The effect of nitrate fertilization on the distribution of exported ^{14}C-photosynthate in nodulated soybean (*Glycine max* (L.) Merr.) plants

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Abstract

Soybean (Glycine max (L.) Merr. cv. Harosoy 63) plants inoculated with Rhizobium japonicum were grown in vermiculite in the presence or absence of nitrate fertilization for up to 6 weeks after planting. Overall growth of nodulated plants was enhanced in the presence of nitrate fertilization, while the extent of nodule development was reduced. Although the number of nodules was not affected by nitrate fertilization when plants were grown at a light intensity limiting for photosynthesis, at light intensities approaching or exceeding the light saturation point for photosynthesis, nitrate fertilization resulted in at least a 30% reduction in nodule numbers.

The mature, first trifoliate leaf of 21 day old plants was allowed to photoassimilate $^{14}CO_2$. One hour after the initial exposure to $^{14}CO_2$, the plants were harvested and the ^{14}C radioactivity was determined in the 80% ethanol-soluble fraction in order to assess the extent of photoassimilate export and the pattern of distribution of exported ^{14}C. The magnitude of ^{14}C export was not affected by the presence of nitrate fertilization. However, there was a significant effect on the distribution pattern, particularly with regard to the partitioning of ^{14}C-photosynthate between the nodules and the root tissue. In the presence of nitrate fertilization, less than 6% of the exported ^{14}C photosynthate was recovered from the nodules, with much larger amounts (approximately 37%) being recovered from the root tissue. In the absence of nitrate fertilization, recovery of exported ^{14}C-photosynthate from the nodules (19 to 27%) was approximately equal to that from the root tissue (24 to 33%).

By initiating or terminating the applications of nitrate at 14 days of age, it was determined that the period from day 14 to day 21 after planting was particularly significant for the development of nodules initiated

earlier. Addition of nitrate fertilization at this time inhibited further nodule development while stimulating plant growth, whereas removal of nitrate fertilization stimulated nodule development. The results obtained are consistent with the hypothesis that nodule development is inhibited by nitrate fertilization through a reduction in the availability of photosynthate to the nodules.

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1. Introduction

Crop rotation is an important factor in many agricultural systems. Not only are different crops subject to different pests and diseases, but also crops vary in their effect on soil structure and soil fertility. From ancient times, leguminous plants have been used for soil-enrichment in crop rotations, even though it was not until the 19th century that the reasons for their beneficial effect on the soil became apparent.

In 1838, Boussingault (cited in 4, 101) demonstrated that legumes such as peas and clover when grown under natural, non-sterile conditions, assimilated more nitrogen than was supplied to them in combined forms, whereas non-leguminous plants such as wheat and oats did not. Recognizing atmospheric nitrogen (N_2) as a source, Boussingault announced that "...the nitrogen of the atmosphere can be assimilated during the life of a plant" (cited in 4). However, this work was criticized by the eminent agricultural chemist, Liebig (cited in 4, 101), and when Boussingault repeated the experiments under sterile conditions in closed containers, his earlier results were not confirmed, thus causing him to abandon the hypothesis. It was not until 1886 that the controversy was settled when Hellriegel and Wilfarth demonstrated that pea plants were able to assimilate (i.e., fix) atmospheric nitrogen only when root nodules were present. They suggested that the nodules, formed as the result of infection by soil bacteria (later identified as belonging to the genus Rhizobium), were the site of nitrogen fixation and non-nodulated leguminous plants required combined nitrogen fertilizers for growth, as did non-leguminous plants such as the cereals (101).

It is now known that nitrogen fixation is accomplished not only by obligate bacterial symbionts such as those found in the legume-Rhizobium associations, but also by a wide range of free-living organisms, including

asymbiotic diazotrophs (N_2-fixing organisms) such as blue-green algae and some bacteria, and associative symbiotic bacteria which occur in the rhizosphere (root zone) and phyllosphere (leaf zone) of some plants (24, 42, 87).

The extent of nitrogen fixation has been estimated at approximately 250×10^6 tonnes (1 tonne = 1000 kg) of N per annum (42), of which industrial fixation and other abiological processes account for about 75×10^6 tonnes (42). Of the 175×10^6 tonnes associated with biological sources, approximately 50% is assigned to agricultural soils (42) with the legume-*Rhizobium* symbiosis as the most important source from an agronomic standpoint. Up to 350 kg of N per hectare per annum may be fixed by the legume symbionts (23), and the total annual N fixation assigned to leguminous crops is estimated at 40×10^6 tonnes for grain legumes with an additional almost 40×10^6 tonnes for forage legumes in permanent meadows (42).

The root nodules on leguminous plants are formed following the infection of the roots by bacteria of the genus *Rhizobium*. The actual species of *Rhizobium* is host specific. For example, roots of the soybean, *Glycine max* (L.) Merr., form nodules only when infected by *Rhizobium japonicum*. In the presence of rhizobial bacteria, curling and deformation of the root hairs is initiated, followed by the development of an infection thread at the "bend" in the root hair. This infection thread grows through the epidermal cells and into the cortex region of the root, where cortical cells are induced to divide and expand, thus initiating nodule development (22). Rhizobial cells pass through the infection thread and enter cortical cells having twice the normal complement of chromosomes, where the bacteria undergo certain biochemical and structural changes in preparation

for their symbiotic role in nitrogen fixation (8, 66). The rhizobial cells, now termed bacteroids, become incapable of further division, and are packed in the cytoplasm of infected parenchyma cells, which together with non-infected parenchyma cells, form the central tissue of the root nodule structure (8, 113).

Besides the central bacteroidal zone of infected and non-infected parenchyma cells, the root nodule also contains an outer nodule cortex consisting of uninfected parenchyma cells, a meristematic region which provides specialized nodule tissue including the nodule cortex, and a vascular system consisting of xylem tracheids, phloem fibres, seive tubes and companion cells (113). These vascular cells are connected to the vascular system of the host plant (88). The protruberant cell walls of the root pericycle tissue increase the surface to volume ratio enormously, and this, together with the presence of plasmodesmata between bacterial cells, pericycle cells, and sieve element cells, provide a phloem source-to-sink transport system able to supply sugars and other essential organic compounds to the bacteroidal tissue (88).

Bacteroids in the root nodules fix atmospheric nitrogen by means of the bacterial enzyme, nitrogenase (7). The triple bond of molecular nitrogen (N≡N) is reduced to ammonia by a reaction which is summarized as follows:

$$N_2 + 6e^- + 8H^+ + nMg\cdot ATP \longrightarrow 2NH_4^+ + nMg\cdot ADP + nP_i \qquad (69)$$

The basic requirements for dinitrogen fixation, besides the nitrogenase enzyme (24, 42), are a source of low potential reductant and electrons, and ATP bound to a divalent ion, Mg^{2+} being the most effective (24, 42, 69). The first stable product of dinitrogen fixation is ammonia (16, 24, 42, 69)

and evidence for intermediates other than ammonia remains questionable (16).

Provided a source of ATP and reductant are available, the nitrogenase enzyme is also capable of reducing several other substrates, including N_2O, H^+, HCN and C_2H_2 (69). The reduction of acetylene (C_2H_2) is of particular importance since ethylene (C_2H_4) is produced which can be readily measured by the technique of gas chromatography. This characteristic has been developed into the widely-used acetylene-reduction assay for N_2- fixing activity (6, 30, 42, 44).

The bacteroids require oxygen to sustain the aerobic respiration which provides the ATP and reducing power required for nitrogen fixation (120). Since the nitrogenase enzyme is readily inactivated by oxygen (8), however, it has been suggested that leghemoglobin, a myoglobin-like molecule found only in nitrogen-fixing legume root nodules (2), functions in a transport mechanism which allows for a high flux of oxygen at low oxygen tension to the bacteroids (2, 7, 8, 42), thus controlling the oxygen concentration about the bacteroids and so preventing inactivation of the nitrogenase enzyme. It has been suggested that the terminal electron acceptor in bacteroidal respiration is oxyleghemoglobin rather than free oxygen (9). It is interesting that leghemoglobin is comprised of a heme moiety synthesized by the bacteroid, and a protein moiety synthesized by the host plant (2), a cooperative synthesis which is essential to the symbiotic relationship.

The ATP and reductant requirements of the nodules are met by bacteroidal respiration of carbohydrate provided by the host plant (14, 36, 48, 70, 71, 74, 120). Carbon skeletons for the removal of fixed nitrogen are also obtained from host photosynthate translocated to the nodules (14, 48, 74). Estimates of carbohydrate requirement for nitrogen fixation range between

7 and 20 grams for each gram of atmospheric nitrogen assimilated (13, 48, 71, 74). Of the carbohydrate translocated to the root nodules of garden pea, Pisum sativum L., more than one-third was utilized in bacteroidal respiration to provide the required ATP and reducing power, while almost one-half was used in the formation of amino compounds to be subsequently exported to the shoot via the xylem (74). Similar values have been obtained for the utilization of carbohydrate supplied to the root nodules of cowpea, Vigna unguiculata (L.) Walp. (48). Predictably, modification of the availability of photosynthate to the root nodules through manipulation of the plant or environment has been shown to modify the rate of nitrogen fixation (37, 38, 41, 60, 61, 62, 63, 70, 71, 117).

Of major agronomic importance has been the discovery that the formation of nodules, and hence the initiation of nitrogen fixing activity, is strongly reduced when combined nitrogen is present in the soil at moderate or high levels (1, 47, 67, 89). Furthermore, although both symbiotically produced and soil combined nitrogen are readily used by the plant (1, 81, 82) it appears that the production of symbiotically combined nitrogen is inversely related to the amount of combined nitrogen available in the soil (1, 101). That is, high levels of applied combined nitrogen (usually as NO_3^-, NH_4^+ or urea), tend to be preferentially utilized by the plant with a concommitant reduction in the rates of N_2-fixation by the bacterial symbiont. It is for this reason that nitrogen fertilization of legume crops has not provided the large increases in productivity which have been observed in cereal production (19, 42). Nevertheless, small amounts of nitrogenous fertilizer are beneficial to overall plant growth, especially during the early stages when the plant passes through a period of nitrogen deficiency prior to the onset of nitrogen fixation (67, 101, 106).

The effect of combined nitrogen in the soil on the rate of nitrogen fixation may, at least in part, be the result of an inadequate supply of photosynthate to the nodules, possibly caused by a reduction in bacterial respiration. Use of nodulating and non-nodulating isolines of soybean (115) indicated that plants dependent solely upon symbiotic nitrogen fixation exported more ^{14}C to the roots and nodules than did plants totally dependent upon nitrate nitrogen, while partially nodulated plants which were dependent upon both symbiotic and combined nitrogen exhibited intermediate translocation patterns to the roots and nodules (92). Respiration associated with nitrogenase activity was decreased by supplying nodulated Pisum plants with ammonium nitrate, while growth and maintenance components of respiration were unaffected (71).

In view of the significant effect that nitrogen fertilization has on root nodule activity, and may have on translocation patterns as well, the present study was conducted to investigate:

(i) the growth and development of nodulated and non-nodulated soybean plants grown in the presence or absence of nitrate fertilization;

(ii) the magnitude of export and the distribution of ^{14}C-photosynthate in nodulated soybeans supplied with fertilizer nitrogen as nitrate for various time periods during the early stages of growth and development.

2. Literature review

In photosynthesis, light energy is utilized in the chloroplasts of leaf mesophyll cells for the production of ATP (adenosine triphosphate) and reducing power in the form of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form). These compounds are subsequently utilized in the reduction of atmospheric CO_2 forming organic compounds, principally sugars (12, 26). Since the illuminated leaf does not require all of the photosynthetically assimilated carbon compounds, the excess is either stored as starch within the chloroplasts of the mesophyll cells, or is translocated out of the leaf to other sites of utilization or storage (77, 114). Consequently, the photosynthesizing leaf is frequently identified as a "source" of assimilates (assimilates referring to the reduced carbon compounds), whereas the alternate sites of utilization or storage are considered to be metabolic "sinks". Some examples of sinks include the shoot apex, developing leaves, reproductive organs, and subterranean parts of the plant. Since sources and sinks are spatially separated, the vascular tissue, especially the phloem, serves as the "conducting pathway" through which assimilates move from the source to the sinks (77).

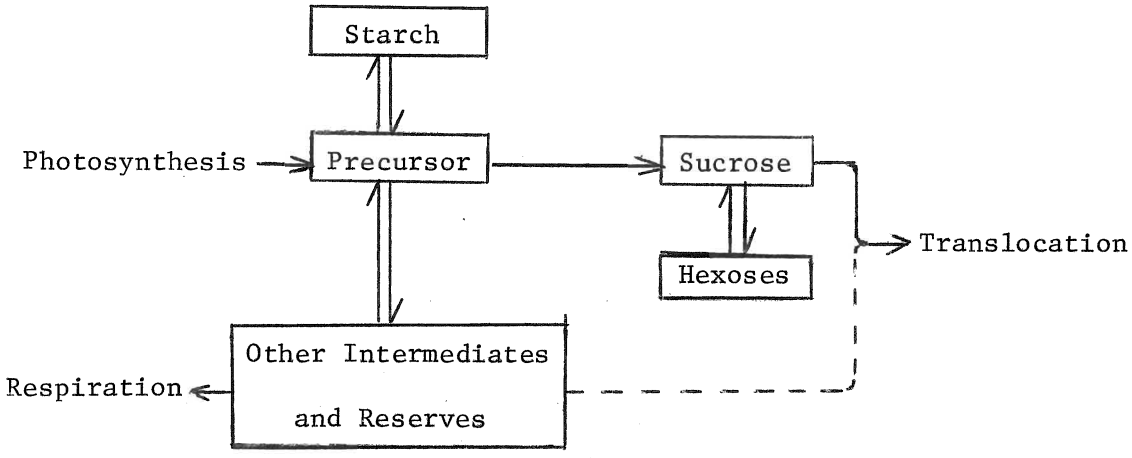
Translocation in plants is the movement of materials from one location to another (i.e., from source to sink) over long distances through specialized conducting tissues at rates faster than can be accounted for by diffusion alone. Although many materials such as water, hormones, inorganic (i.e., mineral) and organic solutes are translocated, it is the distribution of photosynthetically assimilated carbon compounds which is of specific significance to this thesis.

The most abundant and most readily translocated photosynthetically-produced carbon compound in the majority of plants is the non-reducing sugar,

sucrose (3, 54, 56, 75, 80, 104, 109). However, under suitable conditions, other organic compounds including amino acids, organic acids, and other non-reducing sugars such as stachyose and raffinose, may also be translocated (5, 21, 56).

The complex process referred to as translocation consists of, or is dependent upon, several components, which include: the assimilation of CO_2 and subsequent biosynthesis of organic compounds, the localization of metabolic and storage pools, the movement of photosynthate from the leaf mesophyll cells into the phloem, the ensuing longitudinal translocation, and the unloading and eventual utilization or storage of the translocated material in the sink regions (94, 95). Since these components are interrelated, a change in magnitude of one component, affected by either internal or external factors, will eventually have an effect on the other components.

The magnitude of export of photosynthetically reduced (i.e., fixed) carbon out of the source leaf is greatly dependent upon the availability of substrate for export, whether this be recent photosynthate or from the breakdown of storage carbohydrate. The relationship between newly formed photosynthate for translocation and the starch reserves has recently been described in a model presented by Charles-Edwards and Ho (18). In this model, sucrose is identified as the major translocated carbohydrate whose concentration controls the export rate. Photosynthesis provides a precursor pool which acts as a source for the synthesis of both sucrose and starch, while intermediates from respiration or other storage components may either be translocated or converted into sucrose prior to export. An equilibrium exists between sucrose and hexoses, with little exchange between the two pools. This model is shown in Figure 1.



After Charles-Edwards and Ho (18)

Figure 1. The movement of carbon through a leaf.

Ho (50) utilized a range of light intensities to obtain net photosynthesis rates ranging from 0.1 to 4.9 mg $C \cdot dm^{-2} \cdot h^{-1}$. When carbon fixation rates were greater than 2 mg $C \cdot dm^{-2} \cdot h^{-1}$, a proportional relationship between rate of carbon transport and carbon fixation occurred, with 60 to 66% of the recently fixed carbon being exported. Under these conditions, the sucrose concentration was maintained at 1% of the total organic carbon, while starch was 17%. When the rate of carbon fixation was less than 1 mg $C \cdot dm^{-2} \cdot h^{-1}$, the rate of carbon export was maintained at 1 mg $C \cdot dm^{-2} \cdot h^{-1}$, primarily at the expense of starch breakdown, since the concentration of starch decreased to 8% of the total organic carbon, while sucrose concentration fell to 0.5%. These changes in sucrose and starch concentrations were attributed to control mechanisms partitioning fixed carbon between the two carbohydrate pools. The extent of carbohydrate export out of a leaf is therefore controlled by this regulation of sucrose concentration, with starch serving as a potential source of sucrose specifically when CO_2 fixation rates are lower than rates of translocation.

The relationship between rates of photosynthesis and extent of export of photosynthate in two cultivars of Phaseolus vulgaris L. was investigated by Liu et al. (68). The cultivar having a rate of photosynthesis 30% higher than the other cultivar was found to have a higher rate of photosynthate export. Autoradiographs were taken 11 minutes after exposure of illuminated leaves to $^{14}CO_2$. In the cultivar having the lower photosynthetic rate, all the radioactivity (i.e., the ^{14}C label) was present in the mesophyll area of the leaf, the veins and petiole being free from label, while in the cultivar having the higher rate of photosynthesis, the mesophyll area was clearing and label was present in the veins and the petiole, thus indicating a faster rate of vein loading. Also, leaves of the cultivar

having the higher rate of photosynthesis exported over 60% of the photoassimilated ^{14}C within 8 hours of exposure to $^{14}CO_2$, while in the same period, only 40% of the label was exported from leaves of the cultivar with the lower photosynthetic rate.

Stephenson et al. (100) and Hofstra and Nelson (55), utilizing a wide range of plant species, including, for example, millet (Panicum miliaceum L.), tomato (Lycopersicon esculenteum Mill.), sorghum (Sorghum spp.), soybean (Glycine max (L.) Merr.), and radish (Raphanus sativus L.), have shown that those plant species having higher photosynthetic rates were generally found to have higher rates of export of photosynthate, as well as a capacity to export a greater total amount of photoassimilated ^{14}C from the leaf.

By varying light intensity and the concentrations of CO_2 and O_2, Servaites and Geiger (93) obtained increases in the rate of CO_2 fixation and related increases in the rate of photosynthate export from the leaf. The leaf was capable of exporting the excessively produced photosynthate even when the rate of photosynthesis was 4 times greater than the normal rate. They interpreted their data to suggest that the rate of movement of assimilated carbon compounds out of the source leaf (i.e., the mass transfer rate) was not limited by the vein loading capacity of the leaf but instead was limited by the rate of net photosynthesis and in particular by the rate of sucrose synthesis. By using trimmed plants in which only one source leaf was retained, the demand for assimilates by the various sinks was greater than the production of assimilates by the source leaf, thus supporting their interpretation that the extent of starch synthesis was unlikely to affect the rate of sucrose synthesis, although they did not measure the concentrations of carbohydrates in the leaf under the different conditions used in

their study.

Despite the correlation between rate of export of photosynthate and the rate of photosynthesis (55, 68, 93, 100), no evidence has yet been provided for a causal relationship between high rates of photosynthesis and high rates of export, indicating that other factors are also important.

