (5%) different from the pre-irradiated rate.



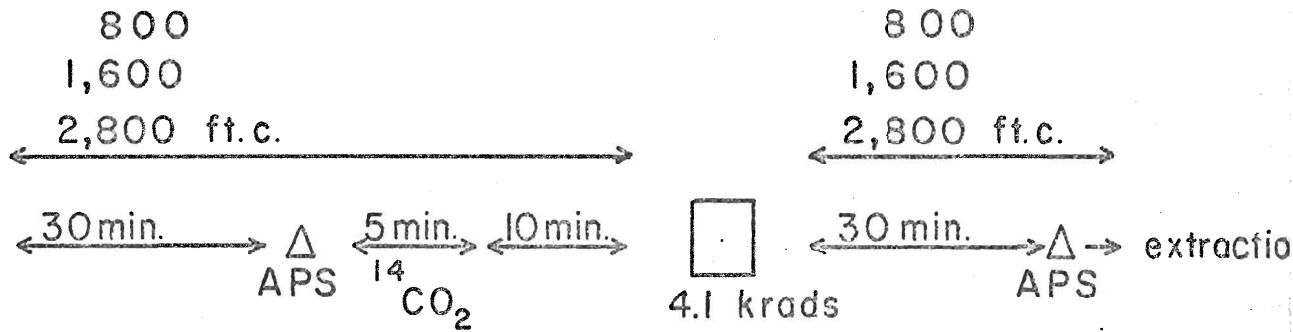
were illuminated at 2,800 foot candles which did not become evident until a dose of 3.94 krads was reached for the plants illuminated with only 1,600 foot candles of light.

## II. RADIATION EFFECTS ON RATES OF PHOTOSYNTHETIC CO<sub>2</sub> UPTAKE, PHOTOPHOSPHORYLATION AND PHOTOASSIMILATE EXPORT

The next set of experiments were designed to study the responses to ionizing radiation of translocation and photosynthesis using the same plant material. For this study, an acute dose of 4.1 krads was used and the responses were studied at three different light intensities. In each of the experiments plants were illuminated for one half hour at either 800, 1,600 or 2,800 foot candles after which time a photosynthetic rate was obtained and then the plant was allowed to photoassimilate <sup>14</sup>CO<sub>2</sub> for 5 minutes followed by a 10 minute translocation period. The plant was then irradiated for a 9 minute period and immediately afterwards returned to the chamber for a 30 minute illumination period at the same light intensity as received prior to irradiation. After this 30 minute period a rate of apparent photosynthesis was determined and then the plant was sacrificed and the <sup>14</sup>C content assayed as described in the Methods. For this study the primary data can be found in Appendix 3 with a summary given in Table 3.

The data in Table 3 shows that when plants were illuminated at 800 foot candles about 7% of the <sup>14</sup>C was exported irrespective of whether the plants were irradiated or not. At an illumination of 1,600 foot candles, although the control plants exported a mean value that is 21% greater than the 6.0% export for irradiated plants, the difference was not significant. At the highest light intensity of 2,800 foot candles the controls exported 8.1% and the magnitude of export for the irradiated plants was only 5.9%, a significant reduction of 27% in translocation 30 minutes following irradiation. The control export values at 800, 1,600 or 2,800 foot candles of light show no significant difference in the per cent of <sup>14</sup>C

Diagrammatic illustration of experimental procedure used to obtain data shown in Table 3.



extraction <sup>14</sup>C products extracted from plant sections in boiling 80% ethanol.

TABLE 3. Export of assimilated  $^{14}\text{C}$  and the rates of photosynthetic  $\text{CO}_2$  uptake by soybean plants illuminated at 800, 1,600 or 2,800 foot candles following an acute dose of 4,100 rads of gamma radiation.

Light Intensity (ft.c)	Treatment	Exported $^{14}\text{C}$ as per cent of $^{14}\text{C}$ recovered from the plant	Per cent change in export	Rates of Photosynthetic $\text{CO}_2$ Uptake (mg $\text{CO}_2$ /hr./fg. fr. wt.)		Per cent change in $\text{CO}_2$ uptake
				Pre-irradiation	Post-irradiation	
800	Control $^{\dagger}n = 7$	7.0 (3.8)	N.S.	8.0 (0.4)	7.9 (0.4)	N.S.
	Irradiated $n = 6$	6.8 (2.9)		7.6 (0.4)	7.3 (0.4)	N.S.
1,600	Control $n = 7$	7.6 (4.2)	N.S.	11.9 (1.0)	12.1 (1.1)	N.S.
	Irradiated $n = 8$	6.0 (2.0)		11.8 (0.8)	11.5 (1.0)	N.S.
2,800	Control $n = 9$	8.1 (2.0)	-27%	12.7 (1.3)	13.1 (0.7)	N.S.
	Irradiated $n = 9$	5.9 (2.0)		13.0 (0.9)	12.3 (0.7)	-5%

$^{\dagger}n$  = sample number

\* Values in parentheses indicate standard deviations

N.S. = not significant at 5% level

exported 49 minutes following the  $^{14}\text{CO}_2$  photoassimilation although numerically the values increase with increasing light intensity. The pre-irradiation photosynthetic rates from Table 3 also show values which increase with increasing light intensity. The control plants show no change in photosynthetic  $\text{CO}_2$  uptake following similar handling as the experimental plants at all three light intensities. Plants illuminated with 800 or 1,600 foot candles and exposed to radiation showed no significant change in the  $\text{CO}_2$  uptake following radiation exposure. However, plants illuminated at 2,800 foot candles of light intensity and exposed to radiation did show a significant reduction of 5% in the rate of photosynthetic  $\text{CO}_2$  uptake.

The data presented in Table 4 comes from experiments in which rates of photophosphorylation by chloroplasts isolated from plants were obtained 15, 60 and 120 minutes following exposure of the plants to radiation. Table 4 shows that control plants (15 and 120 minute) had a mean rate of photophosphorylation of 286  $\mu\text{moles}$  ATP produced per milligram chlorophyll per hour. Photophosphorylation measured 15 or 60 minutes after irradiation had reduced rates which were 53% of the control value. Irradiated plants kept in the light for 120 minutes however showed no reduction in the rate of photophosphorylation.

Another set of experiments studied the extent of assimilate export 45 minutes after a 15 minute photoassimilation period in both control and irradiated plants. The initiation of  $^{14}\text{CO}_2$  photoassimilation occurred either 15 or 120 minutes after exposure of the plants to radiation. As seen in Table 5 the extent of export in the control plants was 21%. In irradiated plants (4.1 krad) assimilating  $^{14}\text{CO}_2$  15 minutes following radiation exposure the magnitude of translocation was reduced to 13.3% a reduction in export of 37%. The plants that were irradiated but remained in the light 120 minutes before photoassimilating  $^{14}\text{CO}_2$  showed a

Diagrammatic illustration of experimental procedure used to obtain the data shown in Table 4.

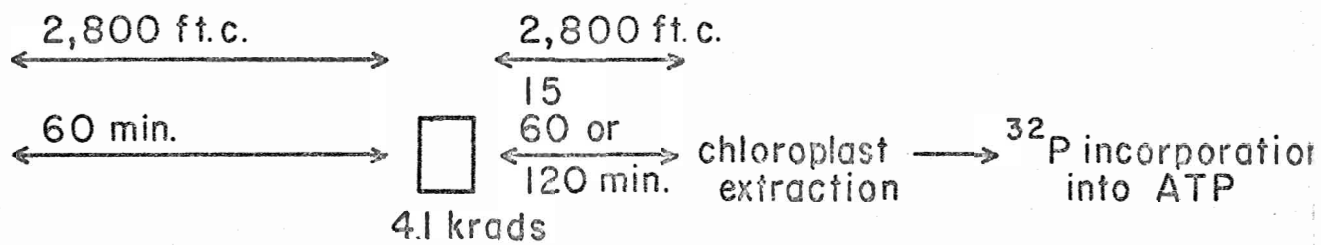


TABLE 4. Rates of photophosphorylation by chloroplasts isolated from soybean plants receiving an acute dose of 4,100 rads of gamma radiation.

Rate of Photophosphorylation ( $\mu$ moles ATP/mg chl./hr.)		Per cent* change following radiation exposure	Rate of photophosphorylation ( $\mu$ moles ATP/mg chl./hr.)		Per cent change following radiation exposure
Non-irradiated (15 & 120 min)	Post-irradiation (15 & 60 min)		post-irradiation (120 min)		
286	151	-47%	271	N.S.	
(66)	(44)		(108)		
<sup>+</sup> n = 7	n = 7		n = 9		

<sup>+</sup>n = sample number

\* per cent of non-irradiated controls

N.S. = not significant at the 5% level

Diagrammatic illustration of experimental procedure used to obtain the data shown in Table 5.

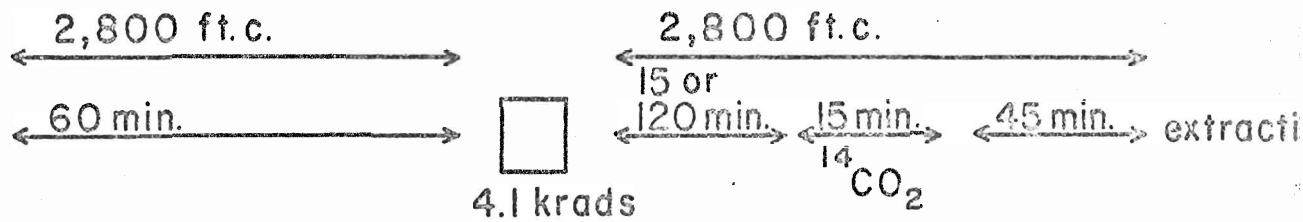


TABLE 5. The magnitude of  $^{14}\text{C}$  photoassimilate export in soybean plants which absorbed an acute dose of 4,100 rads of gamma radiation.

Magnitude of $^{14}\text{C}$ Export (as % of $^{14}\text{C}$ recovered from plant)		Per cent* change following radiation exposure	Magnitude of $^{14}\text{C}$ export (as % of $^{14}\text{C}$ recovered) post-irradiation (120 min)	Per cent change following radiation exposure
Non-irradiated (15 & 120 min)	Post-irradiation (15 min)			
21.0	13.3	-37%	22.5	N.S.
(2.6)	(2.2)		(2.5)	
+n = 7	n = 7		n = 9	

+ sample number

\* per cent of non-irradiated controls

N.S. = not significant at 5% level

magnitude of export of 22.5% a value which was not different than the one obtained for non-irradiated plants.

### III. MAGNITUDE OF PHOTOASSIMILATE EXPORT AT DIFFERENT LIGHT INTENSITIES

The following set of experiments were performed to ascertain the extent that light intensity influences the export of photoassimilated  $^{14}\text{C}$ . The primary data is available in Appendix 6, the summary data has been presented in Tables 6 and 7. The data was obtained from experiments in which the extent of translocation was determined in plants either maintained at constant light intensity during the periods of  $^{14}\text{CO}_2$  assimilation and translocation or in plants in which the illumination prior to and during the  $^{14}\text{CO}_2$  photoassimilation period was different from the light intensity during the subsequent period of  $^{14}\text{C}$  export. As well the free space  $^{14}\text{C}$  content of the fed leaf was determined as described in the Methods.

From Table 6 it can be seen that when the light intensity during translocation was retained constant, the higher levels of export of  $^{14}\text{C}$  were observed when the light intensity was low during photoassimilation. For example, at a light intensity of 2,800 foot candles for translocation and for photoassimilation the per cent export of  $^{14}\text{C}$  was 13.9%, however, if the light intensity during photoassimilation was only 200 foot candles the export was 51.0% of the total  $^{14}\text{C}$  recovered. A similar observation is evident when the intensity of light during translocation was maintained at 800 foot candles with photoassimilate export being 11.3% and 16.4% at photoassimilation light intensities of 2,800 foot candles and 800 foot candles respectively. The same observation is also evident for the plants translocating under illumination of 200 foot candles where the export was 9.2% when the intensity during the period of photoassimilation was 2,800 foot candles but 40.5% if the photoassimilation took place under an illumination of 200 foot candles.

Diagrammatic illustration of experimental procedure used to obtain data shown in Tables 6 and 7.

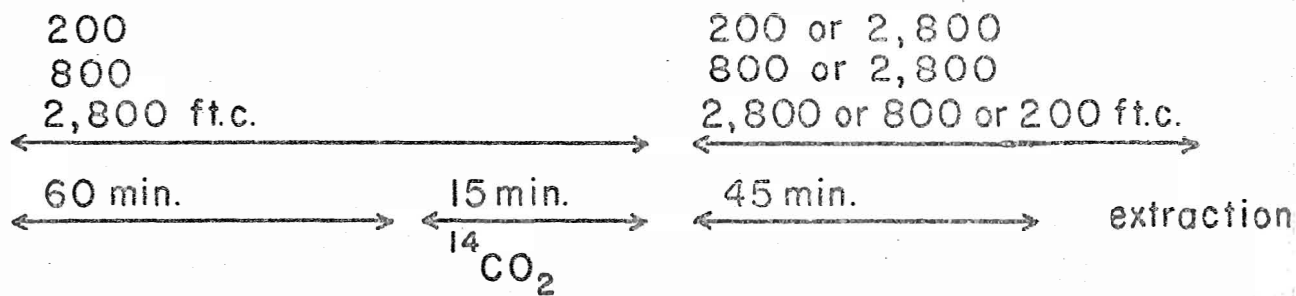


TABLE 6. The magnitude of  $^{14}\text{C}$  photoassimilate export and  $^{14}\text{C}$  free space content in soybean plants photoassimilating at different light intensities.

Light intensity photoassimilation/ translocation (ft.c)	Exported $^{14}\text{C}$ as % of $^{14}\text{C}$ recovered from the whole plant	Total assimilated $^{14}\text{C}$ recovered from the whole plant ( $\mu\text{Ci}$ )	$^{14}\text{C}$ recovered from 80% ethanol extract of leaflets ( $\mu\text{Ci}$ )	$^{14}\text{C}$ recovered from water extract of leaflets ( $\mu\text{Ci}$ )	Free space $^{14}\text{C}$ as % of $^{14}\text{C}$ recovered from leaflets
$^+n = 6$ 2,800/2,800	13.9 <sup>a</sup> (2.4)*	3.69 <sup>a</sup> (.45)	2.43 <sup>a</sup> (.32)	0.72 <sup>a</sup> (.11)	22.9 <sup>a</sup> (3.2)
$n = 6$ 800/2,800	17.4 <sup>a</sup> (5.6)	3.15 <sup>b</sup> (.52)	2.00 <sup>b</sup> (.41)	0.53 <sup>b</sup> (.12)	22.5 <sup>a</sup> (4.1)
$n = 6$ 200/2,800	51.0 <sup>c</sup> (4.7)	2.76 <sup>b</sup> (.24)	1.02 <sup>c</sup> (.11)	0.30 <sup>c</sup> (.07)	22.6 <sup>a</sup> (4.1)
$n = 6$ 2,800/800	11.3 <sup>a</sup> (2.2)	3.39 <sup>a</sup> (.71)	2.31 <sup>a</sup> (.63)	0.62 <sup>a</sup> (.00)	21.2 <sup>a</sup> (4.0)
$n = 6$ 800/800	16.4 <sup>b</sup> (5.0)	2.84 <sup>a</sup> (.49)	1.85 <sup>a</sup> (.30)	0.32 <sup>a</sup> (.07)	21.0 <sup>a</sup> (4.0)
$n = 7$ 2,800/200	9.2 <sup>a</sup> (2.3)	3.53 <sup>a</sup> (.32)	2.81 <sup>a</sup> (.19)	0.72 <sup>a</sup> (.27)	20.1 <sup>a</sup> (6.0)
$n = 6$ 200/200	40.5 <sup>b</sup> (4.1)	2.72 <sup>b</sup> (.33)	1.25 <sup>b</sup> (.12)	0.32 <sup>b</sup> (.07)	20.3 <sup>a</sup> (4.6)

$^+n$  = sample number

\* Values in parenthesis indicate standard deviation

Letters after values show statistical differences (vertically) within a section with translocation light intensity constant; 5% level of significance.

The same data as presented in Table 6 is found in Table 7 but it is arranged such that light intensities during the period of photoassimilation are constant. It is evident that with photoassimilation occurring at a constant intensity there is a greater export of  $^{14}\text{C}$  at the highest or higher light intensity used during the translocation period. For example, with photoassimilation at a light intensity of 2,800 foot candles, the export at the same light intensity is 13.9%, with 11.3% and 9.2% being exported if the light intensity during translocation is 800 and 200 foot candles, respectively. The difference with photoassimilation at 800 foot candles followed by 2,800 and 800 foot candles during translocation is only slight with 17.4% and 16.4% being exported. Greater export of  $^{14}\text{C}$  under a high light intensity during translocation is observed with photoassimilation at 200 foot candles of illumination which gave 51.0% export at 2,800 foot candles and 40.5% export at 200 foot candles of illumination for the translocation period.