In contrast to the data of Servaites and Geiger (93), Nelson (78, 80), who also varied the CO_2 concentration while maintaining a constant light intensity, showed that although the photoassimilation of ^{14}C by the leaf increased by a factor of 3 between the low (0.03%) and high (0.3%) CO_2 concentrations, there was no increase in the amount of ^{14}C exported from the leaf. However, since the concentrations of organic compounds in the leaf were not determined, it is not known whether the extra carbohydrate produced at higher rates of photosynthesis was available for immediate export.

While the illuminated leaf is producing material which is potentially available for export, the ability of the leaf to translocate photoassimilate depends upon the ability of these materials to enter the translocate pathway. The loading of translocatable materials into the vascular tissue is an energy-requiring process (15, 31, 35, 98, 111) and the availability of energy for this purpose is, potentially, a rate limiting step in the export process.

It would appear that the energy for the loading of photoassimilates into the vascular tissues is produced not only during photosynthesis (i.e., the process of photophosphorylation) but is also produced during mitochondrial respiration. Utilizing ionizing radiations to stress the energy requiring processes in young soybean, McCabe (72) showed that at high light intensities, the reduction in export of photoassimilated ^{14}C following exposure of the

whole plant to ionizing radiations (72, 94, 95) was accompanied by a similar reduction in the production of ATP in photosynthesis (i.e., photophosphorylation). He was also able to show that both processes (i.e., ^{14}C-export and photophosphorylation) returned to their pre-irradiation levels following a 2-hour post-irradiation recovery period (72). This relationship suggested that when photosynthesis was light saturated, excess photosynthetically-produced ATP was available and utilized for the vein loading of organic solutes. However, when photosynthesis was limited under low light conditions, the post-irradiation reduction in ^{14}C export was not evident, suggesting that under such low light conditions, the production of ATP through mitochondrial respiration, a process shown to be insensitive to the levels of ionizing radiation used in this study, was utilized for vein loading of photoassimilates (72).

The availability of energy for export of photosynthate may account, at least in part, for the contrasting results of Servaites and Geiger (93) and Nelson (78, 80) presented earlier in this Section. Since Nelson (78, 80) used a constant light intensity of 2000 ft-c. while varying the concentration of CO_2 to affect the rate of net photosynthesis, it is possible that photosynthetically-produced ATP was utilized preferentially in the reduction of assimilated CO_2, especially at the higher rates of net photosynthesis, thus providing an inadequate supply of energy for export and hence no increase in the amount of ^{14}C exported, despite the greater assimilation rate. Since photosynthesis is not light saturated at 2000 ft-c., some of the excessively produced photosynthate may be respired by the mitochondria to supply sufficient ATP to maintain the level of export. In contrast, Servaites and Geiger (93) simultaneously varied both light intensity and CO_2 concentration to increase rates of CO_2 fixation. This could enable the

supply of ATP to be appreciably greater than needed for the reduction of CO_2, even at higher CO_2 levels. Further, since all light intensities utilized by Servaites and Geiger (93) were greater than required to light saturate photosynthesis, the surplus photosynthetically produced ATP would then be available for active export of carbohydrate from the source leaf.

The site of active accumulation of carbohydrates for translocation is thought to be the sieve element-companion cell complex in the minor veins, the veins in closest contact with the photosynthesizing mesophyll cells of the leaf (29, 31, 32, 34, 35, 51). Using the technique of incipient plasmolysis, Geiger et al. (34) demonstrated that solute concentration in the sieve element-companion cell complex of the minor veins was about equal in the sieve elements and the companion cells, and about 3 to 4 times higher than in the adjacent phloem parenchyma cells, while leaf mesophyll cells, the site of photosynthesis, had solute concentrations only 50% greater than the phloem parenchyma cells.

In regards to the movement of translocatable materials from leaf mesophyll cells to the sieve elements of the minor veins, two pathways have been postulated. One pathway consists of movement of the organic solutes through the symplast (i.e., the region of bulk cytoplasm connected by plasmodesmata) from the mesophyll cells to the phloem companion cells. Tyree (110) studied the movement of solutes in various tissues and concluded that the plasmodesmata were of sufficient frequency and diameter to enable the symplastic movement of solutes to be the pathway of least resistance. Cataldo (17) proposed that photosynthetically produced sugars are taken into a transport compartment consisting of the endoplasmic reticulum and rapidly moved via the plasmodesmata to the phloem parenchyma cells bordering the leaf mesophyll cells. Active accumulation into the companion cells subsequently occurs, and sugars are finally deposited in the sieve

elements for long distance transport.

The second pathway consists of an apoplastic movement of organic solutes through the cell walls and intercellular spaces from the mesophyll cells to the minor vein phloem. This involves the expenditure of energy twice: once in crossing the plasmalemma of the mesophyll cells as the solutes exit into the apoplastic region, and secondly when the solutes enter the phloem cells where they are accumulated prior to transport. Brovchenko (15) demonstrated that both sucrose and hexoses are actively exported from mesophyll cells into the apoplast, with sucrose export utilizing photosynthetically produced ATP, whereas the export of hexoses was shown to involve the release of energy through oxidative processes. Furthermore, Brovchenko (15) found that absorption of sucrose and especially hexoses from the apoplast into the minor vein phloem was greatly increased by the addition of ATP, and that sucrose was more strongly retained by the phloem against "leaking back" into the apoplast.

Geiger et al. (35) utilized an isotope trapping technique to provide further evidence of the movement of sucrose into the apoplast prior to vein loading. Unlabelled sucrose was used as the trapping agent in a buffer solution circulating over the abraded upper surface of a leaf whose lower surface was exposed to $^{14}CO_2$. Turnover of the compound in the apoplast was measured by the exchange or trapping of the labelled (i.e. photosynthetically assimilated) sucrose by the unlabelled buffer solution. When the light intensity was increased, the rate of trapping of the ^{14}C-label by unlabelled sucrose increased to the same extent as the increase in translocation rate. Addition of ATP to the external solution increased the rate of translocation by approximately 75%, while trapping of labelled sucrose increased by approximately 66%. The authors concluded that the

turnover rate of sucrose in the free space increased directly with an increase in rate of translocation.

In fact, the possibility also exists that both the apoplast and the symplast may be involved in vein loading. Using data from isotope trapping experiments (35) and from the uptake of exogenous sucrose (98), Sovonick et al. (98) have suggested that assimilates destined for long-distance translocation move via the symplast within the mesophyll region and enter the apoplast at the mesophyll-phloem parenchyma interface prior to active accumulation into the symplast of the seive element-companion cell complex of the minor vein phloem.

The destination of assimilates loaded into the phloem does not occur in a random manner, for specific distribution patterns have been recognized (114). During the early period of development, a leaf, for example, initially imports organic solutes from other parts of the plant, including older leaves and/or storage tissues. Once the leaf reaches between one third and one half of its final size, it becomes self-supporting with respect to its carbon requirements, and subsequently the leaf becomes a net exporter of assimilates to other parts of the plant (40, 96, 105, 107). While the plant is in a vegetative growth phase, export is primarily towards the centres of active growth, such as the shoot apex, developing leaves, and root apices, but as the plant ages and major developmental changes occur, much of the exported assimilates may become diverted into reproductive or storage organs, such as the flowers, fruit, seeds and tubers (40, 114).

The distribution pattern is also influenced by leaf position, with the upper leaves of a plant normally supplying assimilates to the shoot apex, while lower leaves provide assimilates to the roots. Intermediate

leaves may export assimilates to both the shoot apex and the roots (79, 96, 105, 114). Predictably, then, as the plant grows, the young exporting leaf which initially supplies assimilates to the adjacent shoot apex will increase the proportion of exported assimilates which is translocated to the roots (105, 114). These general distribution patterns may also be modified by differences in the internal organization of the vascular system itself (52, 107, 114).

Most importantly, however, it would appear that the pattern of assimilate distribution is determined by the size or demand of the various sinks. Sink demand is largely determined by its metabolic rate, with a high rate of metabolism constituting a larger sink or a greater demand for assimilates. An increase in sink demand may result in a subsequent increase in the magnitude and rate of assimilate translocation, as well as a change in the distribution pattern of exported organic solutes (39, 45, 107, 112, 119). Modification of sink demand or source size through manipulation of either the plant or its environment has been shown to modify not only the magnitude of export from a source leaf, but also the pattern of distribution of photoassimilates between the competing sinks (33, 94, 95, 107, 108, 112, 119).

Seasonal changes have also been shown to have significant effects on the distribution of photoassimilates. In Pinus strobus (112) in spring, the old needles were the major source of ^{14}C photoassimilates, providing photosynthate to the young developing shoots and to the roots. However, by early summer (June), the new shoots had become the major site of photoassimilation, and hence the major source of assimilates for export, particularly to the roots, with translocation to the roots reaching a maximum during late summer and early fall (August to October).

In an attempt to reduce the metabolic activity of a sink, a young developing leaf (i.e., a leaf which is a net importer of assimilates) was shaded, resulting in a reduction in the rate of leaf growth and the final size of the leaf (108). Furthermore, the magnitude of assimilate import was also decreased. The next younger leaf, being unshaded, was a more dynamic sink, and as such was able to attract a greater supply of nutrients. Consequently, the growth rate and final size of the young leaf developing in the light immediately above a shaded developing leaf, was markedly increased. Similar results were obtained by subjecting a developing leaf to reduced CO_2 concentrations in the light, whilst the next younger developing leaf was supplied with normal CO_2 concentrations (108).

Of particular interest to the present study is the distribution of photoassimilates in leguminous plants in which, by virtue of the presence of bacterial nodules on the roots, the active assimilation of atmospheric nitrogen into organic compounds (i.e., the process of nitrogen fixation) constitutes an additional metabolic sink of immense significance.

In the process of nitrogen fixation, the reduction of atmospheric nitrogen to ammonia is catalyzed by the bacteroidal enzyme, nitrogenase. This process utilizes ATP, NADH (nicotinamide adenine dinucleotide, reduced form) and electrons, all of which are generated through bacteroidal respiration utilizing host plant photosynthate translocated to the nodules. Carbon skeletons, also generated by bacteroidal respiration are required in the formation of amino compounds which subsequently are made available to the host plant. Since the nodulated leguminous plant is capable of supplying a considerable proportion of its nitrogen requirements through the symbiotic fixation of atmospheric nitrogen, and since estimates of the carbohydrate required for the fixation process range from at least 7 to possibly 20 g for

each gram of atmospheric nitrogen assimilated (13, 48, 71, 74), it can be seen that the presence of bacterial nodules on the roots constitutes a major metabolic sink for photoassimilates.

Minchin and Pate (74) investigated the carbon and nitrogen budgets in shoots, roots and nodules of garden peas (Pisum sativum L.) during a 9-day period commencing 21 days after sowing. Plants inoculated with the bacterium Rhizobium were grown under conditions in which mineral nitrogen was absent from the nutrient medium, whereas non-inoculated plants were supplied with fertilizer containing nitrogen in the form of nitrate. Over the 9-day period in the nodulated plants, 26% of the photoassimilated carbon was retained in the shoot, 32% was translocated to the nodules and 42% to the roots. Of the carbohydrate translocated to the nodules, almost one third was respired by the bacteroids to generate ATP and reducing power for the assimilation and reduction of atmospheric nitrogen, while almost one half was returned to the shoot, via the xylem, as amino compounds formed following nitrogen fixation. In total, it was calculated that the nodules alone required about 10 g of carbohydrate for each gram of atmospheric nitrogen fixed.

In terms of respiratory efficiency, Minchin and Pate (74) found nodulated roots (i.e., roots plus nodules) fixing atmospheric nitrogen and non-nodulated roots assimilating nitrate-nitrogen to be very similar, each requiring about 15 g of carbyhydrate for each gram of nitrogen assimilated. While this high level of respiratory activity resulted in a considerable increase in CO_2 concentration of the soil atmosphere around the roots, Minchin and Pate did not determine the extent, if any, of dark fixation of CO_2 by the nodules (20, 65, 116), although malate and aspartate, possibly formed in carboxylation reactions in the nodulated roots, were transported to the shoot (74).

The energy requirements for nitrogen fixation in nodulated clover (Trifolium subterraneum L.) plants appear to be similar to the energy requirements for the assimilation of ammonium nitrate by the roots of non-nodulated plants (36). However, during the initial stages of nodule development, nodulated plants had a greater consumption of carbohydrates than did non-nodulated ones, the result being that nodulated plants were significantly smaller, especially with regard to shoot weight.

Using nodulated soybean plants grown with nitrogen-free nutrient solution, and non-nodulated plants regularly supplied with fertilizer nitrogen as nitrate, Nunn (84) also found that shoot weight was significantly reduced in nodulated plants at both 21 and 28 days after planting. While there was no effect on root weight, including nodules when present, at 21 days of age, there was a significant reduction in the fresh weight of roots plus nodules of the nodulated plant at 28 days of age when compared with the root fresh weight of the non-nodulated plants. When the extent of ^{14}C-export was assayed for the one hour period following $^{14}CO_2$ photoassimilation in the 28 day old plants, no difference in magnitude of export was noted between the two populations, with approximately 10% of the recovered ^{14}C being found outside the fed leaf. Although the total recovery from the subterranean parts of the plant was the same in the two populations--about 40% of the exported ^{14}C--in the nodulated population approximately equal amounts of ^{14}C were recovered from the roots and the nodules. Since root fresh weight was much greater than nodule fresh weight, nodular ^{14}C specific activity was very high, indicating that the nodules are indeed a more dynamic sink than the roots.

In contrast to Nunn (84) who reported approximately equal distribution of ^{14}C between the roots and nodules, Bach et al. (5) found that the roots

of soybeans contained almost 3 times as much of the exported ^{14}C as did the nodules. However, Nunn (84) utilized relatively young plants in which the processes of nodule initiation, development and growth were active, whereas Bach et al. (5) utilized much older plants in which nodule initiation and growth were less active. It is likely, therefore, that more of the exported ^{14}C may be incorporated into nodule structure in the younger plants.

It has frequently been observed that the onset of nitrogen fixation in legumes occurs subsequent to the development of the root nodules (36, 58, 76, 103, 106). Visible but inactive nodules are normally present on the roots of soybean plants 14 days after planting, while nitrogen fixation does not commence until 3 to 7 days later (58, 103). On the other hand, one study (76) did report that nodules which first appeared about the time the first trifoliate leaf was fully expanded, already contained considerable nitrogenase activity.

In soybeans, peak nitrogenase activity is normally attained about the time of flowering (5th week after planting) and maintained until about the 10th week after planting, after which there is a rapid decline in activity as the plant and nodules senesce (76). For example, in field grown soybean, Thibodeau and Jaworski (106) noted a rapid increase in nitrogen fixation from the onset of flowering until early pod-fill and, following a short period of peak activity to mid pod-fill, the rate of nitrogen fixation declined rapidly. Nitrogen supply during the early period of growth and development was provided by the assimilation and reduction of soil nitrate, with nitrate reduction and nitrogen fixation being successive events (106). In contrast to the soybean studies, Lawrie and Wheeler (63) found that in peas, the decline in rate of nitrogen fixation commenced around the time of flowering.

In soybeans, the decline in nitrogenase activity (59, 60, 76, 106) occurs at a time when the developing fruits constitute a greater metabolic sink for leaf photoassimilates than do the nodules (57, 60). The nodules being deprived of adequate supplies of carbohydrate, not only for nitrogen fixation, but also for growth and maintenance of their structural integrity, senesce rapidly. The high demands of the fruit for carbon and nitrogen not only contribute to the breakdown of nodule structure but also contribute to the breakdown of leaf protein (106) causing, in fact, the rapid senescence of the whole plant.

Removal of the developing flowers or fruits has been shown to prolong the period of nitrogen fixing activity (41, 60, 63, 91) thus supporting the view that nodule activity is dependent upon the availability of photosynthate. It would appear, in fact, that it is recent photosynthate which is particularly required. For example, although Lawrie and Wheeler (62) found a marked correlation between the accumulation of photoassimilated ^{14}C in the nodules and the rate of acetylene reduction in continuously illuminated pea plants, when the plants were darkened following photoassimilation of $^{14}CO_2$, photosynthate continued to accumulate in the nodules, while nitrogenase activity declined. The data suggested that it is the recent photosynthate which is required for nitrogen fixation and that reserve materials may not significantly affect nitrogenase activity when the supply of photosynthate to the nodules is interrupted. Supporting the above interpretation is the observation that diurnal fluctuations occur in nitrogenase activity, with a significant reduction in activity occurring within two hours of the onset of darkness, in soybean, pea and subterranean clover plants (38, 41, 43, 117).

Consistent with the view that carbohydrate availability to the nodules is essential for nitrogen fixation, is the observation that increasing the supply of carbohydrate to the nodules increases nodule activity. For example, Bach et al. (5) found that supplying excised sliced soybean nodules with sugars enhanced nitrogen fixation by 30 to 60%, irrespective of whether sucrose, glucose or fructose was added.

From measuring rates of photosynthesis, primary productivity (i.e., plant dry weight data) and nitrogenase activity in nodulated pea plants grown at different light intensities, Bethlenfalvay and Phillips (10) found that with increasing light intensity, the plant dry weight, nitrogen content and nodule mass increased. Greater photosynthetic productivity at higher light intensities was correlated with enhanced nitrogenase activity, and both CO_2 uptake and N_2 uptake plots could be extrapolated to the light compensation point. Their data indicated that reduction of N_2 was related directly to concurrent CO_2 uptake and reduction, rather than to total plant productivity.

Increasing the size of the source of photoassimilates by grafting a second shoot onto a nodulated root increased nitrogen fixing activity in the nodules (102). In contrast, reducing source size relative to the sinks by partial or total defoliation reduced nitrogen fixation by the nodules (38, 41, 44). These studies were interpreted to show the dependency of nodule activity on the availability of recent photosynthate.

Recently, Hardy and Havelka (43) have utilized a CO_2-enriched atmosphere to increase photosynthetic production of field grown soybean plants in order to investigate the effect of increased photosynthate supply on nitrogen fixation. Nodulated soybeans were enclosed in open-topped "Mylar" containers and supplied with 800 to 1200 ppm CO_2 during daylight

hours during a period beginning prior to flowering (38 days after planting) and continuing through to maturity (101 days after planting). At the time of harvest, the CO_2-enriched plants had fixed 842 mg of nitrogen, accounting for over 80% of the total nitrogen in the plant, while the plants exposed to normal CO_2 concentrations fixed only 167 mg, being self-sufficient for only 26% of their nitrogen requirement. Nitrogen fixing activity per milligram of nodule tissue, and nodule mass per plant, were approximately doubled in the CO_2-enriched plants, as compared to the controls. More importantly, the period of exponential increase in rate of nitrogen fixation was increased by 8 days and consequently there was a delay in the onset of the rapid decline in nitrogenase activity during the period of fruit maturation.

In a somewhat similar study, Phillips et al. (85) investigated the short-term and long-term effects of CO_2-enrichment on nitrogenase activity in peas (Pisum sativum L.). For the short-term study, plants were grown at 320 ppm CO_2 for 4 weeks and then exposed to 1200 ppm CO_2 for 30 hours. Nitrogenase activity, as determined by the acetylene reduction assay, doubled at about 5 hours after the beginning of enrichment, and this elevated rate was maintained until the experiment was terminated. A second group of plants was exposed to 1200 ppm CO_2 from the time of planting to the age of 4 weeks. Compared to plants grown at 320 ppm CO_2, the 4-week period of CO_2-enrichment resulted in increased growth of the plants and an increase in nodule mass and nodule number per plant. Nitrogenase activity per milligram nodule did not increase, however, with increased CO_2 concentration, although total nitrogen content of the plant increased by almost 60%. Since the increase in nitrogen content was directly proportional to the increase in nodule mass, it would appear that the excess photosynthate

reaching the nodules under the long-term CO_2-enriched conditions was being stored as starch granules rather than being metabolized to increase the specific activity of nitrogenase in the nodules. This interpretation was also verified by morphological studies.

The results of Hardy and Havelka (43) and Phillips et al. (85) appear to be contradictory to some extent. The time of initiation of CO_2-enrichment may contribute to this discrepancy, since Hardy and Havelka (43) initiated CO_2 enrichment at the time of flowering when a pattern of development had already been established, while Phillips et al. (85) began CO_2 enrichment at the time of planting and concluded their experiments within 28 days (i.e., prior to flowering). Phillips et al. (85) also suggested that these differences may have been accounted for by the presence of soil nitrogen which would be expected to inhibit further nodulation of the field-grown soybeans used by Hardy and Havelka (43). Unfortunately, Hardy and Havelka did not record the number of nodules. With peas grown in the absence of soil nitrogen, long-term CO_2-enrichment resulted in an increase in total nitrogenase activity through an increase in nodule number and mean mass per nodule, while short-term CO_2-enrichment enhanced nitrogenase activity of individual nodules (85).

Since the root nodules constitute a major metabolic sink for leaf photosynthate, especially during the vegetative phase of legume growth and development, alterations in source-sink distribution patterns of photo-assimilated ^{14}C may be anticipated in response to factors which affect nodule mass, nodule number, or nodule activity. One of the important environmental factors which has been shown to affect the nodule component, and hence the distribution of photosynthate, is the presence (or absence) of fertilizer or soil nitrogen (47, 59, 76).

The nitrogen requirements of the legume plant may be filled either by the process of nitrogen fixation utilizing the bacterial enzyme nitrogenase, or by the uptake of fixed nitrogen--usually as nitrate (which must then be reduced to ammonia by the enzymes nitrate reductase and nitrite reductase in the plant) but also as ammonium or urea--from the soil. Soil nitrate is a residual component of most agricultural soils, being formed by bacterial breakdown of organic residues, and is often supplemented with fertilizer applications prior to or during the early stages of legume growth and development.

Although well-nodulated legume plants can grow well in the absence of fixed nitrogen, maximum yields are not obtained unless fixed nitrogen is present in the soil. However, the addition of fixed nitrogen tends to substitute for, rather than supplement, symbiotic nitrogen fixation.

For example, Allos and Bartholomew (1) grew several species of legumes supplied with 0 to 800 mg of nitrogen as ammonium over a period of 10 weeks. In all cases, the dry weight of the shoots and roots at the end of the 10 week growth period increased with increasing supply of combined nitrogen, and total nitrogen content of the plant tissues also increased. By labelling the ammonium-nitrogen with ^{15}N, an estimate of the relative contributions of nitrogen fixation and fertilizer uptake was obtained. In all cases, the percent of nitrogen supplied by symbiotic fixation decreased with increasing rates of fertilizer nitrogen. Ammonium-nitrogen supplied in excess of that required by the increase in plant growth directly replaced symbiotically fixed nitrogen. It is interesting to note that with the lowest rate of fertilizer nitrogen, 80 mg over 10 weeks, the increase in plant growth and consequent need for nitrogen resulted in nitrogen fixation being increased over that obtained with the non-fertilized control plants.

Combined nitrogen in the soil is readily available to field-grown plants. In one study comparing rates of nitrate uptake, leaf nitrate reductase activity and nodule nitrogenase activity of soybeans throughout the growing season, Thibodeau and Jaworski (106) observed that nitrate reductase activity was high early in the season when the rate of nitrate uptake from the soil was high, but that about the time of flowering, nitrate reductase activity declined dramatically. At the same time, an equally dramatic increase in nitrogenase activity occurred in the nodules. This reciprocal relationship between nitrate reductase activity and nitrogenase activity suggested that these processes are highly competitive with regard to their energy requirements. As the nodule mass develops, more photosynthate is translocated to the nodules, thus reducing the uptake of soil nitrate by the root and its subsequent reduction in the leaf to ammonia.

Although the enzyme nitrate reductase is primarily localized in the leaf (46, 71, 73, 106), nitrate reductase activity has also been demonstrated in legume root nodules. Rigaud (89) reported that when anaerobic preparations of French bean (Phaseolus vulgaris L.) bacteroids were incubated with nitrate, nitrate reductase activity was observed 10 to 12 hours later. Bacteroids of Rhizobium lupini isolated from lupin (Lupinus luteus L.) plants grown in the absence of fertilizer nitrogen were also shown to reduce nitrate to nitrite during a 10 minute incubation period (90).

Small amounts of fertilizer nitrogen appear to be beneficial to overall plant growth, particularly during the early stages of growth and development prior to the onset of symbiotic nitrogen fixation. Hatfield et al. (47) provided soybean plants, grown from inoculated seed, with nitrate-nitrogen for up to 6 weeks after planting. Although the nodulated plants receiving no nitrate or nitrate only for the first 2 weeks after planting were

significantly lower in stem and leaf dry weights, the nitrogen content of the stems and leaves, when expressed as a percent of the dry weight, was not affected.

Of considerable interest was their observation that when nitrate was provided for only 2 weeks, the number of nodules per plant was 39, whereas non-fertilized control plants and plants supplied with nitrate for 4 weeks had about 26 nodules per plant. In each of these 3 treatments, the mean weight per nodule was about 3 mg. However, when nitrate-nitrogen was supplied for 6 weeks, the number of nodules was reduced to less than 5 per plant and mean nodule weight was less than 2 mg. Since grafting techniques (61) have indicated that the control of nodule initiation lies within the root, while the extent of nodule development, that is, nodule fresh weight, is determined by photosynthate supply from the shoots, it would appear that with the 6 week fertilization period, an alteration in the distribution pattern or extent of export of leaf photosynthate was induced which was not apparent during shorter fertilization periods (47).

To investigate the relationship between nodulation, translocation patterns and fertilization, Russell and Johnson (92) examined the distribution of ^{14}C-photosynthate in 30-day old soybean plants in which the degree of nodulation was affected by the level of ammonium nitrate supplied from the 13th day after planting. At the highest level of fertilizer nitrogen, nodulation was completely inhibited so that this population was completely dependent upon assimilation of inorganic nitrogen from the nutrient solution. Partial inhibition of nodulation was obtained at the intermediate level of fertilizer nitrogen, and nitrogen was obtained through both the symbiotic process of nitrogen fixation and the absorption of nitrogen from the nutrient solution. When no fertilizer nitrogen was supplied, plants were

fully nodulated and were completely dependent upon the symbiotic process for their nitrogen requirements.

In their study (92), the degree of nodulation was found to affect both the magnitude of export and the distribution of ^{14}C-photosynthate. Plant leaves totally dependent upon symbiotically fixed nitrogen exported 14% more ^{14}C-photosynthate than did leaves of plants where nodulation was completely inhibited by application of fertilizer nitrogen, while partial inhibition of nodulation by fertilization resulted in an intermediate level of export. One hour after the termination of $^{14}CO_2$ photoassimilation, the roots and nodules of fully nodulated plants contained 57% and 4%, respectively, of the exported ^{14}C. Where nodulation was partially inhibited by nitrogen fertilization, the roots contained 59% and nodules only 1% of the exported ^{14}C, while the roots of plants where nodulation was completely inhibited contained approximately 43% of the exported ^{14}C. The dry weights of the roots of nodulated plants were significantly higher than in non-nodulated plants, thus accounting, at least in part, for the higher ^{14}C export to the root tissue. Rapid turnover of carbon in the nodules, and subsequent export of amino compounds to aerial sinks, resulted in the recovery of ^{14}C from the nodules being at a relatively constant, but low, level. Unfortunately, rates of nitrogen fixation were not determined.

The effects of fertilizer nitrogen on ^{14}C translocation and on nitrogen fixation, as determined by the acetylene reduction assay, have been investigated by Latimore et al. (59). Fertilizer nitrogen was supplied either as ammonium (NH_4^+) or nitrate (NO_3^-) from the time of planting or at 10 days prior to sampling. $^{14}CO_2$-photoassimilation and acetylene reduction assays were carried out during the vegetative, flowering and pod-fill stages of growth and development. Recovery of ^{14}C from the nodules was reduced by

the application of fertilizer nitrogen either throughout the growing period or at 10 days prior to sampling. However, distribution of ^{14}C amongst the other plant parts was not influenced by the nitrogen fertilizer treatment. The rate of nitrogen fixation (acetylene reduction) was greatly decreased by the presence of inorganic nitrogen, with nitrate-nitrogen generally causing a greater reduction than ammonium-nitrogen. Latimore *et al.* (59) suggested, therefore, that while both sources of inorganic nitrogen were equally effective in reducing the energy flow to the nodules, nitrogenase activity was affected to a greater extent by nitrate-nitrogen than by ammonium-nitrogen. Very little ^{14}C was exported to the nodules, and reduction of acetylene was low, when the developing pods became a significant metabolic sink for leaf photosynthate.

Utilizing nodulated pea (*Pisum sativum* L.) plants, Mahon (70) reported a decrease in the rate of acetylene reduction in the presence of ammonium nitrate, with a concommitant decrease in the rate of root and nodule respiration. By comparing fertilized with non-fertilized plants, Mahon concluded that the component of respiration specifically associated with nitrogenase activity was affected by the presence of fertilizer nitrogen, whereas the growth and maintenance components of respiration were relatively unaffected. Further work (71) confirmed that nitrate was more effective than ammonium in decreasing rates of acetylene reduction and root and nodule respiration. Mahon (71) also reported that while nitrogen fixation occurs in the nodules and consequently contributes to the root and nodule respiration being greater in nitrogen-fixing plants, the reduction of nitrate occurs predominantly in the shoots utilizing reducing equivalents generated either directly or indirectly in photosynthesis. Therefore, not only would the presence of inorganic nitrogen (especially nitrate) have an inhibitory

effect on one metabolic sink (i.e., the root nodules), but would also stimulate another competing sink for photoassimilates and reducing power. This alteration in activity of metabolic sinks would be expected to affect the distribution of photoassimiates within the plant.

While competition for photoassimilated carbon compounds between the processes of nitrate reduction and nitrogen fixation is of major importance, the inhibitory effect of nitrate reductase on nitrogenase activity may also be dependent, at least in part, on competition for common components of the two enzyme systems. Nitrate reductase consists of two sub-units, one being a nitrate-inducible subunit, and the other being a molybdenum-containing protein. This protein is apparently identical to a protein subunit of nitrogenase (27), since functional nitrate reductase in Neurospora crassa has been obtained following substitution of the molybdenum-containing protein subunit with that from soybean bacteroid nitrogenase (28). In the presence of nitrate, the nitrate-inducible subunit may combine preferentially with the available molybdenum-containing subunit, resulting in a reduction in nitrogenase synthesis (28). Also, the synthesis of leghemoglobin, the molecule involved in oxygen transport to the bacteroids, may also be affected, since a recent report (83) has indicated the presence of a heme moeity in nitrate reductase.

Much of the translocation work on nodulated legumes has focussed on assimilate distribution in relatively mature plants in which nodule bacteria are actively fixing atmospheric nitrogen. With such plants, the distribution of photoassimilates and rate of nitrogen fixation are significantly affected by the presence of inorganic combined nitrogen. However, there has been relatively little work done on the effects of fertilizer nitrogen on distribution of photosynthate during the period of nodule initiation and

development up to the initial period of nitrogen fixation. The present study was designed, therefore, to investigate the magnitude of export and distribution of ^{14}C-photosynthate in nodulated soybeans provided with fertilizer nitrogen as nitrate, for various time periods during the early stages of growth and development. Since the earlier studies on legume plants in our laboratory were carried out using non-nodulated soybeans, initially a study was undertaken to analyze the growth and development of both nodulated and non-nodulated soybeans when grown in the presence or absence of fertilizer nitrogen.

3. Materials

The soybean plants (Glycine max (L.) Merr. cv. Harosoy 63) used in this study were grown from seed provided by the Agriculture Canada Research Station, Harrow, Ontario.

Seeds were planted, one to three seeds per 12 cm diameter plastic pot, at a depth of approximately 1.5 cm in "Terra-Lite" horticultural grade vermiculite. To obtain nodulated plants, seeds were coated with a dry, commercially-available inoculum of Rhizobium japonicum (Legume-Aid, Agricultural Laboratories, Inc., Columbus, Ohio, U. S. A.) before sowing. Growth conditions and fertilization procedures are described in the methods section.

Nutrient solutions (0.5 normal) were prepared according to the method of Hoagland and Arnon(53), and were either nitrogen free (-N) or contained nitrogen as nitrate (+N). Stock solutions were prepared from analytical grade reagents and glass distilled water. Glass distilled water was also used in the preparation of the final solution from the stock solutions (see Table 1).

For the experiments on the export and distribution of photosynthetically assimilated carbon compounds, the $Na_2{}^{14}CO_3$ (specific activity 60 mCi/mmole) and the scintillation fluid (Aqueous Counting Scintillant) were both purchased from Amersham-Searle Ltd.

Table 1. Composition of the nitrogen free (-N) and nitrogen containing (+N) nutrient solutions applied to the soybean plants.

Compound	Molecular weight	Concentration of stock solution (M)	Concentration of stock solution (g/l)	Volume of Stock solution per litre of final solution (ml)
A. Nitrogen-free (-N) solution				
K_2SO_4	174.27	0.5	87.14	2.5
$MgSO_4$	120.37	1.0	120.37	1.0
$Ca(H_2PO_4)_2 \cdot H_2O$	252.08	0.05	12.60	5.0
$CaSO_4$	136.14	0.01	1.36	100.0
Fe citrate			5.00	0.5
Combined trace elements*				0.5
B. Nitrogen-containing (+N) solution				
KH_2PO_4	136.09	1.0	136.09	0.5
KNO_3	101.11	1.0	101.11	2.5
$Ca(NO_3)_2 \cdot 4H_2O$	236.15	1.0	236.15	2.5
$MgSO_4$	120.37	1.0	120.37	1.0
Fe citrate			5.00	0.5
Combined trace elements*				0.5

* C. Combined trace elements solution

Compound	Molecular weight	Concentration in stock solution (mM)	Concentration in stock solution (g/l)	Trace element supplied	Final concentration of trace element (µl/l)
H_3BO_3	61.84	46.2	2.86	B	0.250
$MnCl_2 \cdot 4H_2O$	197.91	9.2	1.81	Mn	0.250
$ZnSO_4 \cdot 7H_2O$	287.55	0.8	0.22	Zn	0.025
$CuSO_4 \cdot 5H_2O$	249.71	0.3	0.08	Cu	0.010
$H_2MoO_4 \cdot H_2O$**	(180)	0.1	0.02	Mo	0.005

** Assaying 85% MoO_3

4. Methods

The initial experiments in this study were designed to investigate the effects of nitrate fertilization on the growth and development of nodulated and non-nodulated soybean plants. Subsequent experiments were conducted to investigate the effects of fertilizer nitrate on the magnitude of export of photosynthetically assimilated carbon, and on the distribution of carbon compounds throughout the plant. Rates of net photosynthesis were also determined. The procedures used consisted of:

(i) Assessing the growth and development of the plant through measurements of shoot height, fresh and dry weights, and nodule number.

(ii) Analysis of the export and distribution of ^{14}C following the photo-assimilation of $^{14}CO_2$ by a mature trifoliate leaf.

(iii) Measurement of the rate of net photosynthesis utilizing infra-red gas analysis.

4.1 The growth and development of soybean plants--both inoculated and non-inoculated--in the presence and absence of nitrate fertilization

Inoculated (+R) and non-inoculated (-R) seeds were sown (80 pots of each) as described in the Materials section in September 1976, and placed in a greenhouse at the Horticultural Research Institute of Ontario, Vineland Station, Ontario. The temperature was maintained at 21-24°C both day and night, and a light intensity of approximately 1600 ft-c. at the pot level was provided for a 16 hour daylength. Natural sunlight was supplemented by light from a bank of 30 Gro-Lux fluorescent bulbs. Plants were watered twice daily with tapwater, and, beginning on the seventh day, the plants were fertilized thrice weekly with 0.5 normal nutrient solution (see Materials section) either with (+N) or without (-N)

nitrogen. At weekly intervals, beginning on the seventh day, all plants in each of the four populations (-R-N, -R+N, +R-N, +R+N) were measured for total height. Also beginning on day 7 and continuing weekly thereafter, five plants were selected at random from each population for more detailed examination. After the shoot height was measured, the root was carefully separated from the rooting medium and gently rinsed in tap water, following which the numbers of nodules visible on the roots was determined. The plant was then divided at the level of the uppermost lateral root into shoot and root fractions, and the fresh weights of each determined. Dry weights of the two fractions were obtained by initially placing the plant material in a seed drier which was maintained at a temperature of 30-35°C with a continuous moving air stream for at least two weeks. The plant material was further dried in an oven at 70°C for 12 hours, after which it was allowed to cool in a dessicator immediately prior to weighing. Dry weights reported are the means of two measurements taken at one week intervals. This experiment was terminated at the end of the sixth week by which time flowers were open on plants in all populations except the -R-N population.

In all subsequent experiments, plants were grown from inoculated (+R) seed. The pots were placed in the greenhouse at Brock University for a seven day germination period. During this time, plants were watered with tapwater at least once daily. Natural daylength was supplemented by a row of Sylvania 60 watt fluorescent bulbs which supplied approximately 700 ft-c. at the pot level for a minimum daylength of 14 hours. However, actual daylength, maximum light intensity, and maximum and minimum temperatures varied with the season and prevailing external weather conditions. On the seventh day, plants were transferred to a controlled environment growth

chamber where they were maintained until utilized on day 21.

4.2 Distribution of ^{14}C following the photoassimilation of $^{14}CO_2$ by a mature trifoliate leaf

For the initial translocation study, 7 day old plants were placed in a controlled environment growth chamber (Colmat Environmental System Model 255-6) in which the photoperiod was 16 hours light (0600-2200 h) and 8 hours dark, with temperatures of 25°C and 18°C respectively. The light intensity, measured at the pot level, was approximately 750 ft-c. and illumination was provided by eight 60 watt fluorescent lamps supplemented by two 60 watt rough surface incandescent bulbs. Plants were watered daily with tapwater and four times weekly approximately 150 ml of the appropriate nutrient solution were added to each pot. In this experiment two fertilizer treatments (-N and +N) were applied.