It can also be seen from Table 6 and 7 that the free space  $^{14}\text{C}$  content remained constant under all the experimental conditions.

The major observations from this section can be summarized as follows:

(1) Above a threshold value the rates of photosynthetic  $\text{CO}_2$  uptake were reduced following exposure to ionizing radiation. At lower acute doses the reduction was evident 45 to 60 minutes following irradiation. At higher doses of radiation, the reduction was evident at least 15 minutes following irradiation.

(2) The radiation-induced reduction in rates of photosynthetic  $\text{CO}_2$  uptake that is evident by 60 minutes post-irradiation occurs at both high and low absorbed radiation doses, at both the light intensities of 1,600 foot candles or 2,800 foot candles. The more immediate radiation-induced reductions in photosynthesis, that occur at higher absorbed doses, are evident earlier at the higher light intensity (2,800 ft.c.).

TABLE 7. The magnitude of  $^{14}\text{C}$  photoassimilate export and  $^{14}\text{C}$  free space content in soybean plants translocating at different light intensities.

Light intensity photoassimilation/ translocation (ft.c)	Exported $^{14}\text{C}$ as % of $^{14}\text{C}$ recovered from the whole plant	Total assimilated $^{14}\text{C}$ recovered from the whole plant ( Ci)	$^{14}\text{C}$ recovered from 80% ethanol extract of leaflets ( Ci)	$^{14}\text{C}$ recovered from water extract of leaflets ( Ci)	Free space $^{14}\text{C}$ as % of $^{14}\text{C}$ recovered from leaflets
$^+n = 6$ 2,800/2,800	13.9 <sup>a</sup> (2.4)*	3.69 <sup>a</sup> (.45)	2.43 <sup>a</sup> (.32)	0.72 <sup>a</sup> (.11)	22.9 <sup>a</sup> (3.2)
n = 6 2,800/800	11.3 <sup>b</sup> (2.2)	3.39 <sup>a</sup> (.71)	2.31 <sup>a</sup> (.63)	0.62 <sup>a</sup> (.10)	21.2 <sup>a</sup> (4.0)
n = 7 2,800/200	9.2 <sup>b</sup> (2.3)	3.53 <sup>a</sup> (.32)	2.81 <sup>b</sup> (.19)	0.72 <sup>a</sup> (.27)	20.1 <sup>a</sup> (6.0)
n = 6 800/2,800	17.4 <sup>a</sup> (5.6)	3.15 <sup>a</sup> (.52)	2.00 <sup>a</sup> (.41)	0.53 <sup>a</sup> (.12)	22.5 <sup>a</sup> (4.1)
n = 6 800/800	16.4 <sup>a</sup> (5.0)	2.84 <sup>a</sup> (.49)	1.85 <sup>a</sup> (.30)	0.32 <sup>a</sup> (.07)	21.0 <sup>a</sup> (4.0)
n = 6 200/2,800	51.0 <sup>a</sup> (4.7)	2.76 <sup>a</sup> (.24)	1.02 <sup>a</sup> (.11)	0.30 <sup>a</sup> (.07)	22.6 <sup>a</sup> (4.1)
n = 6 200/200	40.5 <sup>b</sup> (4.1)	2.72 <sup>a</sup> (.33)	1.25 <sup>a</sup> (.12)	0.32 <sup>a</sup> (.07)	20.3 <sup>a</sup> (4.6)

$^+n$  = sample number

\* Values in parentheses indicate standard deviations

Letters after values show statistical differences (vertically) with photoassimilation light intensity constant; 5% level of significance.

(3) Plants exposed to ionizing radiation showed a reduction in the export of photoassimilated  $^{14}\text{C}$  at 2,800 foot candles 30 minutes following radiation exposure. No such reduction was observed in plants maintained at either 1,600 or 800 foot candles.

(4) Although the processes of photophosphorylation and photoassimilate export are significantly reduced 30 minutes following an absorbed dose of 4.1 krads when maintained at 2,800 foot candles of light, the magnitude of both processes are again normal at 2 hours post-irradiation.

(5) More  $^{14}\text{C}$  is exported out of a leaf if  $^{14}\text{CO}_2$  is photoassimilated at low light intensity. There is also an increase of  $^{14}\text{C}$  moving out of the leaf when light intensity during the translocation period is high relative to a translocation light intensity that is low.

## DISCUSSION

The major focus of this work was to study the relationship between the processes of photosynthesis and translocation particularly utilizing the probes of ionizing radiation and light intensity. In fact, it will be shown that the results presented in this thesis are consistent with the hypothesis that photosynthetically produced ATP may be an important source of energy for vein loading and therefore an important factor in determining the magnitude of photoassimilate exported from a leaf.

The data in this thesis shows that exposure of soybean plants to ionizing radiation results in a reduction in the rate of photosynthetic uptake of CO<sub>2</sub> (Tables 1 and 2). A significant reduction in photosynthesis was observed 60 minutes following an acute absorbed dose of only 120 rads and it would appear that this is the lowest reported acute dose to effect a reduction in the rate of apparent photosynthesis. Other studies showing reduced photosynthetic rates following low levels of irradiation include one in which Vicia faba was found to have a radiation-reduced photosynthetic rate 8 to 24 days following absorption of 250 rads of X-irradiation (69) and Pinus strobus which showed a 41% reduction in the photosynthetic capacity 2 days following exposure to 230 rads of ionizing radiation (94). In contrast to those studies in which the reductions were apparent only after 2 days or more, in this thesis data has been presented which shows reductions to be evident within 1 hour following an acute dose of only 120 rads. It may be possible that 60 rads, the lowest dose used in the studies reported in this thesis, may eventually effect a reduction in photosynthesis although such a reduction was not yet evident within the 75 minute post-irradiation period. As one would expect, the higher doses of radiation also reduced the photosynthetic rate. However, the response to these doses was evident at an earlier time once the threshold level

had been reached.

The radiation-induced reductions in photosynthesis, observed in Tables 1 and 2, indicate that there may be more than a single site responsible for the reductions. One radiation sensitive site may be responsible for the reductions in rates of apparent photosynthesis observed within 15 minutes following relatively high radiation doses. At 2,800 foot candles, this was affected at a dose of 1.97 krads and above, at 1,600 foot candles, the effect on this site was not evident until a dose of 3.9 krads was reached. Despite the initial effect on this site, it would appear that some repair or modulation process is operating since the extent of reduction in the rates of apparent photosynthesis became less for a short period thereafter. Eventually however, after 60 or 75 minutes, the reductions in the rates of apparent photosynthesis became larger and indeed at the lower radiation doses such reductions only became evident for the first time. The appearance of reduced rates of apparent photosynthesis at this time may reflect damage to another site which requires a longer time to develop to the point where the apparent photosynthetic rates are significantly affected. A similar pattern of initial reduction in the rate of apparent photosynthesis followed by a partial modulation and then further reduction has also been shown in soybean following a radiation dose of 3.75 krads (53, 95). The damage caused to this second site has been shown to last at least until 4 hours post-irradiation in soybean (53, 95) and others have observed, using different plant species, reduced photosynthetic rates up to and longer than 20 days following radiation exposure (32, 69, 94).

The site of radiation-induced damage responsible for the reduction in apparent photosynthesis at higher radiation doses, as well as the factors responsible for the modulation or repair, are not well understood. The involvement of the chloroplast chromosomes in the photosynthetic response would appear unlikely due to the short time involved even though chromo-

somal radiation-induced damage has been attributed to disruptions in physiological processes within 5 minutes (45). Also the energy dependent repair mechanisms for the chromosome aberrations (5, 6, 45, 101) do not give total repair and hence would not account for the return to normal apparent photosynthetic rates occurring at 2,800 foot candles following the absorbed dose of 1.97 krads. The possibility of protein synthesis being damaged by radiation has been suggested by Sprey (78) who observed a delay in chlorophyll synthesis in 7 to 8 day old etiolated barley leaves at high levels of radiation (100 krads to 500 krads) and related this to delayed RNA synthesis. He also suggested that a reduced incorporation of  $^{14}\text{C}$  into Calvin cycle intermediates indicated a reduced production of all of the necessary enzymes. Chlorophyll and NADP-linked glyceraldehyde phosphate synthesis have also been shown to be reduced by exposure to 20 krads (76). Repair of radiation-induced damage to protein synthesis has also been observed (54). However, the reduction in rates of apparent photosynthesis reported in this thesis are not likely due to an effect on protein synthesis since Shelp (74) has shown that if  $^{14}\text{CO}_2$  photoassimilation is initiated 15 or 30 minutes post-irradiation there is no alteration in the relative amounts of  $^{14}\text{C}$ -labelled photosynthetic products in irradiated or control plants.