In subsequent translocation studies, plants were thinned to one plant per pot. The controlled environment growth chamber (Controlled Environment Chamber Model CEL 37-14) was also maintained on a photoperiod of 16 hours light (0600-2200 h) and 8 hours dark, but the day and night temperatures were 25°C and 20°C, respectively. In this chamber, illumination which was supplied by eighteen Sylvania Cool-White fluorescent bulbs supplemented by twelve 60 watt incandescent bulbs, provided a light intensity of approximately 3000 ft-c. at the pot level. Plants were watered daily with tap water and four times weekly plants were supplied with approximately 150 ml per pot of the appropriate nutrient solution. Four fertilizer treatments were imposed as follows:

In two treatments, plants were fertilized with either +N or -N for the entire period from the 7th to the 21st day. In the other two treatments

the N-containing nutrient solution was applied either from the 7th day to the 13th day, or from the 14th day to the 21st day. The treatments are shown in Table 2. The plants, used at 21 days after planting, consisted of two primary leaves, a fully mature trifoliate leaf and a second developing trifoliate which was approximately one half to two thirds the length of the first mature trifoliate.

4.2.1 Photoassimilation of $^{14}CO_2$

The ^{14}C was introduced into the plant by liberating $^{14}CO_2$ into a closed system for a period of 5 minutes (Expt. 1) or 15 minutes (Expt. 2). The closed system consisted of the following components: a transparent rectangular plexiglass leaf chamber; an electric motor driven diaphragm pump (Universal Electric Co. Model AAIE 122); a gas flow meter (Gilmont Model L95); a 50 ml mixing chamber; and a glass reaction vessel with a rubber cap for the generation of $^{14}CO_2$ from the addition of lactic acid, by syringe through the cap, to $Na_2{}^{14}CO_3$. All of the components were connected with "Tygon" tubing fitted with ground glass joints which were sealed using Dow-Corning stopcock grease and secured with elastic bands. Total volume of the system was approximately 0.25 ℓ and the gases were circulated through the system at a rate of 2.8 ℓ/min.

One hour prior to the liberation of $^{14}CO_2$, plants were removed from the controlled environment growth chamber, watered, and placed under a light intensity of 2800 ft-c. (measured at the level of the mature trifoliate leaf) in an illumination chamber. Illumination was provided by twelve 20 watt Cool-White fluorescent lamps supplemented by twelve 150 watt Reflector Flood incandescent bulbs controlled by adjustable rheostats. The light was filtered through a flowing water shield, 5 cm

Table 2. Patterns of nitrate fertilization utilized.

Treatment	Days after planting		
	0-6	7-13	14-21
-N-N	-N*	-N	-N
-N+N	-N*	-N	+N
+N-N	-N*	+N	-N
+N+N	-N*	+N	+N

* From day 0 to day 6, plants in all populations were supplied with tapwater only. Application of nutrient solutions began on day 7. See text.

in depth. The temperature in the illumination chamber was maintained at approximately 25°C.

The three leaflets and a portion of the petiole of the mature first trifoliate leaf were enclosed in the plexiglass leaf chamber 15 minutes before the liberation of $^{14}CO_2$. For this 15 minute period, the system was open allowing laboratory air to circulate over the enclosed leaf. The system was then closed and immediately 1 ml of 8 M lactic acid was added by syringe to 10 μCi of sodium-^{14}C-carbonate contained in the reaction vessel. The leaf and petiole inside the leaf chamber (later referred to as the fed leaf) were allowed to photoassimilate the liberated $^{14}CO_2$ for 5 or 15 minutes (depending on the experiment), after which time the system was opened and laboratory air flushed through the system for 45 or 55 minutes, again depending on the experiment. The duration of the photo-assimilation and post-assimilation periods will be given with each of the experiments in the Results section.

4.2.2 Extraction of the ethanol-soluble components and determination of the ^{14}C radioactivity

At the conclusion of the post-photoassimilation period, the chamber was removed from the leaf and the plant was immediately sectioned with a razor-blade into the following parts:

Part 1. The stem (shoot) apex, the developing trifoliate leaf and the stem above the node of the fed leaf.

Part 2. The fed trifoliate leaf, including that portion of the petiole present inside the leaf chamber.

Part 3. The remainder of the petiole of the fed leaf.

Part 4. The stem below the node of the fed leaf, including the primary

leaves and the cotyledons, when present.

Part 5. The roots, separated from the stem at the level of the uppermost lateral root.

Part 6. The root nodules.

Each section was weighed using a Mettler Type H6 balance and then immediately immersed in 50 ml of boiling 80% aqueous ethanol (v/v). After boiling until the volume had been reduced by approximately one half, the ethanol solution was decanted and the procedure repeated with an additional 50 ml of boiling 80% ethanol. The volume of the combined extracts for each plant part was measured, and the ^{14}C radioactivity determined by counting duplicate 100 µl aliquots in 15 ml ACS (Aqueous Counting Scintillant) in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3310).

4.2.3 Determination of the magnitude of ^{14}C export and distribution pattern of exported ^{14}C

Once the ^{14}C radioactivity in each part of the plant had been assayed, it was possible to determine the total ^{14}C recovered from outside the fed leaf, and to express this value as a percent of the total ^{14}C recovered from the plant. This, then, represents the percent of recovered ^{14}C exported from the source region. Similarly, the amount of ^{14}C radioactivity contained in each plant part outside the fed leaf, when expressed as a percent of the total exported ^{14}C, was used to give an indication of the distribution pattern of the exported ^{14}C photosynthate.

4.3 Measurement of the rate of net photosynthesis

The rate of net photosynthesis was determined by measuring the rate of depletion of CO_2 in a closed system. In this case, the closed system

consisted of: a transparent rectangular plexiglass leaf chamber; an electric motor driven diaphragm pump; a gas flow meter; and an infra-red gas analyzer (Beckman Model 215-A) to monitor the concentration of CO_2 in the circulating air. The volume of the system was either 260 ml or 174 ml, depending on the size of the leaf chamber utilized. Gases were circulated through the system at a rate of 2.8 ℓ/min.

The infra-red gas analyzer (connected to a Perkin-Elmer recorder) was calibrated with purified nitrogen gas to give a reading of zero, and with gas mixtures containing 270 μℓ/ℓ and 315 μℓ/ℓ CO_2 in air to give the recorder values of 61.5 and 69.0, respectively. All analyzed gases were purchased from the Linde Corporation. The rate of net photosynthesis was then calculated from the rate of depletion of CO_2 from 315 μℓ/ℓ to 285 μℓ/ℓ (i.e., from 69.0 to 64.0 on the recorder chart paper), which represented a mean concentration of 300 μℓ/ℓ.

For each determination, the mature first trifoliate leaf was sealed in the leaf chamber and laboratory air was flushed through the system for 10 minutes. A light intensity of 2800 ft-c. was provided by the illumination chamber described earlier. Following the 10 minute preconditioning period, the system was closed and depletion of CO_2 was monitored and recorded. A minimum of four depletions were carried out, with an average of the last three serving as the reported value. Between depletions, the system was flushed with laboratory air. Upon completion of the four depletions, the test leaf and petiole enclosed within the leaf chamber were excised and the fresh weight determined. In this thesis, the rate of net photosynthesis is reported in units of mg CO_2 removed per hour per gram leaf fresh weight. When the rate of net photosynthesis was determined on the same plants that were subsequently used in translocation studies, the

rate was determined 10 minutes after the leaf was sealed in the leaf chamber and hence immediately prior to the liberation of $^{14}CO_2$.

4.4 Statistical analysis

The data obtained were analyzed using the standard analysis of variance for factorial experiments. This analysis allows for the testing of the main effects of each factor (e.g., factor A, factor B) as well as the interaction between factors (i.e., A x B interaction). The interaction represents the extent to which the effects of one factor change at the different levels of another. The mean standard error was determined using the formula:

$$S_{\bar{x}} = \left(\frac{E.M.S.}{n} \right)^{\frac{1}{2}}$$

where $S_{\bar{x}}$ is the mean standard error, E.M.S. is the error mean square derived from the analysis of variance, and n is the number of observations in the mean. The $S_{\bar{x}}$ value was used in Duncan's New Multiple Range Test (99) for calculating a "...set of significant differences of increasing size, the size [depending] upon the closeness of the means after ranking, with the smallest value for adjacent means and the largest for the extremes". Each treatment mean was compared with every other treatment mean, and significant differences between means were identified.

Since in the Duncan's Test all treatments and components are considered together, differences between components with small values are overwhelmed by those between components having larger values. This is especially true in the case of nodule fresh weights. Consequently, the nodule fresh weight component was analyzed using Student's 't' test (99) to test the hypothesis that $\mu_1 = \mu_2 = \ldots = \mu_n$, where $\mu_1, \mu_2, \ldots, \mu_n$ are the means of the

populations from which the samples are drawn.

All calculations were performed on a Wang 720C programmable calculator. In this thesis, significance is reported at the 5% level.

5. Results

The results presented in this thesis come from three groups of experiments. The initial results were obtained from an experiment in which soybean plants, either inoculated or non-inoculated with Rhizobium japonicum, were grown either with the addition of fertilizer nitrogen as nitrate, or with nitrogen-free nutrient solution. In this experiment, the extent of growth and development was monitored as well as the extent of nodulation. In the second group of experiments, the magnitude of export of photoassimilated ^{14}C and the distribution pattern of ^{14}C were determined in 21 day old soybean plants grown from Rhizobium-inoculated seeds in the presence or absence of nitrate fertilization. The final results were obtained from a group of experiments in which the magnitude of ^{14}C export and the distribution pattern were determined in nodulated 21 day old soybean plants in which the dates of initiation and termination of nitrate fertilizer applications were varied. Rates of net photosynthesis were also determined.

5.1. The growth and development of soybean plants--both inoculated and non-inoculated--in the presence and absence of nitrate fertilization

In this experiment, 4 populations of plants were grown in the greenhouse for a 42 day period in the fall of 1976. Two of the populations were inoculated with Rhizobia (+R) and two were not (-R). As well one of the inoculated populations and one of the non-inoculated populations received a fertilizer solution containing nitrogen as nitrate (+N). The other population in each case received nitrogen-free nutrient solution (-N). The four populations are identified as -R-N (non-inoculated and without fertilizer nitrogen), -R+N (non-inoculated and with nitrate fertilization), +R-N (inoculated and without fertilizer nitrogen), and +R+N (inoculated and

with nitrate fertilization). Measurements were taken at weekly intervals, commencing of the 7th day after sowing, and terminating on the 42nd day, at which time flowers were open on plants of all populations except the non-inoculated, non-nitrogen-fertilized population (i.e., -R-N).

In Figure 2, the height of the sampled plants is recorded through a 42-day period of growth after planting. These data, as well as an indication of those differences statistically significant at the 95% level of confidence (i.e., $p = 0.05$), are also presented in Table 3.

It can be seen from Figure 2 and Table 3 that, during the first two weeks, the mean height of plants in the four populations were not significantly different. After the second week, however, the mean height of plants in the -R-N population was always significantly less than plants in the other three populations. It is also interesting to note that the mean heights of plants receiving nitrate fertilization (+N) were not significantly different during the last four weeks of the experiment, irrespective of whether the plants were inoculated (+R) or not (-R), and that during the third and fourth weeks, the height of nitrate-fertilized non-nodulated plants (-R+N) was not significantly different from the nodulated plants which did not receive fertilizer nitrogen (+R-N). The population with the largest mean height at the termination of this experiment was the nodulated, non-nitrogen-fertilized population (i.e., +R-N).

A second assessment of vegetative growth was obtained from measurements of root and shoot weights. Fresh and dry weight data for the shoot and root fractions are presented graphically in Figure 3 and in tabular form in Tables 4 and 5.

From Figure 3A, it can be seen that the plants in the -R-N population showed the lowest shoot fresh weights after the second week (Table 4), and

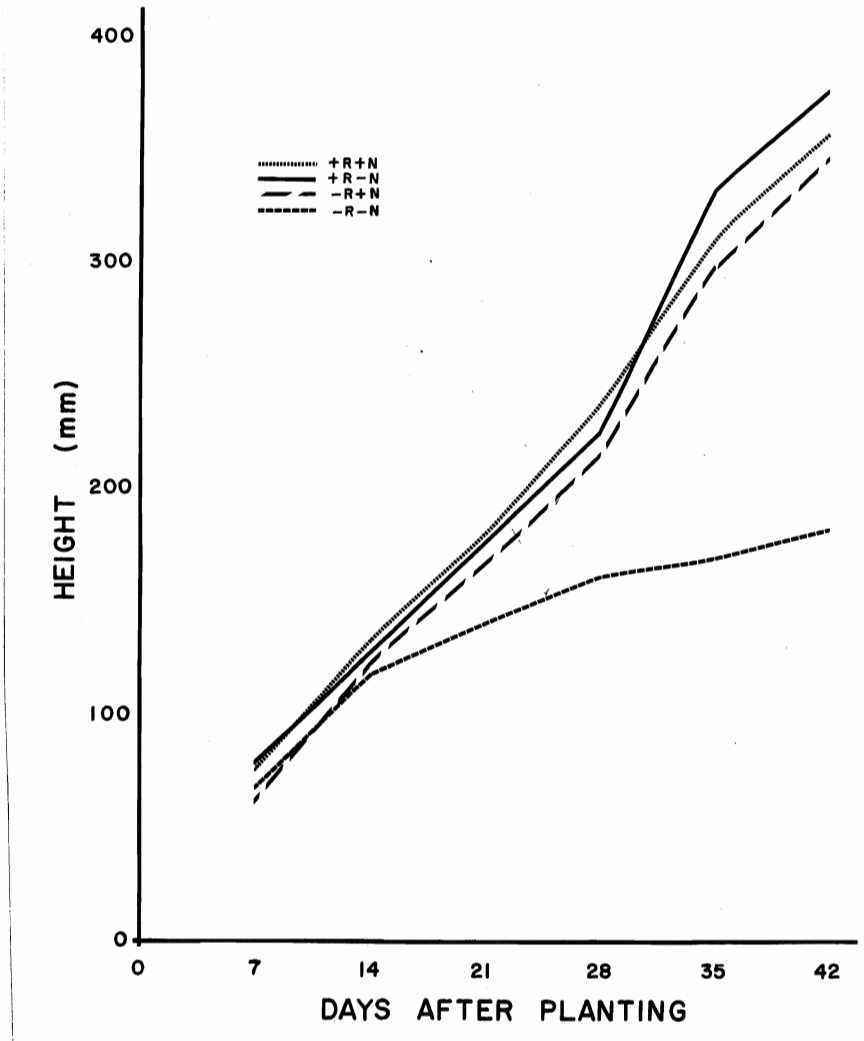


Figure 2. Height of soybean plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

Table 3. Height of soybean plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

Days after planting	Height (mm)			
	-R-N	-R+N	+R-N	+R+N
7	67.4 a (9.3)	61.4 a (13.3)	78.2 a (5.0)	75.8 a (4.9)
14	117.6 a (15.5)	123.4 a (4.7)	126.0 a (8.4)	132.4 a (5.0)
21	139.4 a (18.1)	166.4 b (16.3)	175.4 b (4.1)	179.8 b (9.5)
28	160.0 a (7.6)	213.6 b (14.4)	224.6 b (36.5)	235.0 b (27.3)
35	168.3* a (17.8)	297.8 b (27.7)	331.8 c (34.0)	310.0 bc (34.0)
42	180.8 a (11.8)	346.2 b (28.5)	375.8 c (34.5)	356.8 bc (22.9)

1. Values in parentheses indicate standard deviations.
2. Letters after values indicate statistical differences (horizontally); values not sharing the same letter are significantly different at the 5% level.
3. n = 5, except * where n = 3.

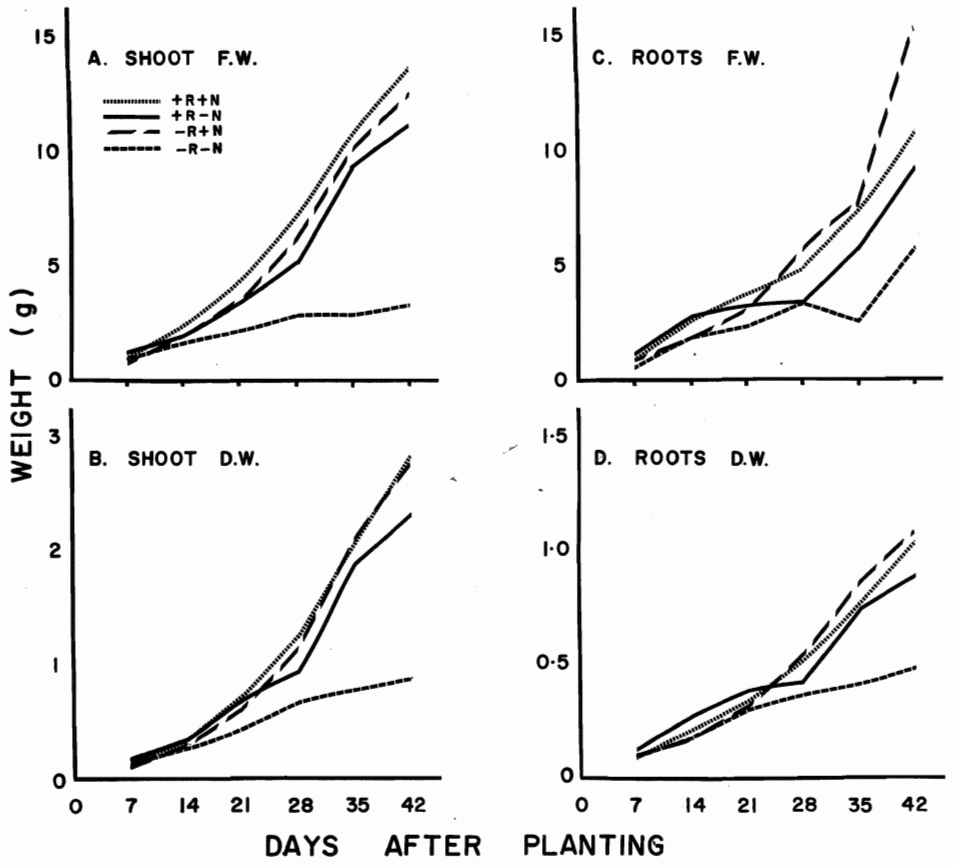


Figure 3. Shoot and root fresh and dry weights of soybean plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

Table 4. Shoot and root fresh weights of soybean plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

Days after planting	Fresh weight (g)			
	-R-N	-R+N	+R-N	+R+N
Shoots				
7	0.9991 a (0.0923)	0.8926 a (0.1916)	1.2326 a (0.2033)	1.1275 a (0.0478)
14	1.6742 a (0.5078)	1.8915 a (0.1726)	1.9370 a (0.1635)	2.3362 a (0.2965)
21	2.1736 a (0.3904)	3.6584 b (0.5210)	3.4960 b (0.2469)	4.4128 b (0.5438)
28	2.8630 a (0.4783)	6.1303 bc (0.6585)	5.1490 b (1.2994)	7.0561 c (1.5270)
35	2.8978* a (0.7290)	10.0340 bc (1.3318)	9.5033 b (1.9266)	10.8442 c (1.3443)
42	3.3321 a (0.2896)	12.4465 c (1.4300)	11.1011 b (2.0411)	13.6042 c (1.6402)
Roots				
7	0.7039 a (0.1847)	0.8224 a (0.2017)	1.1029 a (0.2797)	0.8475 a (0.1630)
14	1.8726 a (0.3128)	1.8690 a (0.2126)	2.8124 a (0.2927)	2.6117 a (0.3613)
21	2.4751 a (0.5000)	3.1666 ab 0.4479)	3.2950 ab (0.3432)	3.7795 b (0.6355)
28	3.4346 a (0.4805)	5.7340 b (0.4922)	3.3356 a (0.5677)	4.7973 b (0.8124)
35	2.5840* a (0.3658)	7.8601 c (1.6683)	5.8472 b (0.7632)	7.3766 c (1.3280)
42	5.8112 a (0.6731)	15.2266 d (1.6931)	9.2583 b (1.7213)	10.9646 c (1.6236)

1. Values in parentheses indicate standard deviation.
2. Letters after values indicate statistical differences (horizontally); values not showing the same letter are significantly different at the 5% level.
3. n = 5, except * where n = 3.

Table 5. Shoot and root dry weights of soybean plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

Days after planting	Dry weight (g)			
	-R-N	-R+N	+R-N	+R+N
Shoots				
7	0.1383 a (0.0141)	0.1196 a (0.0237)	0.1501 a (0.0239)	0.1417 a (0.0074)
14	0.2446 a (0.0543)	0.2627 a (0.0179)	0.2888 a (0.0177)	0.3128 a (0.0353)
21	0.4359 a (0.0877)	0.5973 ab (0.0923)	0.6093 ab (0.0338)	0.7286 b (0.1100)
28	0.6746 a (0.0870)	1.1368 bc (0.1116)	0.9297 b (0.2270)	1.2625 c (0.2663)
35	0.7815* a (0.1689)	2.1282 b (0.2495)	1.8856 b (0.3374)	2.0617 b (0.2983)
42	0.8685 a (0.0794)	2.7420 c (0.2905)	2.3041 b (0.4700)	2.8010 c (0.3508)
Roots				
7	0.0841 a (0.0136)	0.0899 a (0.0201)	0.1215 a (0.0364)	0.0815 a (0.0132)
14	0.1712 a (0.0311)	0.1650 a (0.0255)	0.2724 b (0.0414)	0.2105 ab (0.0309)
21	0.2895 a (0.0570)	0.3077 a (0.0499)	0.3756 a (0.0432)	0.3318 a (0.0452)
28	0.3664 a (0.0315)	0.5432 b (0.0416)	0.4123 a (0.0718)	0.5037 b (0.0818)
35	0.4172* a (0.0417)	0.8543 c (0.1411)	0.7451 b (0.0939)	0.7674 bc (0.1241)
42	0.4804 a (0.0552)	1.0748 c (0.0633)	0.8884 b (0.1693)	1.0258 c (0.1058)

1. Values in parentheses indicate standard deviation.
2. Letters after values indicate statistical differences (horizontally); values not sharing the same letter are significantly different at the 5% level.
3. n = 5, except * where n = 3

significantly lower shoot dry weights were apparent following the third week (Figure 3B, Table 5). Shoot fresh weights for the remaining three populations were not significantly different from each other at the third week. However, by the 6th week, the two populations receiving nitrate fertilization (+N) showed significantly higher shoot fresh weights, irrespective of whether the plants were inoculated or not (Table 4). The shoot dry weights (Figure 3B, Table 5) of the four populations showed essentially the same trends as were obtained from the fresh weight data (Figure 3A, Table 4).

Root fresh weights (Figure 3C, Table 4) were not significantly different for the first two weeks of this experiment, and at the third week, significant differences were noted only between the two populations -R-N and +R+N. In the 4th and 5th weeks, plants in those populations supplied with fertilizer nitrogen as nitrate had significantly higher root fresh weights than those plants which did not receive fertilizer nitrogen. Again, the root dry weights (Figure 3D, Table 5) of the four populations showed similar trends to those observed for the fresh weight data (Figure 3C, Table 4).

Data showing the number of nodules visible on the roots of inoculated plants are presented in Figure 4. Nodules first became discernible at day 14. Throughout weeks 2 to 6, the roots of inoculated plants that did not receive fertilizer nitrogen (population +R-N) showed significantly higher numbers of nodules than those nodulated plants which were nitrate-fertilized (population +R+N). By the 6th week, the +R-N population averaged 37 nodules per root, whereas the +R+N population averaged only 16 nodules per plant, a decrease of over 50%. No nodules were present on the roots of non-inoculated (-R) plants.

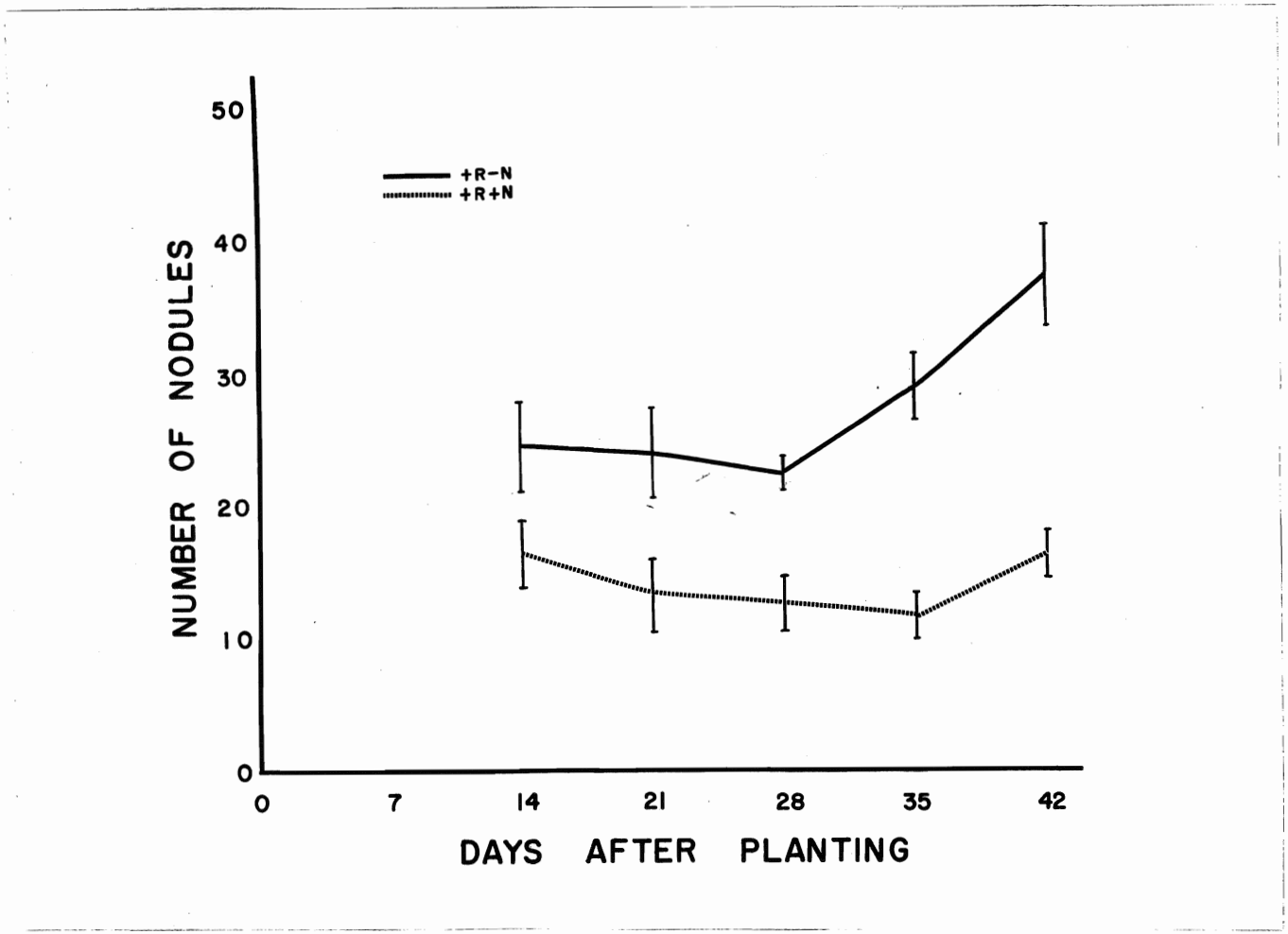


Figure 4. Nodulation of soybean plants grown in the presence (+N) or absence (-N) of nitrate fertilization. (Note: vertical bars are standard deviations.) Data refer to number of nodules per plant.

In addition to the measurable growth data already presented, a number of observations were also made throughout the course of this experiment, and these are presented in Table 6. Seedling vigour (i.e., the initial growth rate of the seedlings from emergence to the unfolding of the primary leaves) was greater in plants grown from inoculated (+R) seeds than in those grown from non-inoculated (-R) seeds. By the second week, differences between populations were less noticeable. However, by the third week after planting, plants in the -R-N population were showing signs of chlorosis and stunting. At this time, plants in the -R-N population had one fully expanded trifoliate leaf, one approximately half-expanded trifoliate and the third trifoliate was just visible. At the same age, plants in the other populations had two fully expanded trifoliate leaves and an additional two or three expanding trifoliate leaves. Plants in the -R-N population became increasingly chlorotic and comparatively more stunted during the remainder of this experiment. Axillary bud development was evident in plants in the other three populations by the 4th week. Flower clusters were visible but not open by the 5th week, and flowers started to open on day 37. When the experiment was terminated at the 6th week (day 42), there was no axillary development in plants in the -R-N population, and leaf petioles of plants in this population had developed a red colour.

5.2. Translocation studies with nodulated 21 day old soybean plants grown in the presence or absence of nitrate fertilization

In this study, plants were grown in a controlled environment growth chamber at a light intensity of approximately 750 ft-c. at the pot level. On day 21, the mature first trifoliate leaf was allowed to photoassimilate $^{14}CO_2$ for 5 minutes after which an additional 55 minute period in laboratory

Table 6. Visual observations made during the growth and development of plants grown from inoculated (+R) and non-inoculated (-R) seeds in the presence (+N) or absence (-N) of nitrate fertilization.

	-R-N	-R+N	+R-N	+R+N
Seedling vigour	+	+	++	++
Chlorosis	evident after week 3	none	slight at week 3 none at week 4	none
Stunting	evident by week 3	none	none	none
Number of trifoliate leaves week 3	3	4	4	4
week 6	5	10	10	10
Axillary bud development	none	evident by week 4	evident by week 4	evident by week 4
Flowers open	none	day 37	day 37	day 37
Petiole colour week 6	red	green	green	green

air was allowed for further translocation. Light intensity during the preconditioning, photoassimilation and translocation periods was 2800 ft-c. at the level of the fed leaf. Plants were then harvested to determine the distribution pattern of ^{14}C within the plant. Translocation data are presented in Figure 5 and Table 7, while growth and development data are presented in Figure 6 and Table 8.

5.2.1. Translocation

The extent of photoassimilate export was determined by expressing the content of ^{14}C found outside the fed leaf as a percent of the total ^{14}C recovered from the plant. From Table 7, it is evident that the magnitude of export was about 17% in both the population which received nitrate fertilization (+N) and the population which did not receive fertilizer nitrogen (-N).

The distribution pattern of exported ^{14}C was determined by expressing the amount of ^{14}C radioactivity contained in each plant part outside the fed leaf as a percent of the total exported ^{14}C. In contrast to the extent of export, differences in the distribution pattern of exported ^{14}C were observable between the two populations of plants (Table 7). Translocation to the shoot apex and portion of the plant above the node of the fed leaf was significantly higher in plants which did not receive fertilizer nitrogen (16%) than in those plants which did receive nitrate fertilizer (7%). Of further interest is the observation that in the nitrate-fertilized plants, only 3% of the exported ^{14}C was recovered from the nodules, whereas in plants which did not receive fertilizer nitrogen, 24% was recovered from the nodules. In contrast, the nitrate-fertilized plants showed 36% of the ^{14}C export in the roots, whereas the roots of plants which did not receive fertilizer nitrogen contained only 19% of the ^{14}C export. Despite the

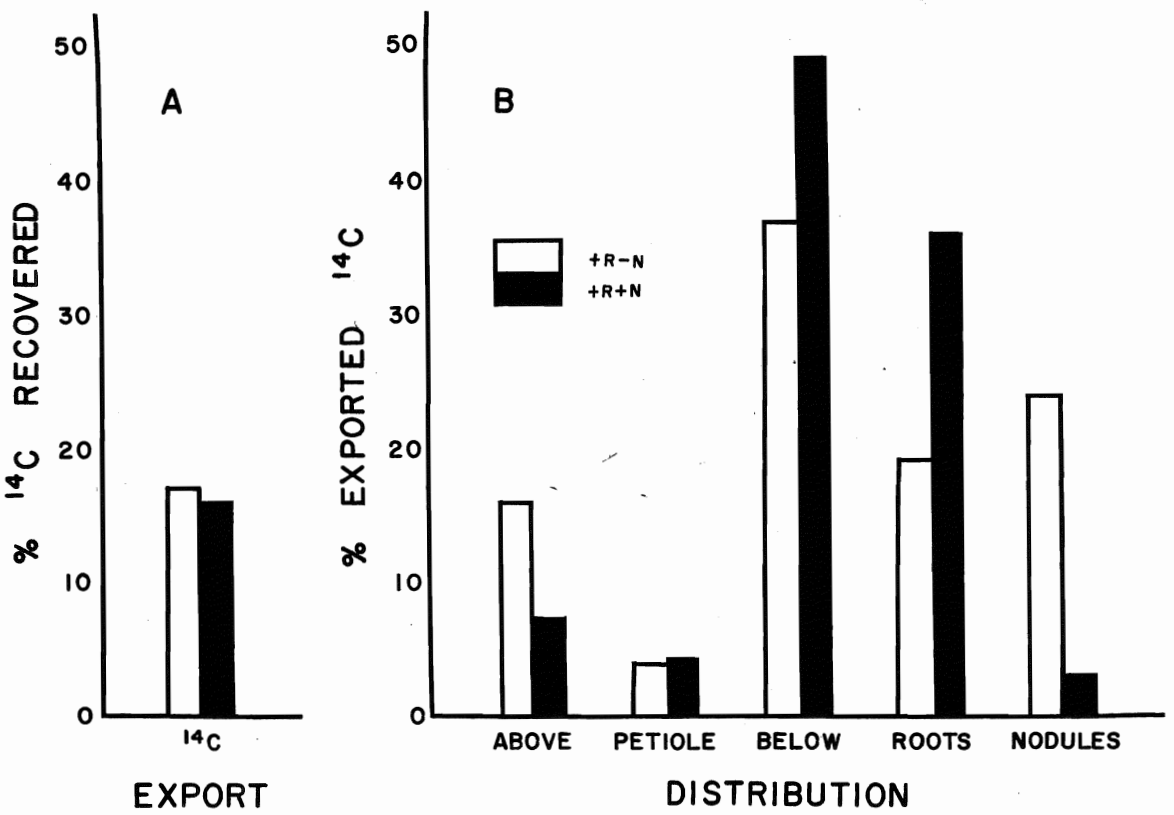


Figure 5. Magnitude of ^{14}C export and distribution of exported ^{14}C in nodulated 21 day old soybean plants grown in the presence (+N) or absence (-N) of nitrate fertilization.

Table 7. The magnitude of ^{14}C export and distribution pattern of exported ^{14}C in nodulated (+R) 21 day old soybean plants grown in the presence (+N) or absence (-N) of nitrate fertilization.

Treatment	Total ^{14}C recovered (μCi)	Exported ^{14}C (% ^{14}C recovered)	% Exported ^{14}C				
			Above	Petiole	Below	Roots	Nodules
-N	1.84 (0.40)	17.0 (4.5)	16.0 (6.3)	4.0 (1.3)	36.9 (5.2)	19.2 (4.1)	23.8 (5.7)
+N	1.80 (0.49)	16.2 (6.2)	7.5 (2.6)	4.4 (0.7)	49.1 (3.4)	36.0 (2.6)	3.0 (2.7)
Significant difference p = 0.05	n.s.	n.s.	sig.	n.s.	sig.	sig.	sig.

1. Values in parentheses are standard deviations.
2. n.s. = not significant at the 5% level.
3. n = 5

differences in ^{14}C allocation between the roots and the nodules, in both populations about 40% of the ^{14}C exported from the source (fed) leaf was in the subterranean parts of the plant within the 60 minute period of photo-assimilation and translocation.

5.2.2. Growth and development

For the plants used in this study, data was also obtained regarding the extent of nodulation (Table 8) as well as the fresh weights of the various parts of the plant (Figure 6, Table 8). As seen from Table 8, plants receiving nitrate fertilization had a mean total weight of 4.6 g, whereas the population not receiving fertilizer nitrogen had a mean total weight of only 3.5 g. Significantly larger fresh weights were observed for the above, below and roots fractions of the nitrate-fertilized plants than for plants not receiving fertilizer nitrogen. However, nodule fresh weights were greater in those plants receiving no nitrate fertilization (-N) than in the nitrate-fertilized plants (+N).

Although the mean number of nodules per plant was the same for both populations (approximately 25), the mean fresh weight per nodule in the population which did not receive fertilizer nitrogen was 5.1 mg whereas in the population receiving nitrate fertilization, the value was 0.9 mg (Table 8).

5.3. Translocation studies with nodulated 21 day old soybean plants in which the dates of initiation and termination of fertilizer nitrate application were varied

For this study, soybean plants were grown in a controlled environment growth chamber which provided a light intensity of approximately 3000 ft-c.

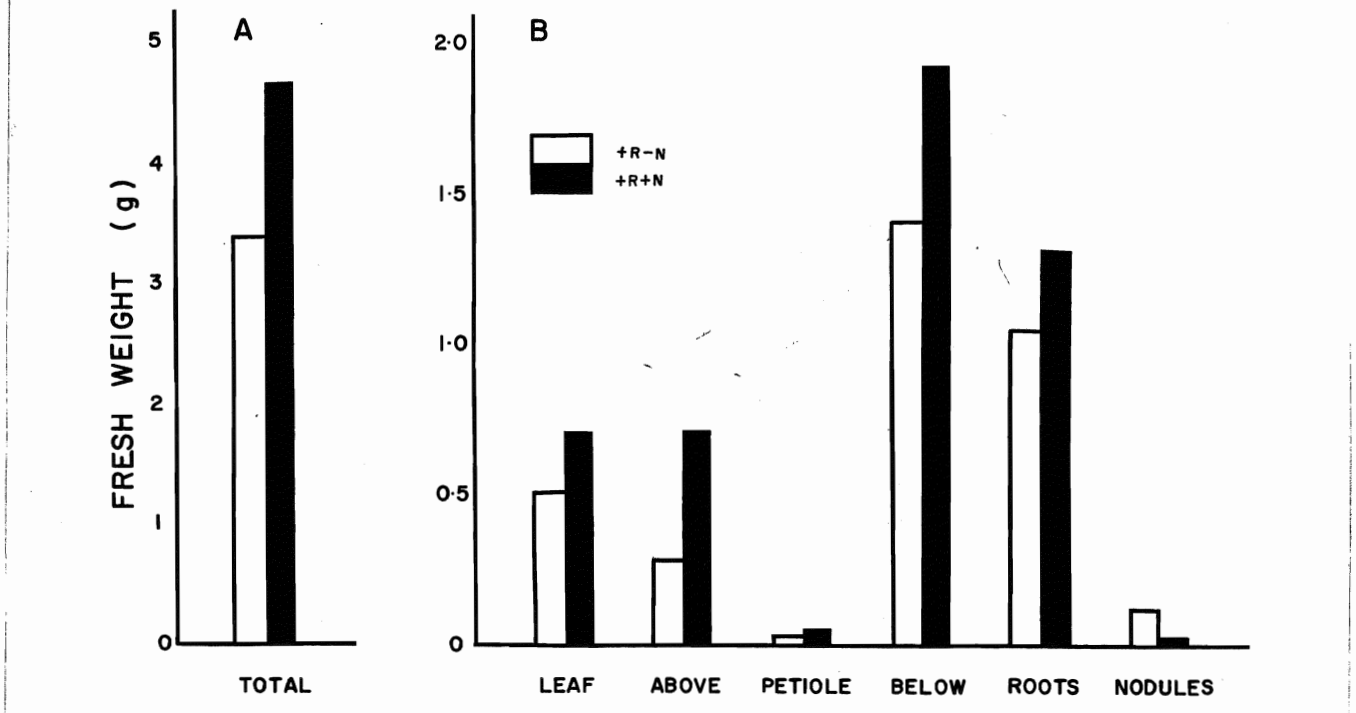


Figure 6. Fresh weights of nodulated 21 day old soybean plants grown in the presence (+N) or absence (-N) of nitrate fertilization.