The second site of radiation-induced damage to photosynthesis appears to require more time before reductions in rates of apparent photosynthesis are evident. Although the cause of the reduction in apparent photosynthesis is not known, processes that have been studied and shown not to be responsible include stomatal diffusive resistance (73), dark respiration (73), photorespiration (53, 73, 95) and relative levels of leaf metabolites (74). Although the work with soybean has not shown the reductions in apparent photosynthesis to be due to stomatal resistances or metabolic changes, others have correlated increased diffusive resistance and altered

metabolic processes with reduced CO<sub>2</sub> uptake (42, 69, 78, 102).

The extent of the observed effect on photosynthesis caused by radiation exposure is influenced by the intensity of illumination during the measurement of CO<sub>2</sub> uptake (Tables 1 and 2). The radiation-induced reduction following an absorbed dose of 120 rads for both light intensities, 1,600 foot candles and 2,800 foot candles, occurs 60 minutes following radiation exposure. Quite clearly the induction of this response was not affected by the light intensity. However at higher radiation doses the intensity of illumination is important. The appearance of the reduction in apparent photosynthesis, occurring 15 minutes post-irradiation, occurs at a dose of 1.97 krads at 2,800 foot candles whereas the reduction is not observed, at 1,600 foot candles, until 3.9 krads. Not only is the radiation threshold for the reduction of apparent photosynthetic rate lower at the higher light intensity but the extent of the reductions in photosynthesis are greater under the higher illumination as well.

Different magnitudes of photosynthetic reductions have been reported for Sorghum at different light intensities during a chilling stress (84, 85). The reductions were greater at the highest light intensities used and the increase in damage was attributed to the greater retention of the photosynthetic products produced at high light which caused conformational changes in the stroma and thylakoids of the chloroplast. Whether or not chloroplast conformation changes, due to retention of photosynthetic products, are increasing damage at the high light intensity used in this study is not known. However, radiation absorbed doses of 3 krads have been shown to cause ultrastructural damage and to cause swelling of the chloroplasts of Osmundas regalis within 2 hours post-irradiation (1). If photosynthetic products were being retained by the chloroplasts this could account for the lowering in the photosynthetic rate as initially suggested by Boussingault (60).

In summarizing the effects of radiation on the rates of apparent photosynthesis in the soybean plants in this study it is apparent that:

- (1) radiation above a threshold reduces the photosynthetic rate,
- (2) radiation-induced photosynthetic damage likely occurs at two sites with one site very sensitive to low doses as well as high, and the second site sensitive only to relatively higher levels of radiation,
- (3) light intensity influences both the magnitude of the observed response as well as the threshold dose at which a reduction in apparent photosynthesis occurs following the higher radiation levels.

In addition to ionizing radiation inducing a reduction in rates of apparent photosynthesis it also reduces the extent of photoassimilate exported from a soybean leaf as shown in Table 3. The data presented in Table 3 has been derived from measurements of rates of photosynthetic CO<sub>2</sub> uptake and the magnitude of export monitored in the same plant following exposure to ionizing radiation. In this experiment plants photoassimilated <sup>14</sup>C<sub>2</sub> prior to radiation exposure so that <sup>14</sup>C labelled photosynthetic products would be available for export during the illumination period following irradiation. It should be noted that <sup>14</sup>C radioactivity in the 80% ethanol-soluble fraction only was determined since previous studies (74) have shown that exposure of soybean plants to radiation does not alter the relative distribution of <sup>14</sup>C between the ethanol-soluble and -insoluble fractions.

At 2,800 foot candles illumination, the extent of <sup>14</sup>C photoassimilate export following an absorbed dose of 4.1 krads of ionizing radiation was 73% of the export value (8.1%) in non-irradiated plants whereas the net photosynthetic rate 30 minutes following irradiation was only 5% less than controls. Both reductions were significant at the 5% level of significance. In a similar experiment Shelp observed a 32% decrease in the magnitude of export (74) and other studies have reported approximately

5% reductions of photosynthesis 30 minutes after a dose of 3.75 krads (53, 95).

It is unlikely that the small reductions in the rates of photosynthesis are directly responsible for the larger reductions in photoassimilate export. Ho (38) has suggested that the increase in export observed with increasing light intensity is due to a greater production of sucrose whereas Servaites and Geiger (72) suggest that export is a constant amount of the increase in CO<sub>2</sub> uptake so that total assimilation controls the export rate. The data in Table 3 which shows a reduction in photosynthesis and export occurring only at the highest light intensity would indicate that either the radiation damage is common to both the processes of photosynthetic CO<sub>2</sub> uptake and export or that damage to one has affected indirectly the other.

Since the stress of ionizing radiation induced a greater reduction in photoassimilate export than the reduction in the rate of photosynthetic CO<sub>2</sub> uptake and because the effects on both processes were more pronounced under high light intensities the process of photophosphorylation was measured in chloroplasts isolated from normal plants and plants exposed to radiation stress. Photophosphorylation was examined because the utilization of ATP in the process of vein loading has been reported (25, 46, 77, 91).

The data in Table 4 shows that there is a reduction in the rates of photophosphorylation following radiation exposure. A reduction of 47% was evident 15 minutes following an absorbed dose of 4.1 krads and the reduction was still evident 60 minutes post-irradiation. The observed reduction in photophosphorylation following radiation exposure is confirmed by work in vitro (75) and in vivo (62) although considerably higher doses of radiation are required to alter rates of photophosphorylation when chloroplasts are irradiated 'in vitro'.

It can also be seen (Table 4) that the initial reduction in the rates of photophosphorylation were no longer evident if the rates of photophosphorylation were measured 120 minutes post-irradiation.

Evidence for the utilization of ATP in the process of vein loading has come particularly from experiments in which the addition of exogenous ATP promoted the rate of vein loading (25, 46, 77, 91) as well as the rate of translocation (25). Although the 'in vivo' source of ATP for vein loading is not definitely known there is evidence that the process of photophosphorylation may play a contributory role (34, 55, 67). The most recent evidence for the involvement of photosynthetically produced ATP in vein loading comes from experiments in which  $K^+$ -deficient plants were shown to export less photoassimilated  $^{14}C$  (55). Mengel and Haeder suggested that since  $K^+$  affects rates of photophosphorylation positively (66) their data indicated that the decrease in photophosphorylation was responsible for the decrease in the vein loading process leading to reduced translocation. Amir and Reinhold (2) found that  $K^+$ -deficient plants exported amounts similar to the controls in the dark, but in the light the  $K^+$ -deficient plants exported less.

The transfer of photosynthetically produced ATP from the chloroplast into the cytoplasm has been well documented (4, 35, 36, 52, 80, 81) and recently a model showing an indirect transfer of ATP has been developed (37). In this model (Fig. 3) it can be seen that photosynthetically produced dihydroxyacetone phosphate (DHAP) is transported across the membrane by the 'phosphate translocator', isomerized in the cytoplasm to glyceraldehyde phosphate (GAP) which is then oxidized to 1,3 diphosphoglyceric acid (1,3 DiPGA) forming reduced nicotinamide adenine dinucleotide (NADH). The 1,3 DiPGA is then converted to phosphoglyceric acid (PGA), the exergonic step which produces ATP from the ADP and  $P_i$  in the cytoplasm. The PGA is then transported back into the chloroplast (phosphate translocator) to become

reduced again to GAP and DHAP. Other workers have suggested a direct exchange of ATP and ADP across the chloroplast membrane (4, 80, 81). Irrespective of the method utilized to transport ATP it is apparent that the light energy initially converted and stored in ATP via photophosphorylation can enter the cytoplasm rapidly to be used in processes other than photosynthesis (52). It is therefore possible for photosynthetically produced ATP to participate in vein loading or possibly as an energy source for transport of translocatable material across the parenchyma cell plasmalemma into the apoplast (free space) from where it can eventually be loaded into phloem tissue.

It has been shown (Table 4) that the photophosphorylation ability of the chloroplasts is reduced following radiation exposure. Although reductions in photophosphorylation were not accompanied by large reductions in rates of apparent photosynthesis the process of assimilate export clearly was affected. Table 5 indicates that at the same time there was a 47% reduction in rates of photophosphorylation, the magnitude of export was reduced by 37%. Also, if the photoassimilation of  $^{14}\text{CO}_2$  was initiated 120 minutes post-irradiation the plant exported the same amount of  $^{14}\text{C}$  as did the plants not receiving radiation. The photophosphorylation data (Table 4) indicates that this process is also repaired by 120 minutes post-irradiation.

The observations that both the rates of photophosphorylation and magnitude of photoassimilate export showed large reductions following radiation exposure and were capable of returning to normal at the same time suggests a close relationship between these two processes. Furthermore it shows a closer relationship between photosynthetically produced ATP and photoassimilate export than between export and the rate of  $\text{CO}_2$  uptake which is still reduced 4 hours post-irradiation (53, 95). This close relationship between export and photophosphorylation is exhibited at least during the

relatively short periods of time used in this study. Eventually the rates of CO<sub>2</sub> uptake may drop to a level where photosynthesis limits the availability of material for export as observed by Ho (38) but that does not appear to have happened in this study.

The observation (Table 3) that the radiation-induced damage to export is apparent only at the high light intensity would indicate that the role of photophosphorylation in the vein loading process varies with light intensity and is in fact more significant at high light intensities. The other source of ATP would presumably be respiratory and would be more important in vein loading at low light intensities and in the dark (21). It has been shown that there is no change in dark respiration 15 minutes following a dose of 4.1 krads of radiation (unpublished) and Schefski (73) also did not observe any change 2 hours following exposure to a similar dose. The observation that the radiation-induced reduction in export occurs only at the higher light intensity is likely due to the fact that the major source of ATP for vein loading is from photophosphorylation a process shown in this thesis to be radiation-sensitive. It has been shown, to the satisfaction of some that in such high intensity illumination, there is an inhibition of mitochondrial respiration presumably the result of an increase in the ATP levels in the cytoplasm thus altering the ATP/ADP ratio and hence the availability of ADP and Pi in the mitochondria.

To further assess the participation of photo-induced ATP production in the process of vein loading, the final set of experiments were conducted in which light intensity was varied during the periods of <sup>14</sup>CO<sub>2</sub> photo-assimilation and translocation. Utilizing light of different intensities was suggested from work in which isolated chloroplasts produce more ATP with increasing light intensity (7, 70, 89) as well as increasing the amount of CO<sub>2</sub> fixed (89). Light intensities of 200, 800 and 2,800 foot candles were chosen for use in this experiment because these intensities

represented conditions of near light-compensation (200 ft.c.), light-limiting (800 ft.c.) and light-saturation (2,800 ft.c.) for soybean.

The data (Table 6) indicates that when the light intensity is kept constant during the translocation period there is a significant increase in the export of  $^{14}\text{C}$  when the light intensity during the period of photoassimilation is low. For example plants illuminated at 2,800 foot candles during translocation exported 14.1% of the  $^{14}\text{C}$  recovered when photoassimilation of the  $^{14}\text{CO}_2$  occurred at 2,800 foot candles. When the light intensity during the photoassimilation period was only 800 foot candles or 200 foot candles the extent of export increased significantly to 17.4% and 51.0%, respectively. The apparent increase in export at the lower light intensity during photoassimilation could be explained by an increase in the amount of  $^{14}\text{C}$  incorporated into sucrose and therefore readily available for translocation.

The export of  $^{14}\text{C}$  sucrose from a leaf has been shown to be directly proportional to its specific activity (26) consequently more  $^{14}\text{C}$  is exported if the specific activity of the sucrose produced is increased and this is believed to happen under conditions of low light intensity. A preferential production of sucrose, at low light intensity, has been reported by Wardlaw (96, 97) who found that at low light intensities a greater percentage of the products was in a translocatable form.

Of more importance to this study, however, is the observation that in addition to affecting the amount of exportable material available for translocation, light intensity also affects the magnitude of photoassimilate export. Although the data supporting this statement is in Table 6, the same data has been reorganized for better presentation in Table 7. The data in Table 7 shows that when the intensity of illumination was maintained constant during the period of photoassimilation higher light intensities during the period of export increased the amount of  $^{14}\text{C}$  exported out of the leaf. For example, if the light intensity during photoassimilation was

2,800 foot candles a light intensity of 2,800 foot candles during export induced an export of 14.1%, a significantly higher value than the 11.3% export observed at 800 foot candles and the 9.2% exported at 200 foot candles. The effect of the higher light intensity is likely attributed to the increase supply of ATP, produced photosynthetically, increasing the vein loading or possibly the apoplast loading as well and hence the increase in export. Differences in magnitude of export observed when the light intensity during the period of translocation was varied were not due to changes in the amount of  $^{14}\text{CO}_2$  photoassimilated, for at each of the three light intensities used during the period of assimilation, the same amount of  $^{14}\text{C}$  was photoassimilated.

This data (Table 7) agrees with the work of Wardlaw (96, 97) who has shown using Lolium that export or mass transfer of carbon increases with light intensities. When Lolium was exposed to light of high energy ( $96 \text{ Wm}^{-2}$ ) throughout the periods of photoassimilation and translocation the plant exported 12.2 ( $\text{mg dry weight dm}^{-2} \text{ hr}^{-1}$ ). However if the light intensity was reduced to  $20 \text{ Wm}^{-2}$  during the translocation period, the export value dropped to 7.7. If photoassimilation was occurring at a low light intensity the export was 9.3 and was increased to 17.5 if the light intensity during translocation was increased to  $96 \text{ Wm}^{-2}$ . They interpreted their data as showing an increase in ATP availability for vein loading at the higher levels of light energy.

Both Tables 6 and 7 show data for the free space  $^{14}\text{C}$  content ( $\text{H}_2\text{O}$  soluble) expressed as a per cent of the total ethanol soluble  $^{14}\text{C}$  in the leaf. It can be seen that light intensity does not affect the free space content regardless of the amount of  $^{14}\text{C}$  fixed or the amount of  $^{14}\text{C}$ -photoassimilate exported. This may indicate that the free space concentration, particularly sucrose is kept constant relative to all other compounds being

produced or to the total  $\text{CO}_2$  fixed and that changes in export reflect changes in the rate of active vein loading from the free space. However since  $^{14}\text{C}$  label in the free space may not necessarily reflect the absolute levels of organic compounds, it is unfortunate that the specific activity of the sucrose found within the free space and total production of photoassimilates was not determined. Such information would have given a clearer picture in regards to our understanding of the apoplast and vein loading phenomena.

SUMMARY

One of the important physiological processes occurring in plants is the transfer of organic molecules from their site of synthesis in the green cells of the leaf to the specialized vascular tissue of the phloem for movement from the leaf to other parts of the plant. According to one of the prominent, contemporary hypotheses, the movement of these molecules involves an initial transfer from the cytoplasm of the green cells into the extracellular space (apoplast) and ultimately re-entry from the apoplast into the cytoplasm of the phloem tissue. Both exit from the symplasm and the re-entry into the symplasm (vein or phloem loading) requires the movement of the organic solutes, principally sucrose, across the plasmalemma of cells, with at least one process for which an energy requirement has been demonstrated. Although the data in this thesis has been discussed in relationship to such concepts as radiosensitivity, threshold, radiation modulation and light influenced rates of assimilation and translocation, the data has been 'woven together' to focus on the energy source for the movement of organic solutes into the phloem, the process of photoassimilate export.

The data in this thesis has been interpreted as suggesting that at high light intensities the energy for vein loading is supplied by photophosphorylation whereas at low intensities or darkness, the source of energy is supplied by the mitochondria. This suggestion is supported by the observation that in plants irradiated with ionizing radiation, there occurs a reduction in the export of  $^{14}\text{C}$ -translocate out of a leaf which has photoassimilated  $^{14}\text{CO}_2$  as well as a concomitant reduction in the ability of chloroplasts isolated from the leaves to produce ATP. Both of these processes return to normal by 120 minutes post-irradiation. Furthermore it was demonstrated that there was a higher level of export occurring

under high light intensities than found at the lower light intensities. At low light intensities it has been shown that the export rate is not affected by radiation and it has also been observed that the dark respiration rates are unaffected by exposure to radiation.

The reduced export is unlikely due to the reduction in the rates of photosynthetic CO<sub>2</sub> uptake observed following radiation exposure since export was shown to be reduced to a considerably greater extent than photosynthesis. Furthermore, the greater reduction in rates of photophosphorylation following irradiation than in photosynthesis, may suggest that the reduced photosynthetic CO<sub>2</sub> uptake is probably the result of damage to some other factor rather than to the production of ATP in the chloroplasts. This is supported by the finding that photophosphorylation returns to its normal capacity 120 minutes post-irradiation while photosynthesis is found to be still reduced 4 hours post-irradiation (53, 95). It is possible that retention of photosynthetic products in the chloroplast may cause a build up of materials which in turn reduces photosynthesis as hypothesized by Bossingault (60).

The data presented in this thesis is unable to give the answer to where the energy made available by photophosphorylation is used but it has shown that this energy may play an important role in determining the amount of export that does occur from a leaf. Even though the idea that photophosphorylation participates in the vein loading or export process is not new, the utilization of low doses of ionizing radiation as a means of attacking the problem is novel and has been shown to be a fruitful approach.