Table 8. Fresh weight and nodule data for nodulated (+R) 21 day old soybean plants grown in the presence (+N) or absence (-N) of nitrate fertilization.

Treatment	Fresh weight (g)							Nodulation	
	Total	Above	Leaf	Petiole	Below	Roots	Nodules	Number of nodules	Weight per nodule (mg)
-N	3.3926 (0.4717)	0.2818 (0.0825)	0.5159 (0.0487)	0.0295 (0.0070)	1.4020 (0.2558)	1.0432 (0.1736)	0.1202 (0.0193)	25.8 (10.3)	5.13 (1.56)
+N	4.6443 (0.7311)	0.6451 (0.2412)	0.7137 (0.1020)	0.0525 (0.0178)	1.8920 (0.3702)	1.3164 (0.2026)	0.0246 (0.0130)	25.4 (9.9)	0.90 (0.33)
Significant difference (p = 0.05)	sig.	sig.	n.s.	n.s.	sig.	sig.	sig.	n.s.	sig.

1. Values in parentheses are standard deviations
2. n.s. = not significant at the 5% level.
3. n = 5

at the pot level. These plants were treated in one of four ways (Table 2). One population did not receive fertilizer nitrogen for the entire 21 day period post-sowing (-N-N), a second population was regularly supplied with fertilizer nitrogen as nitrate from day 7 to day 21 (+N+N), a third population received nitrate fertilization only for the period from day 7 to day 13 (+N-N), while a fourth population only received nitrate fertilization from day 14 to day 21 (-N+N). For the translocation study, the source trifoliate leaf was allowed to photoassimilate $^{14}CO_2$ for 15 minutes, followed by an additional 45 minute translocation period, after which the plants were harvested to determine the distribution pattern of ^{14}C within the plant. Light intensity, measured at the level of the source (fed) trifoliate leaf, was 2800 ft-c. throughout the preconditioning, photoassimilation and translocation periods. Rates of net photosynthesis were also determined immediately prior to the $^{14}CO_2$ photoassimilation period. Translocation data are presented in Figure 7 and Table 9, growth and development data in Table 10, and photosynthesis rate data in Table 11.

5.3.1. Translocation

As seen in Table 9, the magnitude of export was approximately 11% of the total recovered ^{14}C in all four populations. In contrast to the extent of export, differences in the distribution pattern were observable between the four populations of plants (Figure 7, Table 9). In the population receiving fertilizer nitrate for the entire fertilization period (population +N+N), only 6% of the exported ^{14}C was recovered from the nodules, whereas in plants which did not receive any fertilizer nitrogen (population -N-N), 33% of the ^{14}C export was recovered from the nodules. In contrast, the nitrate-fertilized plants showed 39% of the exported ^{14}C in

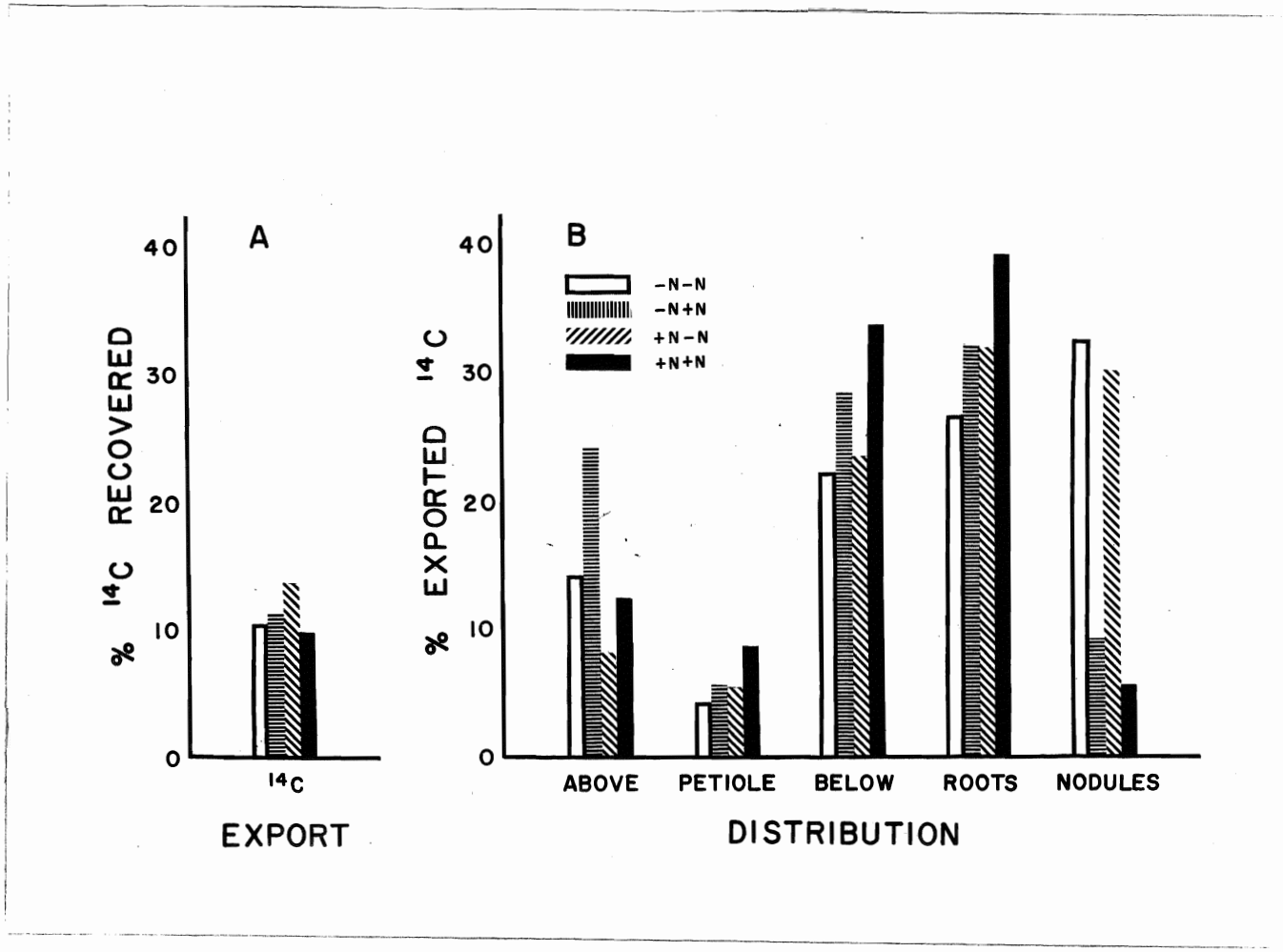


Figure 7. Magnitude of ^{14}C export and distribution pattern of exported ^{14}C in nodulated 21 day old soybean plants grown with various patterns of nitrate fertilization.

Table 9. Effect of time of initiation and termination of nitrate fertilization on the magnitude of ^{14}C export and distribution pattern of exported ^{14}C in nodulated (+R) 21 day old soybean plants.

Treatment	Total ^{14}C recovered (μCi)	Exported ^{14}C (% ^{14}C recovered)	% Exported ^{14}C				
			Above	Petiole	Below	Roots	Nodule
-N-N	2.30 a (0.42)	10.3 a (6.7)	14.1 a (8.3)	4.2 a (1.7)	22.2 a (5.2)	26.7 a (3.1)	32.7 a (4.7)
-N+N	1.77 a (0.61)	11.5 a (2.6)	24.4 b (10.8)	5.6 a (2.5)	28.5 ab (5.4)	32.3 a (9.7)	9.3 b (4.6)
+N-N	2.27 a (0.45)	13.8 a (5.3)	8.3 a (3.0)	5.5 a (3.0)	23.7 a (4.7)	32.2 a (5.4)	30.3 a (2.8)
+N+N	2.15 a (0.70)	9.7 a (5.1)	12.5 a (5.9)	8.7 a (2.4)	33.9 b (6.2)	39.0 b (10.8)	5.8 b (3.4)

1. Values in parentheses are standard deviations.
2. Letters after values indicate statistical differences (vertically); values not sharing the same letter are significantly different at the 5% level.
3. n = 7

the roots, while the roots of plants not receiving any fertilizer nitrogen contained only 27% of the ^{14}C export. Within the 60 minute period of photoassimilation and translocation, 60% of the ^{14}C exported from the source (fed) leaf was recovered from the subterranean parts of the plants which did not receive any fertilizer nitrogen, whereas only 45% was recovered from the subterranean parts of plants which did receive fertilizer nitrate for the entire fertilization period. Nevertheless, the nitrate fertilized (+N+N) plants did show 34% of the exported ^{14}C in the stem below the fed leaf, whereas only 22% was recovered from the stem below the fed leaf in plants receiving no fertilizer nitrogen (-N-N). In both populations, however, about 80% of the exported ^{14}C was recovered from those parts of the plant below the node of the source (fed) leaf.

When nitrate fertilizer was supplied only during the period from day 7 to day 13 (population +N-N), the values of percent ^{14}C in the various parts of the plant were not statistically different from those values obtained from plants which did not receive any fertilizer nitrogen during the entire fertilization period (population -N-N). However, when fertilizer nitrate was supplied only during the period from day 14 to day 21 (population -N+N), the distribution pattern of exported ^{14}C was different from that obtained where no fertilizer nitrogen was supplied (population -N-N). In the -N+N population, the values of percent ^{14}C recovered from the shoot apex and the stem above the node of the source leaf (24%) and from the nodules (9%) were significantly different from the corresponding values obtained in the -N-N population (14% and 33%, respectively).

When nitrate fertilizer was supplied only during the period from day 14 to day 21 (population -N+N), the distribution pattern of exported ^{14}C obtained was different not only from that obtained where no fertilizer

nitrogen was supplied (population -N-N), and hence also of that obtained where nitrate fertilization was applied only from day 7 to day 13 (population +N-N), but also from that obtained where nitrate fertilizer was supplied throughout the fertilization period (population +N+N). The percent of ^{14}C present above the node of the fed leaf and in the roots of plants in the -N+N population (24% and 33%, respectively) were significantly different from the corresponding values obtained from the +N+N population (13% and 39%, respectively). However, the amount of ^{14}C recovered from the nodules in both populations (-N+N and +N+N) was about 8%, and from the subterranean parts (i.e., roots + nodules) of the plant about 43%.

5.3.2. Growth and development

Data were also obtained regarding the extent of nodulation as well as the fresh weights of the various parts of the plants used in this study. As seen in Table 10, the total fresh weight and fresh weight values of the plant parts except for the nodules, are significantly less in the plants where no fertilizer nitrogen was provided (population -N-N) than the corresponding values obtained from plants in the other three populations. Plants which received fertilizer nitrogen only from day 7 to day 13 (population +N-N) had fresh weight values which were higher than for the plants which received fertilizer nitrogen only during the day 14 to day 21 period (population -N+N). The highest fresh weight values were obtained from those plants which had received fertilizer nitrogen throughouth the entire fertilization period (population +N+N). In contrast, this population (+N+N) showed the lowest nodule weight per plant. The total weight of nodules per plant was highest in those populations which did not receive fertilizer nitrogen during the period from day 14 to day 21 (populations -N-N, +N-N).

Table 10. Effect of time of initiation and termination of nitrate fertilization on fresh weight and extent of nodulation in nodulated (+R) 21 day old soybean plants

Treatment	Fresh weight (g)							Nodulation	
	Total	Above	Leaf	Petiole	Below	Roots	Nodules	Number of nodules	Mean nodule weight (mg)
-N-N	5.2527 a	0.4291 a	0.7882 a	0.0400 a	1.4738 a	2.1715 a	0.3502 a	*68.4 a	*5.08 b
	(0.9170)	(0.1909)	(0.1925)	(0.0106)	(0.3069)	(0.4793)	(0.0692)	(8.6)	(1.13)
-N+N	6.7309 b	0.7814 b	1.1308 b	0.0586 a	1.6397 a	2.9252 b	0.1952 b	72.3 a	2.72 c
	(1.3185)	(0.4125)	(0.1391)	(0.0114)	(0.2821)	(0.5852)	(0.0568)	(18.2)	(0.63)
+N-N	8.6312 c	0.8262 b	1.1892 b	0.0789 a	2.1512 b	4.0092 c	0.3764 a	66.2 a	5.87 a
	(1.2752)	(0.3300)	(0.1944)	(0.0183)	(0.2383)	(0.7052)	(0.0909)	(16.2)	(1.51)
+N+N	9.2255 c	0.8571 b	1.5924 c	0.1116 a	2.2801 b	4.2651 c	0.1191 c	47.1 b	2.55 c
	(1.3634)	(0.3680)	(0.2301)	(0.0338)	(0.3261)	(0.7501)	(0.0602)	(15.9)	(1.00)

1. Values in parentheses are standard deviations.
2. Letters after values indicate statistical differences (vertically); values not sharing the same letter are significantly different at the 5% level.
3. n = 12 except * where n = 11.

From Table 10, it can also be seen that plants receiving nitrogen fertilizer throughout the entire fertilization period (population +N+N) had 47 nodules per plant, which was significantly lower than the approximately 69 nodules per plant for the other three populations. The mean weight per nodule was about 2.6 mg in the two populations receiving fertilizer nitrogen during the period from day 14 to day 21 (populations -N+N, +N+N), while the values for the populations which did not receive any fertilizer nitrogen (-N-N) or received fertilizer nitrogen only during the period from day 7 to day 13 (+N-N) were 5.1 mg and 5.9 mg per nodule, respectively. These differences were statistically significant, as indicated in Table 10.

5.3.3. Rates of net photosynthesis

The rate of net photosynthesis was determined on the mature first trifoliate leaf immediately prior to the liberation of $^{14}CO_2$ for the 15 minute photoassimilation period, and is reported here in units of mg CO_2 removed per hour per gram of leaf fresh weight. The results are presented in Table 11, together with the values of the magnitude of ^{14}C export and of the nodule weight as a percent of the total plant weight. As seen from Table 11, plants which received no fertilizer nitrogen during the period from day 14 to day 21 (populations +N-N, -N-N) showed the highest rates of net photosynthesis (6.5 mg CO_2 removed.hr^{-1}.g leaf fresh wt^{-1}). The plants which received fertilizer nitrogen throughout the fertilization period (population +N+N) showed the lowest rates of net photosynthesis (3.8 mg CO_2.hr^{-1}.g leaf fresh $weight^{-1}$), as well as the lowest values for both the magnitude of export of ^{14}C (9.7% of the total ^{14}C recovered) and the nodule weight as a percent of the total plant fresh weight (1.3%).

Table 11. Effect of time of initiation and termination of nitrate fertilization on apparent net photosynthesis, magnitude of ^{14}C export and extent of nodulation in nodulated (+R) 21 day old soybean plants.

	-N-N	-N+N	+N-N	+N+N
Rate of net photosynthesis ($mg\ CO_2 \cdot h^{-1} \cdot g\ leaf\ FW^{-1}$)	6.49 a (1.44)	5.08 b (1.53)	6.50 a (0.79)	3.77 c (0.85)
n =	8	9	8	7
^{14}C export as % total ^{14}C recovered	10.3	11.5	13.8	9.7
Nodule weight as % of total plant weight	6.7	2.9	4.4	1.3

1. Values in parentheses show standard deviations
2. Letters after photosynthesis rate values indicate statistical differences; values not sharing the samw letter are significantly different at the 5% level.
3. ^{14}C export data from Table 9.
4. Nodule weight data from Table 10.

6. Discussion

In this study, the effects of nitrate-nitrogen fertilization on the growth and development of nodulated soybean plants, and on the translocation of photosynthetically assimilated ^{14}C compounds, were investigated. The study intentionally focussed on:

1. nodulated plants, since the survival of sufficient *Rhizobia* to effectively nodulate non-inoculated plants has been demonstrated under field conditons, even with intervals in excess of 10 years between soybean crops (25), and

2. the early stages of growth and development of the plant, a time during which the initiation and development of root nodules occurs leading to the onset of the symbiotic process of nitrogen fixation.

Evident in this study is the observation that when nodulated soybean plants are deprived of fertilizer nitrogen, and hence must depend entirely on symbiotic nitrogen fixation once the cotyledonary reserves are exhausted, there is a reduction in the extent of plant growth. Shoot fresh weights in non-nitrogen-fertilized plants were reduced between 20 and 44%, and root fresh weights between 13 and 43% at 21 days after planting when compared to nodulated plants which did receive nitrate-nitrogen fertilization (Tables 4, 8 and 10). Even at 42 days after planting, shoot and root fresh weights were reduced by 18 and 16%, respectively (Table 4). Under greenhouse conditions, the height of the plants was not affected by the presence or absence of fertilizer nitrogen (Table 3).

Shoot and root growth of nodulated legumes grown in the absence of combined nitrogen in the nutrient solution have been shown to be less than when fertilizer nitrogen was supplied (36, 47, 58, 84). For example, Hatfield *et al.* (47) reported a reduction in shoot and root dry weights of

about 60 and 40%, respectively, when nodulated soybean plants were grown for 6 weeks under greenhouse conditions in the absence of nitrate-nitrogen. When plants were grown in a growth chamber at 2600 ft-c. in the absence of nitrate fertilization, Laing (58) noted a 50% reduction in both shoot and root fresh weights as compared to nitrate fertilized plants, which is in close agreement with the approximately 44% reduction reported in this study (Table 10). The reduction in shoot and root growth observed in nodulated clover (Trifolium subterraneum L.) plants grown in the absence of combined nitrogen has been attributed by Gibson (36) to a greater respiratory consumption of photosynthetically assimilated carbohydrate by the nodulated root during the early stages of nodule development, resulting in nodulated plants having smaller fresh weights, despite a subsequent recovery in growth rates.

In the present study utilizing nodulated soybean (Glycine max (L.) Merr. cv. Harosoy 63) plants grown under greenhouse conditions, the absence of fertilizer nitrogen resulted in smaller fresh weights of the shoot and root components only following the 21st day after planting, as compared to plants receiving combined nitrogen (Table 4). Further, there was no increase in root fresh weight of non-fertilized plants during the period from the 21st to the 28th day after planting (Table 4). It would appear, therefore, that the developing nodules and the growing root both compete for available photosynthate during the same period after planting, a period in which Laing (58) observed a 2½ fold increase in the rate of nitrogen fixation. This observation has also been made by Nunn (84) who showed that the rate of root growth decreased during the phase of rapid growth and development of the nodules. Following the 28th day after planting, the rate of root growth again increased; however, the period of

retarded root growth resulted in root fresh weights of non-nitrate-fertilized plants being subsequently lower than those of fertilized plants (Table 4).