Additional experiments which would complement the work presented in this thesis could include:

- (1) Determination of a dose/response curve for photophosphorylation

and total leaf ATP levels so that a comparison to the dose/response results for translocation could be made following radiation exposure.

(2) Utilization of irradiated isolated leaf disks to measure the uptake of exogenously supplied  $^{14}\text{C}$ -sucrose to determine if the photophosphorylation ATP is being used in the active accumulation step from the free space.

(3) Determination of  $^{14}\text{C}$  uptake in leaf disks from radiation stressed plants in which sulfhydryl groups are protected prior to irradiation. The sulfhydryl groups on the membranes adjacent to the free space have been shown to be important in vein loading (28) and membrane sulfhydryl groups are also known to be radiosensitive (45). Such an experiment would indicate if export damage is solely due to reduced ATP available for vein loading.

(4) Analysis of the effects of irradiation exposure on transport across the chloroplast membrane and into the free space to determine the effect of radiation on export across the chloroplast membrane and to provide information about apoplast loading. The experiments would have to be done with a knowledge of the specific activities and total concentrations of materials entering and leaving different leaf tissues and regions.

(5) Relative contribution of mitochondrion and chloroplast organelles as energy sources for  $^{14}\text{C}$  export at different light intensities.

APPENDIX 1. Photosynthetic CO<sub>2</sub> uptake of soybean leaves prior to and at various times following varying acute doses of gamma radiation at a light intensity of 2,800 foot candles.

Absorbed dose	Ratio of developing leaf <sup>+</sup> length to fully developed leaf length	Leaf weight (gm)	Rates of apparent CO <sub>2</sub> uptake (mg CO <sub>2</sub> /hr/gr. fr. wt.)							
			Pre	15	20	25	30	45	60	75
<u>0 Rads</u>										
	0.50	.4712	12.3	11.7	12.0	12.3	12.3	12.3	13.0	12.3
	0.56	.5414	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3
	0.53	.5797	11.9	11.9	11.9	12.0	11.9	11.9	11.9	12.7
	0.52	.5905	13.3	13.3	13.3	13.3	13.2	13.3	13.2	13.2
	0.60	.5205	13.2	13.1	13.2	13.2	13.7	13.1	13.2	13.2
	0.58	.4673	11.8	11.7	11.8	11.8	11.8	11.9	11.9	12.6
<u>60 Rads</u>										
	0.56	.4843	12.0	12.0	12.6	13.0	12.6	12.0	11.7	12.0
	0.56	.4510	11.9	11.9	12.2	12.2	12.2	11.9	12.2	12.2
	0.53	.5525	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.2
	0.52	.4462	13.0	12.4	12.4	13.0	13.0	13.0	13.0	13.0
<u>120 Rads</u>										
	0.52	.4310	11.6	11.6	11.6	11.8	12.2	11.4	11.1	9.5
	0.57	.3819	12.8	12.8	13.1	13.1	13.1	13.1	12.3	12.3
	0.52	.4654	12.5	12.3	12.5	12.3	12.5	12.5	11.3	11.0
	0.61	.4760	14.0	13.6	14.0	14.0	14.0	14.0	12.2	12.9

APPENDIX 1 (cont'd)

<u>250 Rads</u>										
0.52	.4577	12.0	11.9	12.4	12.7	12.7	12.0	11.2	11.0	
0.55	.4888	14.1	14.1	13.3	14.1	14.1	13.3	13.0	12.5	
0.54	.4072	14.3	13.5	14.3	13.5	14.3	13.5	13.2	12.6	
0.72	.5088	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.5	
<u>490 Rads</u>										
0.59	.4050	13.0	13.0	13.0	13.0	13.0	11.6	11.3	10.7	
0.56	.5202	12.5	12.3	12.5	12.5	12.5	11.8	11.8	11.3	
0.55	.4784	12.5	12.5	12.5	12.5	12.7	11.5	11.8	11.4	
0.53	.3527	13.9	13.6	13.9	13.9	13.9	13.0	13.0	12.5	
<u>990 Rads</u>										
0.51	.5761	11.3	11.3	11.3	11.3	11.6	10.9	9.8	9.6	
0.52	.5096	11.3	11.3	11.3	11.3	11.1	11.3	10.8	10.3	
0.56	.5168	13.3	13.3	13.2	13.3	12.9	12.9	12.6	10.7	
0.58	.5547	12.4	12.4	13.3	12.4	12.4	11.7	11.4	10.5	
<u>1970 Rads</u>										
0.52	.5591	12.3	12.0	12.0	12.2	12.3	12.3	12.2	11.6	
0.57	.5934	11.8	11.3	11.3	11.3	11.6	11.6	10.6	9.5	
0.51	.5085	12.0	11.8	12.0	12.0	12.0	11.7	10.8	10.1	
0.51	.5519	12.5	11.8	12.3	12.5	12.5	12.3	12.0	11.8	

APPENDIX 1 (cont'd)

2960 Rads

0.57	.5264	12.3	11.6	11.6	12.1	12.1	10.5	9.5	8.9
0.61	.6710	13.7	11.9	12.8	12.9	13.2	12.9	11.9	10.3
0.52	.4935	11.6	9.9	10.3	11.1	11.2	11.5	10.4	9.2
0.52	.5248	9.6	8.5	8.3	9.1	9.6	8.2	8.6	8.2

3940 Rads

0.51	.7053	11.1	10.0	10.4	10.4	11.1	11.1	10.4	10.0
0.52	.5753	10.6	9.6	10.1	10.5	10.5	9.7	9.4	8.7
0.54	.4709	13.0	11.6	11.7	12.7	12.7	12.9	11.7	10.7
0.55	.4833	10.9	9.9	10.1	10.1	10.6	9.9	8.2	8.2

4930 Rads

0.50	.5892	13.4	11.0	11.2	11.7	12.3	12.1	10.7	9.4
0.53	.5412	12.4	10.9	11.1	11.3	12.0	11.6	10.6	8.9
0.52	.5582	14.1	11.8	12.4	12.4	12.7	12.4	12.4	11.3
0.60	.5169	12.5	11.5	11.9	12.2	12.2	12.2	11.9	10.7

+ Plastochron index

Plants were illuminated 30 minutes prior to obtaining the pre-irradiation rate after which the plant was irradiated.

APPENDIX 2. Photosynthetic CO<sub>2</sub> uptake of soybean leaves prior to and at various times following varying acute doses of gamma radiation at a light intensity of 1,600 foot candles.

Absorbed Dose	Ratio of developing <sup>+</sup> leaf length to fully developed leaf length	Leaf Weight (gm)	Photosynthetic Rates (mg CO <sub>2</sub> /hr/gr. fr. wt.)							
			Minutes Post-radiation							
			Pre	15	20	25	30	45	60	75
<u>0 Rads</u>										
	0.62	.5742	10.7	10.7	10.7	10.7	11.1	10.4	11.1	10.7
	0.52	.4751	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.8
	0.51	.5471	10.3	10.3	10.3	10.3	10.3	10.3	10.6	10.9
	0.53	.6123	10.9	10.9	10.9	10.9	10.9	11.1	10.9	10.9
<u>60 Rads</u>										
	0.58	.4635	11.3	11.3	11.3	11.7	11.7	11.3	11.3	11.1
	0.60	.5832	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
	0.54	.4901	11.9	11.9	12.2	12.2	12.5	11.9	11.9	11.9
	0.59	.5910	10.1	10.1	10.1	10.4	10.4	10.1	10.4	10.1
<u>120 Rads</u>										
	0.64	.5610	11.6	11.6	11.6	11.6	11.6	11.9	10.9	10.9
	0.57	.5313	12.2	12.2	12.2	12.6	13.0	12.2	11.5	11.5
	0.56	.5027	12.9	12.2	12.5	12.5	12.9	12.5	12.5	12.5
	0.58	.5572	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0

## APPENDIX 2 (cont'd)

<u>250 Rads</u>										
0.56	.5775	11.2	11.6	11.2	11.9	11.6	11.6	11.2	10.9	
0.58	.4890	12.0	12.3	12.0	12.0	12.0	11.7	11.7	11.4	
0.59	.4962	11.7	11.7	12.0	11.7	11.7	11.1	11.1	11.1	
0.61	.5800	10.6	10.6	10.6	11.2	10.9	10.6	10.3	10.3	
<u>490 Rads</u>										
0.51	.5618	10.3	10.3	10.3	10.1	10.3	10.9	10.3	10.1	
0.65	.5405	11.3	11.3	11.0	11.3	11.3	10.7	10.7	10.5	
0.58	.4009	9.0	9.2	9.3	9.2	9.2	9.2	8.9	8.7	
0.68	.7329	10.7	10.0	10.4	10.7	10.4	10.2	10.4	9.3	
<u>990 Rads</u>										
0.63	.7541	10.4	10.4	10.4	10.8	10.4	10.4	10.4	10.4	
0.63	.6239	12.6	11.8	11.8	11.8	12.2	12.0	11.4	11.4	
0.59	.7808	9.7	9.7	9.7	9.7	9.7	9.1	9.4	9.1	
0.54	.4407	12.5	12.2	12.5	12.5	12.5	12.3	11.9	11.6	
<u>1970 Rads</u>										
0.59	.6082	10.1	9.8	10.1	10.1	10.5	10.1	9.8	9.8	
0.58	.6788	10.5	10.5	10.5	10.5	10.8	10.8	10.2	10.2	
0.55	.6482	10.3	10.3	10.3	10.3	10.3	10.6	11.0	10.3	
0.50	.6492	11.3	11.3	11.7	11.7	11.7	11.3	11.3	11.0	

APPENDIX 2 (cont'd)

2960 Rads

0.53	.5326	11.5	11.5	11.5	11.2	11.5	10.9	10.6	10.4
0.58	.7940	10.7	10.7	11.1	10.7	10.7	10.7	10.3	9.9
0.55	.5786	11.9	10.6	11.2	11.7	11.7	11.6	11.2	11.2
0.51	.4991	12.3	11.0	10.8	11.5	10.5	10.8	10.5	10.5

3940 Rads

0.52	.5580	10.7	9.9	9.4	9.7	9.9	10.1	10.1	9.6
0.63	.5083	11.1	10.6	10.6	10.3	10.8	9.6	9.9	9.2
0.60	.6715	11.0	9.7	10.0	10.3	10.6	9.7	9.7	9.1
0.55	.6651	10.0	9.8	9.8	9.5	9.8	9.2	9.5	9.2

4930 Rads

0.53	.6070	11.7	11.0	10.7	11.0	11.4	11.0	11.0	11.0
0.55	.4683	11.8	10.5	11.0	11.2	11.8	11.6	10.7	10.5
0.64	.5834	10.5	9.5	9.2	9.0	8.8	9.5	9.5	9.5
0.65	.5963	10.9	10.0	10.0	9.7	10.3	10.0	9.7	9.0

+ Plastochron index

Plants were illuminated 30 minutes prior to the pre-irradiation rate after which the plant was irradiated.

APPENDIX 3. Magnitude of  $^{14}\text{C}$  export and photosynthetic  $\text{CO}_2$  uptake of soybean leaves at 800, 1,600 and 2,800 foot candles 30 minutes following exposure to 4,100 rads of gamma radiation.

Treatment and Plastochron Index	Leaf weight (gm)	Total assimilated $^{14}\text{C}$ recovered ( $\mu\text{Ci}$ )	Exported $^{14}\text{C}$ as % of total recovered from whole plant	Rates of apparent $\text{CO}_2$ -uptake (mg $\text{CO}_2$ /hr/gr. fr. wt.)	
				Pre-irradiation	Post-irradiation
800 Foot candles					
<u>Non-irradiated</u>					
0.63	-	2.52	14.3	-	-
0.59	0.5851	2.28	7.9	8.4	8.0
0.57	0.6009	2.57	3.1	7.3	7.2
0.61	0.5318	2.53	6.3	7.8	7.8
0.61	0.4715	2.51	4.8	8.1	8.1
0.54	0.5065	2.73	8.8	8.5	8.7
0.59	0.5526	2.46	4.1	7.9	7.7
<u>Irradiated</u>					
0.54	0.5886	2.16	6.9	8.1	6.9
0.57	0.6050	2.89	9.3	7.8	7.8
0.54	0.5993	2.25	4.5	7.4	7.2
0.61	0.6116	2.18	11.0	7.2	7.2
0.57	0.4256	1.65	5.5	8.0	7.9
0.55	0.6554	1.78	3.4	7.0	7.0

APPENDIX 3 (cont'd)

1,600 Foot candles

Control

0.55	0.5964	2.77	7.6	11.2	11.2
0.58	0.5224	2.28	5.3	12.4	12.8
0.59	0.6105	1.86	4.3	12.0	12.0
0.57	0.5276	2.58	4.7	10.7	10.7
0.55	0.5533	1.85	11.9	11.7	11.1
0.56	0.6715	1.84	4.4	12.6	13.1
0.52	0.5318	2.62	14.9	13.5	13.5

Irradiated

0.64	0.6211	2.34	7.3	12.7	12.7
0.55	0.6290	2.74	5.5	12.5	11.7
0.58	0.5896	2.12	9.0	12.5	12.5
0.59	0.6033	3.38	4.4	12.2	12.2
0.66	0.6524	3.70	6.8	10.6	9.9
0.58	0.5953	3.08	2.3	11.1	10.3
0.53	0.7468	2.28	5.7	11.6	11.1
0.55	0.6602	2.41	7.1	11.3	11.3

2,800 Foot candles

Control

0.53	0.5327	0.96	8.3	14.8	14.3
0.51	0.5797	2.80	9.6	12.7	12.7

APPENDIX 3 (cont'd)

2,800 Foot candles

Control (cont'd)

0.67	0.4623	2.29	6.1	10.4	12.6
0.60	0.5038	2.89	7.3	14.1	14.1
0.57	0.4428	3.10	8.4	12.5	13.1
0.52	0.5763	2.56	10.9	12.8	13.2
0.60	0.7205	1.96	10.2	12.8	12.8
0.57	0.6208	4.06	7.6	11.8	12.3
0.55	0.5290	4.18	4.5	12.8	12.6

Irradiated

0.54	0.6093	2.88	3.5	12.9	12.1
0.54	0.4165	3.20	3.1	13.6	12.7
0.50	0.4818	3.12	4.2	12.7	11.7
0.52	0.6574	2.26	7.1	12.9	12.9
0.54	0.6343	2.20	8.2	12.9	12.9
0.56	0.5285	1.65	7.9	14.9	13.2
0.58	0.7028	1.77	7.9	13.1	13.1
0.57	0.8678	4.28	6.1	11.6	11.6
0.56	0.6924	4.56	5.5	12.3	11.4

Plants were allowed to photoassimilate  $^{14}\text{CO}_2$  prior to irradiation. The post-irradiation photosynthetic rate was obtained 30 minutes following irradiation after which the plant was sectioned and extracted in 80% ethanol.

APPENDIX 4. Photophosphorylation rate in chloroplasts isolated from soybean leaves maintained under 2,800 foot candles 15, 60 and 120 minutes post-irradiation.

Treatment and Chlorophyll Concentration per Reaction ( $\mu\text{g Chl}$ )	Total Counts ( $^{32}\text{P}$ ) (1)	Dark Counts ( $^{32}\text{P}$ ) (2)	Light Counts ( $^{32}\text{P}$ ) (3)	Photophosphorylation Rate ( $\mu\text{moles ATP/hr/mg Chl}$ )
<u>Non-irradiated 15 &amp; 120 min</u>				
24.1	$1.64 \times 10^5$	$8.51 \times 10^1$	$5.43 \times 10^3$	$2.20 \times 10^2$
19.1	$2.51 \times 10^5$	-	$9.01 \times 10^3$	$3.76 \times 10^2$
15.9	$2.82 \times 10^5$	-	$6.07 \times 10^3$	$2.26 \times 10^2$
35.1	$2.52 \times 10^5$	$1.29 \times 10^2$	$1.15 \times 10^4$	$2.32 \times 10^2$
17.3	$3.07 \times 10^5$	$3.39 \times 10^2$	$1.01 \times 10^4$	$3.30 \times 10^2$
12.3	$8.38 \times 10^4$	$2.15 \times 10^2$	$2.26 \times 10^3$	$3.57 \times 10^2$
12.7	$7.96 \times 10^4$	-	$1.52 \times 10^3$	$2.71 \times 10^2$
<u>Post-irradiation 15 &amp; 60 min</u>				
14.5	$2.57 \times 10^5$	$1.27 \times 10^3$	$4.73 \times 10^3$	$1.67 \times 10^2$
20.9	$2.50 \times 10^5$	-	$3.62 \times 10^3$	$1.24 \times 10^2$
14.1	$2.49 \times 10^5$	$1.14 \times 10^3$	$3.51 \times 10^3$	$1.21 \times 10^2$
23.4	$2.21 \times 10^5$	-	$5.69 \times 10^3$	$1.98 \times 10^2$
19.1	$2.30 \times 10^5$	$3.96 \times 10^2$	$5.74 \times 10^3$	$2.19 \times 10^2$
11.4	$2.31 \times 10^5$	$1.74 \times 10^2$	$1.63 \times 10^3$	$1.00 \times 10^2$
14.1	$1.96 \times 10^5$	$3.72 \times 10^2$	$3.29 \times 10^3$	$1.28 \times 10^2$