Concurrent with the increase in respiration associated with the development of the nodulated root (36), a period of nitrogen stress following the depletion of cotyledonary reserves and prior to the onset of symbiotic nitrogen fixation, may also contribute to the observed reduction in plant growth. In the present study, chlorosis of the leaves of the non-fertilized plants was apparent 21 days after planting although not noticeable 7 days later, indicating chlorophyll synthesis was adversely affected in the absence of an adequate supply of combined nitrogen. Furthermore, the cotyledons of non-fertilized plants were distinctly more chlorotic and abscissed at an earlier age than those of the nitrate-fertilized plants. A similar stage of "nitrogen hunger" has been reported previously for well-nodulated soybean plants growing in the absence of combined nitrogen, while plants supplied with ammonium nitrate developed normally (118).

The role of photosynthate availability to the nodules for the development and maintenance of the nitrogen-fixing enzyme system has been reported to be of major significance (10, 43, 61, 62, 85). While the initiation of nodules is controlled by the roots, the subsequent development is determined by or dependent upon an adequate supply of photosynthetically assimilated carbon compounds from the shoot (61). An approximately 2 fold enhancement of nitrogen fixation, as assessed by the reduction of acetylene to ethylene, has been achieved when the photosynthate supply from the shoots has been increased either by CO_2 enrichment (43, 85), reduced photorespiration (86), increasing source size through grafting (102) or by increased light

intensities (10, 60). Not only is nitrogen fixing activity enhanced, but also the growth and development of the nodules are stimulated as well (10, 60, 85).

In the growth chamber studies reported in this thesis, two light intensities were used, with the temperature regime and photoperiod being held constant. When nodulated soybean plants were grown at 750 ft-c. in the absence of fertilizer nitrogen, about 26 nodules per plant were present on the roots 21 days after planting (Table 8). At a higher light intensity of 3000 ft-c., the number of nodules at 21 days was 68 per plant (Table 10). Despite the differences in nodule number at both light intensities, however, the mean weight per nodule was about 5 mg. At least part of this increase in nodule number, and hence nodule mass per plant, may be accounted for by the larger roots of plants growing at the higher light intensity thus providing a larger surface for infection by the inoculum. At the lower light intensity (750 ft-c.), the nodules represented 3.5% of the total plant fresh weight, while at 3000 ft-c., the nodules constituted over 6.5% of the plant on a fresh weight basis. Bethlenfalvay and Phillips (10) reported a similar increase in the nodule component, from 3.3% of the plant dry weight when light intensity was severely limiting (200 μEi) to over 8% at a saturating light intensity of 800 μEi. Thus, not only is the plant grown at a higher light intensity able to provide a greater root area for nodulation, but also through an increase in availability of photo-assimilated carbohydrate, is able to provide for an equally rapid rate of growth and development of the greater number of nodules.

Although nitrate fertilization enhanced the growth and development of soybean plants, applications of fertilizer nitrogen did reduce the development of the nodule component (Tables 8 and 10). Whereas in the non-

fertilized plants grown at 750 ft-c. the nodules represented 3.5% of the total plant fresh weight, in plants supplied with nitrate fertilization the nodule component was only about 0.5% of the plant fresh weight. Mean weight per nodule was reduced from 5 mg to less than 1 mg by the presence of nitrate in the nutrient solution (Table 8). Similarly at 3000 ft-c., the nodule component was reduced by nitrate fertilization from 6.7% to 1.3% of the total plant fresh weight, while mean weight per nodule was reduced from 5 mg to about 2.5 mg (Table 10). Latimore et al. (59) have reported a similar reduction in the nodule component at 30 days after planting from 6% of the total plant weight in the absence of nitrogenous fertilizer, to 3% in the presence of ammonium (NH_4^+) or nitrate (NO_3^-), while Laing (58) observed a reduction with nitrate fertilization from 5% to less than 1% of the total plant weight at 42 days after planting.

Although the presence of nitrate fertilization consistently reduced the total nodule mass per plant as well as the mean weight per nodule, the effect on nodule number was less definitive. In plants grown at 750 ft-c., the number was not affected by nitrate fertilization (Table 8). At 3000 ft-c., however, nitrate fertilization reduced the number of nodules at 21 days after planting from 68 per plant in the absence of nitrates, to 47 where nitrate fertilization was applied (Table 10). Similarly, under greenhouse conditions where light intensity was maintained at a minimum of 1600 ft-c., nitrate fertilization reduced the number of nodules from 24 to 13 at 21 days of age and from 37 to 16 at 42 days after planting (Figure 4, Appendix 1.6). It would appear, therefore, that nitrate fertilization is effective in reducing nodule number when plants are

grown at light intensities approaching or exceeding the light saturation point for photosynthesis, whereas when photosynthesis is severely light limited, control of the infection process which results in nodulation is not affected by applications of nitrate fertilizer. Evidence for a similar effect of light intensity on the reduction in nodule number associated with nitrate fertilization does not appear to be available.

Despite the large differences in nodule number and mean weight, nodule mass per plant and plant size , the presence of nitrate fertilization did not affect the extent or magnitude of ^{14}C photoassimilate export from the source leaf during a one hour period following the initial liberation of $^{14}CO_2$. For plants grown at 750 ft-c. during May and June 1977, the magnitude of export was 16 to 17% of the total recovered ^{14}C (Table 7), while 10 to 14 % of the recovered ^{14}C was found outside the fed leaf of plants grown at 3000 ft-c. during the period from July to September 1977. A light intensity of 2800 ft-c at the level of the source trifoliate leaf was utilized for all $^{14}CO_2$ photoassimilation experiments and for the subsequent period allowed for translocation of ^{14}C-labelled photoassimilates.

The observed difference in magnitude of export for plants grown at the two light intensities may reflect differences in the sucrose-to-starch ratio in the source leaf which in turn regulates the magnitude of carbohydrate export (18, 50). The sucrose-to-starch ratio in leaves of plants grown at 3000 ft-c. is not significantly affected during the 2 hour period at 2800 ft-c. from the start of preconditioning to harvest of the plant, and hence starch production remains an important fate of the photoassimilated ^{14}C. In contrast, for plants grown at 750 ft-c., the period at 2800 ft-c. results in a much higher sucrose-to-starch ratio resulting in more of the ^{14}C-labelled photosynthate being available for export (72, 121).

The difference in magnitude of export between plants grown at low and at high light intensities may not, however, be only due to the growing conditions. Utilizing non-nodulated soybean plants grown under uniform conditions, Shelp (95) noted that the magnitude of ^{14}C export varied seasonally, with less than 8% of the total recovered ^{14}C exported in October and more than 21% exported in March and April.

Although the magnitude of ^{14}C export was unaffected by the various treatments utilized in this study, large differences were observed in the pattern of distribution, reflecting the relative metabolic activities of the various sinks. As shown in Tables 7 and 9, one hour after the initial liberation of $^{14}CO_2$ for photoassimilation, between 25 and 33% of the exported photoassimilated ^{14}C was recovered from the nodules of soybean plants grown in the absence of nitrate fertilization, while only 3 to 6% of the exported ^{14}C was recovered from the nodules of nitrate fertilized plants. In contrast, the roots of nodulated, nitrate-fertilized plants contained 36 to 39% of the exported ^{14}C, whereas the roots of nodulated plants grown in the absence of fertilizer nitrogen contained only 19 to 27% (Tables 7 and 9). It is apparent that when fertilizer nitrogen is supplied, a far greater proportion of the exported ^{14}C is recovered from the roots than from nodules, whereas in plants grown in the absence of nitrate fertilization there is an approximately equal partitioning between the root and nodule components.

The observation that there is a reduction in recovery of exported ^{14}C photoassimilates from the nodules of nitrate-fertilized plants is consistent with other recent reports investigating the effects of nitrate and/or ammonium fertilization on photosynthate distribution (59, 92, 97). For example, Russell and Johnson (92) noted that with 30 day old soybean plants

in which nodulation was partially inhibited by application of ammonium nitrate, only 1% of the exported ^{14}C was recovered from the nodules one hour after exposure to $^{14}CO_2$, compared to 4% from the nodules of plants which received no fertilizer nitrogen. The corresponding values for recovery of ^{14}C from the roots were 59 and 57%, respectively.

It has been suggested that the reduction in demand for photosynthate by the nodules associated with the presence of fertilizer nitrogen is the result of an inhibition of nodule respiration (70, 71, 97). Recently, Mahon (70) has reported that the presence of fertilizer nitrogen inhibits nodule respiration, specifically the component of respiration associated with nitrogenase activity. He further noted that nitrate had a greater inhibitory effect than ammonium-nitrogen fertilizers (71).

Russell and Johnson's (92) observation that only 4% of the exported ^{14}C was recovered from the nodules of 30 day old plants is considerably less than the 23 to 33% observed in the nodules at 21 days of age (Tables 7 and 9). Their relatively low value of 4% may be accounted for by the rapid utilization of photosynthate in nitrogen fixation, and the subsequent redistribution of the ^{14}C label to other parts of the plant. In contrast to 30 day old plants where little accumulation of the ^{14}C label in the nodules can be anticipated, the development of nodule structure and activity at 21 days after planting utilizes a greater proportion of the recent photosynthate within the nodule with little redistribution, consequently recovery of the ^{14}C label is much greater.

When a carbon budget for a 9 day period from the 21st to the 30th day after planting was determined for nodulated garden pea (Pisum sativum L.) plants grown in the absence of fertilizer nitrogen, Minchin and Pate (74) calculated that 74% of the photoassimilated carbon was translocated to

the subterranean parts of the plant while the remaining 26% was incorporated directly into shoot dry matter. Of the carbon translocated to the nodules, only about one-sixth was incorporated into nodule structure, about one-half was returned to the shoot in the form of amino compounds generated in nitrogen fixation, while the remaining one-third was utilized in respiration for nodule maintenance and nitrogen fixation. They further noted that during the first 3 days of this period (i.e., days 21 to 24) nearly 25% of the carbon translocated to the nodules was utilized for nodule growth, while during the last 3 day period (days 27-30), nodule growth utilized less than 5% of the carbon translocated to the nodules. Conversely, the utilization of carbon translocated to the nodules for the return of assimilated atmospheric nitrogen to the shoot increased from 40% during the first 3 day period to almost 60% during the last 3 day period. Throughout each 3 day period under investigation, nodule respiration utilized approximately one third of the photosynthate received.

Further indication of the nodules being a dynamic sink for photosynthate is the observation that nodule ^{14}C specific activity, that is, the amount of ^{14}C label per gram of tissue, was between 6 and 11 times greater than root ^{14}C specific activity within one hour of the initial liberation of $^{14}CO_2$ for photoassimilation. Bach et al. (5) also noted that nodule specific activity was about twice as great as that of the roots within 19 hours of the exposure of soybean plants to $^{14}CO_2$, while Lawrie and Wheeler (64) reported nodule ^{14}C specific activity was greater than that of any other plant part with the exception of the photoassimilating leaves.

The application of fertilizer nitrogen also affected the ^{14}C specific activities in the various sinks, particularly in the nodules. Nodule ^{14}C specific activity was about 50% greater in plants not receiving fertilizer

nitrogen when compared to those plants grown with nitrate fertilization, while root specific activities were little affected by the presence or absence of nitrate in the nutrient solution. The specific activity of the nodulated root (i.e., considering roots and nodules together) was between 28 and 140% greater for plants grown without application of fertilizer nitrogen. Similarly, Nunn (84) reported the ^{14}specific activity in nodulated roots grown in the absence of fertilizer nitrogen was 42% greater than non-nodulated roots dependent upon the uptake of nitrate from the nutrient solution.

It is apparent from the results of this study that during the initial 3 week period of plant growth, important processes such as the establishment and development of nodules, the extent of organ growth and patterns of photoassimilate distribution can be affected by nitrate-fertilization during this period. Of further importance was the observation that the addition or removal of nitrate fertilization at the 14th day after planting can significantly modify the trends previously established. For example, the removal of nitrogen fertilization following the second week of growth (Treatment +N-N) resulted in significantly greater nodule weights, and indeed nodule numbers, than in plants which were provided with fertilizer nitrogen during the entire fertilization period (Treatment +N+N). From Table 10, it can be seen that in the presence of continuous nitrate fertilization (+N+N), there were 47 nodules per plant with a total fresh weight of about 0.12 g, whereas in the population where nitrate fertilization was discontinued at the 14th day after planting (+N-N), 66 nodules per plant were present with a total fresh weight of almost 0.38 g. In fact, nodule fresh weight per plant in the +N-N population was not significantly different from the value for plants to which no nitrate fertilization was

applied during the entire 21 day period of growth following planting (population -N-N, Table 10).

Removal of nitrate fertilization at day 14 also caused a modification in the distribution pattern of photosynthetically assimilated ^{14}C as compared to plants which received continuous nitrate fertilization. In fact the distribution of ^{14}C to any fraction of the plant, and especially to the nodules, in the two populations grown in the absence of nitrate fertilization subsequent to the 14th day after planting (populations +N-N and -N-N) were not significantly different (Table 9). About 30 to 33% of the exported ^{14}C was recovered from the nodules in both these populations, while less than 6% was recovered from the nodule component of plants supplied with fertilizer nitrogen throughout the entire fertilization period (population +N+N, Table 9). It is apparent that removal of nitrate fertilization at day 14 reversed the effect of nitrate on ^{14}C-photosynthate distribution to the nodules. The resumption of an adequate supply of photosynthate to the nodules was also accompanied by a reversal of the nitrate-induced inhibition of nodule development and especially of nitrogen fixation (acetylene reduction) observed by Laing (58).

Alternative to the removal of nitrate fertilization at day 14 after a 7 day period of fertilization, was the procedure where fertilizer nitrogen was initially applied 14 days after planting. When nitrate fertilization commenced following the second week of growth (population -N+N), the number of nodules per plant was not affected (Table 10). Both the -N-N and -N+N populations had about 70 nodules per plant. However, the application of nitrate fertilization at day 14 greatly reduced the nodule weight per plant and the mean weight per nodule (Table 10), indicating that photosynthate supply to the nodule component was reduced.

In fact, on the 21st day, less than 10% of the exported ^{14}C was recovered from the nodules of these plants (population -N+N), whereas in the -N-N population, over 30% of the exported ^{14}C was translocated to the nodules. Not only was the nodule mass affected by the application of nitrate fertilization commencing 14 days after planting, but also the capacity of the nodules to reduce acetylene was reduced by over 50% (58). It would appear, therefore, that under the conditions of temperature and photoperiod used in this study, the period from the 14th to the 21st day after planting is critical for the growth and development of nodules established earlier.

The data for ^{14}C-photosynthate distribution shown in Table 9 has been re-expressed and is shown in Table 12. All values presented in Table 12 are the means of two populations. For example, means of the data for the -N-N and -N+N populations (Table 9) are presented in Table 12 as -N±N, while the +N+N and +N-N means are designated +N±N. Similarly, the -N-N and +N-N date is represented as ±N-N and the +N+N and -N+N data as ±N+N.

From Table 12, it is more apparent that the presence of fertilizer nitrogen during the first half of the fertilization period resulted in less ^{14}C being recovered from the shoot apex and stem above the node of the fed leaf (10%) and more from the roots (36%) than when no fertilizer nitrogen was supplied during this time (19 and 29%, respectively, Table 12). There was no significant effect of the presence or absence of fertilizer nitrogen during this period with respect to recovery of ^{14}C from the stem below the fed leaf, the nodules, or the subterranean parts of the plant.

Different results, however, were obtained when nitrate fertilization was applied during the period from day 14 to day 21 (Table 12). The presence of fertilizer nitrogen at this time resulted in an increased recovery of ^{14}C from above the fed leaf (18% as compared to 11% in the absence of nitrate fertilization), the stem below the fed leaf (31% with

Table 12. Effect of time of nitrate fertilization on distribution pattern of exported ^{14}C in nodulated (+R) 21 day old soybean plants.

Treatment Day 7-13	Day 14-21	% exported ^{14}C				
		Above	Petiole	Below	Roots	Nodules
-N	±N	19.3 (10.7)	4.9 (2.2)	25.4 (6.1)	29.5 (7.5)	21.0 (13.0)
+N	±N	10.4 (5.0)	7.1 (3.1)	28.8 (7.5)	35.6 (8.9)	18.0 (13.1)
		sig.	sig.		sig.	
±N	-N	11.2 (6.7)	4.8 (2.5)	22.9 (4.8)	29.4 (5.1)	31.5 (3.9)
±N	+N	18.5 (10.4)	7.2 (2.9)	31.2 (6.2)	35.6 (10.5)	7.5 (4.3)
		sig.	sig.	sig.	sig.	sig.

1. Values in parentheses are standard deviations.
2. Significant differences indicated are at the 5% level.
3. From Table 9, Appendix 3.1. Values presented here are the means of two populations.

nitrate, 23% without) and the roots (36 and 29%, respectively), and greatly reduced the recovery of ^{14}C from the nodules (8% in the plants receiving nitrate compared to 32% in those plants not receiving nitrate fertilization) and from the subterranean parts of the plant (43 and 61%, respectively). Clearly, then, the application of nitrate-fertilizer during the 14 to 21 day period after planting had a far greater effect on the distribution of ^{14}C-photosynthate than did nitrate fertilization during the 7 to 13 day period.

While application of nitrate fertilizer resulted in increased growth of the plant (Tables 8 and 10), a stimulation in net rate of CO_2 uptake was not apparent. In fact, the rate of apparent photosynthesis of the mature first trifoliate leaf was significantly less in those plants that received nitrate fertilization during the period from the 14th to the 21st day after planting (Table 11). Such a reduction in the rate of photosynthetic CO_2 uptake may reflect a diversion of light-stimulated ATP and NADPH production for nitrate reduction to ammonia in the leaves (46, 71, 73, 106) with a resultant decrease in availability of these compounds for CO_2 reduction (49).

Indeed a consistently lower rate of photosynthetic CO_2 uptake in pea (Pisum sativum L.) plants grown in the presence of nitrate as compared with plants grown in nitrogen-free nutrient solution has recently been reported (71). Similarly, Bethlenfalvay and Phillips (11) noted higher CO_2 uptake rates in nitrogen-fixing pea plants as compared to those plants receiving fertilizer nitrogen. In the present study, addition of nitrate fertilization at the 14th day after planting (-N+N) resulted in a 25% reduction in rate of CO_2 uptake when measured on the 21st day after planting as compared to those plants which received no nitrate fertilization

(-N-N), while plants which were supplied with nitrate fertilization throughout the entire fertilization period (+N+N) exhibited rates of apparent photosynthesis over 40% lower than the non-fertilized plants (Table 11). Furthermore, when nitrate fertilization was discontinued after the 14th day following planting (+N-N), there was an increase in rate of photosynthetic CO_2 uptake so that rates were not different from the population which did not receive nitrate fertilization (-N-N).

While differences in magnitude of export were not statistically significant, it is interesting to note that the population with the lowest rate of CO_2 uptake (population +N+N) also exhibited the lowest rate of export (Table 11). Competition for photosynthetically produced ATP may also have resulted in an inadequate supply of energy for the active accumulation of photosynthetically assimilated carbon compounds into the minor vein phloem prior to long distance translocation (15, 31, 35, 98, 111).