APPENDIX 4 (cont'd)

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Post-irradiation 120 min

14.5	$1.18 \times 10^5$	$2.32 \times 10^2$	$9.12 \times 10^2$	$1.24 \times 10^2$
15.9	$9.79 \times 10^4$	-	$1.48 \times 10^3$	$2.26 \times 10^2$
13.7	$1.27 \times 10^5$	-	$8.67 \times 10^2$	$1.39 \times 10^2$
10.9	$1.25 \times 10^5$	-	$3.56 \times 10^3$	$4.97 \times 10^2$
15.9	$1.03 \times 10^5$	-	$2.57 \times 10^3$	$2.82 \times 10^2$
17.7	$9.10 \times 10^4$	$13.9 \times 10^2$	$1.70 \times 10^3$	$1.75 \times 10^2$
10.9	$8.03 \times 10^4$	-	$1.30 \times 10^3$	$2.68 \times 10^2$
14.1	$2.57 \times 10^5$	-	$6.26 \times 10^3$	$3.11 \times 10^2$
17.3	$2.48 \times 10^5$	-	$6.41 \times 10^3$	$2.69 \times 10^2$

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- (1) Total dpm  $^{32}\text{P}$  activity introduced into the reaction minxute.
- (2) Total dpm  $^{32}\text{P}$  activity incorporated into ATP fraction in the light.
- (3) Total dpm  $^{32}\text{P}$  activity incorporated into ATP fraction in the dark.

Chloroplasts were isolated and allowed to form  $\text{AT}^{32}\text{P}$  for 2 minutes in the light and dark.

APPENDIX 5. Magnitude of  $^{14}\text{C}$  export from soybean leaves 15 and 120 minutes following an acute exposure to 4,100 rads of gamma radiation.

Treatment and Plastochron Index	Leaf Weight (gm)	Total Assimilated ( $\mu\text{C}$ )	% Export
<u>Non-irradiated</u>			
0.57	0.6556	1.91	23.0
0.57	0.7725	2.27	19.8
0.57	0.5058	1.67	17.6
0.56	0.6924	1.86	20.4
0.60	0.8134	3.21	24.0
<u>15 minute Post-irradiation</u>			
0.58	0.5642	2.84	14.1
0.57	0.7498	2.48	9.3
0.54	0.6944	1.66	14.5
0.62	0.7392	3.52	14.5
0.61	0.5966	2.85	14.0
<u>120 minute Post-irradiation</u>			
0.57	0.7100	3.09	24.3
0.63	0.7229	1.32	25.0
0.55	0.6469	1.45	22.1
0.63	0.7218	1.42	20.4
0.63	0.7272	2.23	20.6

Plants photoassimilated  $^{14}\text{CO}_2$  for 15 minutes, followed by 45 minute period for export, 15 and 120 minutes post-irradiation.

APPEXDIX 6. Magnitude of  $^{14}\text{C}$  photoassimilate export and free space  $^{14}\text{C}$  content in soybean plants photoassimilating and exporting at varying light intensities.

Light treatment and Plastochron Index	Leaf weight (gm)	Total assimilated $^{14}\text{C}$ ( $\mu\text{Ci}$ )	Exported $^{14}\text{C}$ outside of the source leaf ( $\mu\text{Ci}$ )	80% ethanol soluble $^{14}\text{C}$ -extracted from leaflets ( $\mu\text{Ci}$ )	Water soluble $^{14}\text{C}$ -extracted from leaflets ( $\mu\text{Ci}$ )	Total extracted $^{14}\text{C}$ from leaflets ( $\mu\text{Ci}$ )	$^{14}\text{C}$ -extracted from the petiole inside plexiglass chamber ( $\mu\text{Ci}$ )
2,800/2,800							
0.56	0.6142	3.80	0.42	2.26	0.70	3.36	0.02
0.58	0.6413	3.09	0.43	1.96	0.68	2.64	0.02
0.62	0.5183	4.28	0.65	2.78	0.82	3.60	0.03
0.59	0.5238	4.09	0.67	2.62	0.76	3.38	0.04
0.63	0.4855	3.42	0.38	2.19	0.83	3.02	0.02
0.57	0.4994	3.45	0.55	2.34	0.54	2.88	0.02
2,800/800							
0.58	0.5803	4.36	0.52	3.13	0.67	3.80	0.04
0.58	0.5929	3.94	0.39	2.95	0.57	3.52	0.03
0.56	0.4584	3.60	0.52	2.39	0.67	3.06	0.02
0.57	0.5659	3.09	0.29	2.06	0.72	2.78	0.02
0.57	0.5734	2.85	0.22	1.50	0.45	1.95	0.01
0.59	0.5600	2.48	0.38	1.84	0.62	2.46	0.01

APPENDIX 6 (cont'd)

2,800/200								
0.57	0.4783	4.26	0.39	2.85	1.00	3.85	0.02	
0.59	0.5578	4.27	0.31	2.82	1.12	3.94	0.02	
0.60	0.6858	3.93	0.39	2.80	0.71	3.51	0.03	
0.60	0.6021	3.32	0.24	2.52	0.53	3.05	0.03	
0.59	0.4973	3.75	0.51	2.71	0.51	3.22	0.02	
0.59	0.5579	3.83	0.27	3.15	0.39	3.54	0.02	
0.55	0.4105	4.00	0.40	2.80	0.78	3.58	0.02	
800/800								
0.59	0.6005	3.52	0.60	2.11	0.77	2.88	0.04	
0.59	0.5672	3.18	0.34	2.10	0.70	2.80	0.04	
0.58	0.6785	3.00	0.66	1.92	0.37	2.29	0.05	
0.56	0.5941	2.76	0.29	2.00	0.44	2.44	0.03	
0.56	0.7344	2.32	0.50	1.43	0.36	1.79	0.03	
0.57	0.5508	2.28	0.37	1.52	0.37	1.89	0.02	
800/2,800								
0.60	0.4456	3.38	0.70	1.94	0.71	2.65	0.03	
0.57	0.5408	3.82	0.77	2.49	0.53	3.02	0.03	
0.59	0.3767	3.41	0.79	2.17	0.41	2.58	0.04	
0.58	0.5865	3.10	0.33	2.23	0.51	2.74	0.03	
0.63	0.7272	2.85	0.36	1.84	0.63	2.47	0.02	
0.52	0.5542	2.33	0.59	1.31	0.41	1.72	0.02	

## APPENDIX 6 (cont'd)

200/2,800								
0.53	0.5795	2.86	1.40	1.18	0.24	1.42	0.04	
0.54	0.5664	3.06	1.56	1.05	0.39	1.44	0.06	
0.60	0.4523	2.70	1.35	1.05	0.27	1.32	0.03	
0.55	0.5039	2.95	1.67	0.94	0.31	1.25	0.03	
0.56	0.5058	2.52	1.10	1.02	0.37	1.39	0.03	
0.53	0.5879	2.44	1.36	0.85	0.21	1.06	0.02	
200/200								
0.57	0.6084	3.03	1.31	1.38	0.30	1.68	0.04	
0.58	0.4626	2.43	0.91	1.22	0.28	1.50	0.02	
0.60	0.5884	3.14	1.43	1.38	0.28	1.66	0.05	
0.60	0.6105	2.78	1.10	1.23	0.40	1.63	0.05	
0.58	0.6330	2.63	1.12	1.23	0.23	1.46	0.05	
0.56	0.5305	2.32	0.80	1.07	0.41	1.49	0.03	

Plants were maintained at photoassimilation light intensity for 1 hour and photoassimilated  $^{14}\text{CO}_2$  for 15 minutes at the same light intensity followed by a 45 minute period of export, where light intensity was maintained the same or changed.

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Addendum

The increase in the rate of photosynthetic CO<sub>2</sub> uptake observed at 1,600 foot candles following a 60 rad absorbed dose of radiation was not mentioned in the thesis for several reasons. The first was that the increase was observed in this set of experiments only and was not evident in any other treatment. If an initial reduction (15 minutes post-irradiation) had been observed, then the increase at 25 and 30 minutes post-irradiation could have been argued as being an overshoot by a repair mechanism. However no damage was evident. Lastly the increase was only a marginal one in the photosynthetic ability and not likely to affect the translocation process, the primary focus of this work.