On the basis of the data presented in this thesis, it is evident, therefore, that the presence of nitrate in the soil environment of a nodulated soybean plant may have a significant influence on such important processes as plant growth, nodule development, photosynthetic CO_2 uptake and the distribution of photoassimilated carbon. The following conclusions more specifically summarize the important findings in this work.

1. The growth of nodulated soybean plants, as evidenced by fresh weight, is enhanced in the presence of nitrate fertilization. In the absence of nitrate fertilization, chlorosis becomes apparent during the third week of growth following planting, which is likely indicative of a period of nitrogen stress following the depletion of seed nitrogen reserves and prior to the onset of symbiotic nitrogen fixation.

2. The application of nitrate fertilizer limits the extent of

nodulation (nodule number) in plants grown at light intensities approaching or exceeding the light saturation point for photosynthesis, while at severely limiting light intensities for photosynthesis nodule number is unaffected by the presence or absence of fertilizer nitrogen. However, nodule development (i.e., nodule fresh weight per plant and mean weight per nodule) is inhibited by the presence of nitrate fertilization at all light intensities.

3. The presence of fertilizer nitrogen does not affect the magnitude of export of photoassimilated ^{14}C from a fed leaf, but there is a significant effect on the distribution pattern, particularly with regards to the partitioning of photosynthate between the nodules and root tissue. With the reduction in nodule activity in the presence of nitrate fertilizer, more photosynthate is available for the increased growth and development of other plant components.

4. Under the growth conditions used in this study, the period from day 14 to day 21 is particularly important in the development of nodules initiated earlier. The addition of nitrate fertilization at day 14 after planting inhibits further growth and development of the nodules while stimulating overall plant growth. Removal of nitrate fertilization at this time stimulates nodule growth and development.

5. Based on the results of this study, the optimal fertilization strategy for nodulated soybeans would be to provide sufficient nitrogenous fertilizer during the early stages of plant growth and development for an enhanced rate of plant growth, but subsequently discontinuing the application of combined nitrogen fertilizer to allow the plant to assimilate atmospheric nitrogen through the symbiotic process. Under field conditions, however, this may be difficult to achieve.

6. There appears to be a relationship between nitrate application, nitrate reduction in the leaves, and leaf photosynthesis, which supports the hypothesis that the supply and utilization of photosynthate is a major limiting factor in the growth of nodulated soybeans.

Appendix I. Growth and development of soybean plants grown from inoculated and non-inoculated seed in the presence or absence of nitrate fertilization.

Table A1.1 Total Height (mm)

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
-R-N		72	107	162	166	188	170
		59	104	152	170	--	173
		56	133	137	155	149	178
		77	136	130	157	--	183
		73	108	116	152	168	200
	Mean	67.4	117.6	139.4	160.0	168.3	180.8
	S.D.	9.3	15.5	18.1	7.6	17.8	11.8
-R+N		62	122	184	206	270	336
		56	125	168	220	269	333
		79	130	154	196	308	363
		67	123	180	234	309	386
		43	117	146	212	333	313
	Mean	61.4	123.4	166.4	213.6	297.8	346.2
	S.D.	13.3	4.7	16.3	14.4	27.7	28.5
+R-N		70	124	178	251	306	420
		81	118	170	245	300	365
		77	122	178	204	317	338
		82	140	179	170	361	353
		81	126	172	253	375	403
	Mean	78.2	126.0	175.4	224.6	331.8	375.8
	S.D.	5.0	8.4	4.1	36.5	34.0	34.5
+R+N		78	135	177	226	275	345
		72	137	191	231	343	350
		71	132	175	260	310	397
		83	134	188	262	345	340
		75	124	168	196	277	352
	Mean	75.8	132.4	179.8	235.0	310.0	356.8
	S.D.	4.9	5.0	9.5	27.3	34.0	22.9

Table A1.2 Fresh Weight (g) of Shoots

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
-R-N		1.1105	1.3261	2.3454	2.6534	3.4097	3.0881
		1.0033	1.2663	2.5330	2.7130	--	3.3771
		0.9897	1.9014	2.3276	2.5606	2.9918	3.2869
		0.8586	2.4643	2.1418	2.7069	--	3.1061
		1.0358	1.4129	1.5204	2.6811	2.2919	3.8024
	Mean	0.9991	1.6742	2.1736	2.8630	2.8978	3.3321
	S.D.	0.0923	0.5078	0.3904	0.4783	0.7290	0.2896
-R+N		0.6579	2.0403	4.4 228	5.9891	8.6769	13.0254
		0.8379	1.6392	3.7190	6.3799	8.8996	12.3663
		1.0952	1.8329	3.4143	5.4944	10.3854	13.0895
		1.0847	2.0648	3.7354	7.1390	10.2225	13.7162
		0.7874	1.8803	3.0007	5.6494	11.9856	10.0352
	Mean	0.8926	1.8915	3.6584	6.1303	10.0340	12.4465
	S.D.	0.1916	0.1726	0.5210	0.6585	1,3318	1.4300
+R-N		1.1376	2.0261	3.2875	6.5131	7.8499	13.7406
		1.5460	1.8957	3.4107	5.3657	8.1187	10.7609
		1.1088	1.7737	3.7369	4.2169	8.9967	8.8465
		1.3236	2.1739	3.7815	3.4519	9.9232	9.5792
		1.0474	1.8157	3.2635	6.1974	12.6280	12.5784
	Mean	1.2326	1.9370	3.4960	5.1490	9.5033	11.1011
	S.D.	0.2033	0.1635	0.2469	1.2994	1.9266	2.0411
+R+N		1.1437	2.2633	4.3251	6.4370	9.0924	12.7421
		1.0455	2.3578	4.7129	7.8758	12.2123	12.1793
		1.1372	2.4900	4.8912	7.3478	11.4990	16.3778
		1.1399	2.6834	3.5112	8.8184	9.7537	13.0628
		1.1715	1.8868	4.6238	4.8016	11.6637	13.6593
	Mean	1.1275	2.3362	4.4128	7.0561	10.8442	13.6042
	S.D.	0.0478	0.2965	0.5438	1.5270	1.3443	1.6402

Table A1.3 Fresh Weight (g) of Roots

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
-R-N		0.9955	1.6066	2.0829	2.8857	2.2481	5.2554
		0.7418	1.6773	2.8807	4.1787	--	6.3122
		0.5721	2.1134	2.9262	3.2667	2.9738	5.7957
		0.5231	2.2997	2.6737	3.2775	--	5.0557
		0.6874	1.6662	1.8123	3.5647	2.5301	6.6373
	Mean	0.7039	1.8726	2.4751	3.4346	2.5840	5.8112
	S.D.	0.1847	0.3128	0.5000	0.4805	0.3658	0.6731
-R+N		0.5303	2.1601	3.3234	5.6106	7.5135	17.3361
		0.8649	1.7248	3.5847	5.1220	5.4329	13.3317
		0.7841	2.0335	3.3460	5.4697	7.6804	14.1468
		1.0945	1.7067	3.1683	6.3059	8.7333	16.6311
		0.8384	1.7201	2.4107	6.1618	9.9407	14.6873
	Mean	0.8224	1.8690	3.1666	5.7340	7.8601	15.2266
	S.D.	0.2017	0.2126	0.4479	0.4922	1.6683	1.6931
+R-N		1.4326	3.1600	3.2736	3.5846	5.5779	9.9980
		1.1016	2.3813	3.4283	3.5144	5.7872	9.3214
		0.7534	2.6926	3.1607	3.0562	4.8190	7.0977
		1.3165	2.9380	3.7824	2.5176	6.1552	8.2471
		0.9105	2.8902	2.8600	4.0052	6.8971	11.6274
	Mean	1.1029	2.8124	3.2950	3.3356	5.8472	9.2583
	S.D.	0.2797	0.2927	0.3432	0.5677	0.7632	1.7213
+R+N		1.1113	2.5662	3.2660	4.4852	6.7950	9.6893
		0.7698	2.6995	3.6172	4.9636	7.8658	9.5694
		0.7940	3.0648	4.1905	5.2780	7.1624	13.1509
		0.6833	2.6681	3.1630	5.6857	5.7431	12.2256
		0.8792	2.0603	4.6612	3.5744	9.3167	10.1880
	Mean	0.8475	2.6117	3.7795	4.7973	7.3766	10.9646
	S.D.	0.1630	0.3613	0.6355	0.8124	1.3280	1.6236

Table A1.4 Dry Weight (g) of Shoots

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
-R-N		0.1410	0.2102	0.4768	0.6438	0.9463	0.8029
		0.1434	0.2004	0.5263	0.8290	--	0.8724
		0.1501	0.2726	0.4644	0.6155	0.7898	0.8677
		0.1136	0.3279	0.4178	0.6459	--	0.8022
		0.1138	0.2120	0.2946	0.6392	0.6086	0.9976
	Mean	0.1383	0.2446	0.4359	0.6746	0.7815	0.8685
	S.D.	0.0141	0.0543	0.0877	0.0870	0.1689	0.0794
-R+N		0.0817	0.2874	0.7297	1.1085	1.8849	2.8427
		0.1186	0.2376	0.6445	1.1114	1.9254	2.7617
		0.1404	0.2684	0.5453	1.0565	2.1795	2.8853
		0.1392	0.2594	0.5768	1.3323	2.1409	2.9792
		0.1181	0.2610	0.4905	1.0755	2.5105	2.2413
	Mean	0.1196	0.2627	0.5973	1.1368	2.1282	2.7420
	S.D.	0.0237	0.0179	0.0923	0.1116	0.2495	0.2905
+R-N		0.1400	0.2938	0.6173	1.1265	1.6086	2.9150
		0.1888	0.2802	0.5974	0.9740	1.6793	2.2572
		0.1358	0.2751	0.5979	0.7655	1.7904	1.8013
		0.1576	0.3178	0.6624	0.6310	1.8920	1.9169
		0.1286	0.2772	0.5717	1.1519	2.4579	2.6305
	Mean	0.1501	0.2888	0.6093	0.9297	1.8856	2.3041
	S.D.	0.0239	0.0177	0.0338	0.2270	0.3374	0.4700
+R+N		0.1416	0.3190	0.6714	1.1509	1.6335	2.6017
		0.1290	0.3107	0.7842	1.5145	2.3300	2.5928
		0.1434	0.3226	0.8250	1.3010	2.2538	3.4035
		0.1455	0.3550	0.5622	1.4820	1.8680	2.5858
		0.1493	0.2568	0.8004	0.8643	2.2235	2.8216
	Mean	0.1417	0.3128	0.7286	1.2625	2.0617	2.8010
	S.D.	0.0074	0.0353	0.1100	0.2663	0.2983	0.3508

Table A1.5 Dry Weight (g) of Roots

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
-R-N		0.0957	0.1464	0.3085	0.3629	0.4642	0.4585
		0.0923	0.1577	0.3441	0.4216	--	0.4924
		0.0677	0.2068	0.3154	0.3496	0.4038	0.4832
		0.0704	0.2030	0.2852	0.3557	--	0.4076
		0.0944	0.1423	0.1943	0.3426	0.3838	0.5603
	Mean	0.0841	0.1712	0.2895	0.3664	0.4172	0.4804
	S.D.	0.0136	0.0311	0.0570	0.0315	0.0417	0.0552
-R+N		0.0579	0.1963	0.3266	0.5252	0.8010	1.0468
		0.0975	0.1492	0.3830	0.4800	0.6440	1.0385
		0.0856	0.1868	0.2985	0.5562	0.8813	1.1459
		0.1116	0.1354	0.2787	0.5861	0.9276	1.1383
		0.0971	0.1577	0.2519	0.5689	1.0176	1.0049
	Mean	0.0899	0.1650	0.3077	0.5432	0.8543	1.0748
	S.D.	0.0201	0.0255	0.0499	0.0416	0.1411	0.0633
+R-N		0.1796	0.3206	0.4098	0.4369	0.6752	1.0582
		0.1077	0.2210	0.4063	0.4372	0.7142	0.8472
		0.0845	0.2773	0.3317	0.3642	0.6579	0.7081
		0.1322	0.3026	0.4057	0.3189	0.7940	0.7554
		0.1039	0.2405	0.3249	0.5043	0.8846	1.0731
	Mean	0.1215	0.2724	0.3756	0.4123	0.7451	0.8884
	S.D.	0.0364	0.0414	0.0432	0.0718	0.0939	0.1693
+R+N		0.1005	0.2231	0.3066	0.4612	0.6182	1.0265
		0.0889	0.2139	0.3459	0.5700	0.8478	0.9547
		0.0710	0.2481	0.3556	0.5391	0.8370	1.2028
		0.0675	0.2043	0.2677	0.5685	0.6485	0.9357
		0.0799	0.1631	0.3836	0.3801	0.8857	1.0095
	Mean	0.0815	0.2105	0.3318	0.5037	0.7674	1.0258
	S.D.	0.0132	0.0309	0.0452	0.0818	0.1241	0.1058

Table A1.6 Number of Nodules

Treatment		Time After Planting (weeks) 1	2	3	4	5	6
+R-N		0	23	37	23	24	41
		0	15	26	18	26	30
		0	34	21	22	26	37
		0	23	16	25	35	49
		0	28	20	24	34	30
	Mean	0.0	24.6	24.0	22.4	29.0	37.4
	S.D.	0.0	7.0	8.1	2.7	5.1	8.0
+R+N		0	13	16	16	10	18
		0	19	18	19	14	15
		0	16	18	12	17	22
		0	24	6	7	10	13
		0	10	9	9	8	14
	Mean	0.0	16.4	13.4	12.6	11.8	16.4
	S.D.	0.0	5.4	5.6	4.9	3.6	3.6

Appendix II. Translocation studies with nodulated 21 day old soybean plants grown in the presence or absence of nitrate fertilization.

Table A2.1 Magnitude of ^{14}C export and distribution of exported ^{14}C

Treatment		Total ^{14}C recovered (μCi)	Exported ^{14}C (% ^{14}C recovered)	% of exported ^{14}C				
				Above	Petiole	Below	Roots	Nodules
-N		1.52	15.1	8.7	4.3	39.1	21.7	26.1
		1.47	17.0	16.0	4.0	36.0	12.0	32.0
		2.10	21.9	23.9	4.3	30.4	19.6	21.7
		1.70	10.6	11.1	5.6	44.4	22.2	16.7
		2.40	20.4	20.4	2.0	34.7	20.4	22.4
	Mean	1.84	17.00	16.02	4.04	36.92	19.18	23.78
	S.D.	0.40	4.48	6.31	1.30	5.22	4.14	5.69
+N		1.17	18.8	4.5	4.5	54.5	36.4	0
		1.97	12.2	8.3	4.2	50.0	37.5	0
		1.39	13.7	10.5	5.3	47.4	31.6	5.3
		2.12	10.4	9.1	4.5	45.5	36.4	4.5
		2.33	25.8	5.0	3.3	48.3	38.3	5.0
	Mean	1.80	16.18	7.48	4.36	49.14	36.04	2.96
	S.D.	0.49	6.22	2.62	0.72	3.41	2.61	2.72

Table A2.2 Fresh weight and nodule data.

Treatment	Fresh weight (g)							Number of Nodules	Mean Nodule Weight (mg)
	Total	Above	Leaf	Petiole	Below	Roots	Nodules		
-N	3.9705	0.3043	0.5638	0.0233	1.6321	1.3132	0.1338	21	6.3714
	3.3282	0.4130	0.4804	0.0257	1.3551	0.9103	0.1437	40	3.5925
	3.7324	0.2634	0.5664	0.0412	1.6347	1.1201	0.1066	33	3.2303
	2.7757	0.2181	0.4580	0.0301	1.0126	0.9605	0.0964	15	6.4267
	3.1561	0.2102	0.5110	0.0273	1.3754	0.9119	0.1203	20	6.0150
Mean	3.3926	0.2818	0.5159	0.0295	1.4020	1.0432	0.1202	25.80	5.1272
S.D.	0.4717	0.0825	0.0487	0.0070	0.2558	0.1736	0.0193	10.33	1.5794
+N	5.3186	0.5751	0.7256	0.0425	2.5096	1.4455	0.0203	21	0.9667
	3.5752	0.3454	0.5693	0.0438	1.5174	1.0952	0.0041	12	0.3417
	4.5405	0.6111	0.6936	0.0423	1.8778	1.2874	0.0283	24	1.1792
	4.4434	0.6804	0.7247	0.0499	1.7880	1.1643	0.0361	33	1.0939
	5.3436	1.0133	0.8555	0.0839	1.7674	1.5895	0.0340	37	0.9189
Mean	4.6443	0.6451	0.7137	0.0525	1.8920	1.3164	0.0246	25.40	0.9001
	0.7311	0.2412	0.1020	0.0178	0.3702	0.2026	0.0130	9.91	0.3287

Appendix III. Translocation studies with nodulated 21 day old soybean plants in which the dates of initiation and termination of fertilizer nitrate application were varied.

3.1 Magnitude of ^{14}C export and distribution of exported ^{14}C.

3.2 Fresh weight and nodule data.

3.3 Photosynthetic CO_2 uptake.

Table A3.1 Magnitude of ^{14}C export and distribution of exported ^{14}C

Treatment		Total ^{14}C Recovered (μ Ci)	Exported ^{14}C (% ^{14}C Recovered)	% Exported ^{14}C				
				Above	Petiole	Below	Roots	Nodules
-N-N		2.80	3.2	14.4	4.4	26.7	23.3	31.1
		2.38	12.2	6.2	6.6	24.8	25.2	37.2
		2.29	23.5	18.2	2.6	13.2	30.4	35.6
		1.95	11.4	28.7	3.1	16.6	26.9	24.7
		2.82	9.4	4.1	4.1	23.3	30.8	37.6
		2.18	4.4	10.5	6.3	26.3	23.2	33.7
		1.69	7.7	16.9	2.3	24.6	26.9	29.2
	Mean	2.30	10.26	14.14	4.20	22.21	26.67	32.73
	S.D.	0.42	6.73	8.30	1.71	5.21	3.07	4.70
-N+N		0.73	9.3	11.8	5.9	25.0	47.1	10.3
		2.78	14.9	18.1	4.1	21.5	40.1	16.2
		2.02	12.1	18.0	6.5	33.1	32.2	10.2
		1.79	7.7	41.3	5.1	34.8	16.7	2.2
		1.56	12.8	26.5	10.5	31.5	27.0	4.5
		1.82	10.1	18.9	3.8	31.4	34.1	11.9
		1.72	13.8	36.3	3.0	22.4	28.7	9.7
	Mean	1.77	11.53	24.41	5.56	28.53	32.27	9.29
	S.D.	0.61	2.59	10.81	2.50	5.43	9.73	4.65
+N-N		1.61	5.2	3.6	12.0	30.1	25.3	28.9
		2.53	10.6	7.8	5.9	24.2	36.8	25.3
		2.88	13.2	11.3	2.9	24.2	27.1	34.5
		2.24	17.2	8.0	5.2	19.9	35.8	31.1
		1.87	12.0	6.7	4.9	29.0	27.2	32.1
		2.66	21.7	8.0	3.8	21.0	37.3	30.0
		2.06	16.4	12.7	3.8	17.2	36.1	30.2
	Mean	2.27	13.76	8.30	5.50	23.66	32.23	30.30
	S.D.	0.45	5.30	2.98	3.04	4.72	5.38	2.85
+N+N		1.51	3.4	7.7	11.5	36.5	36.5	7.7
		1.72	19.7	13.7	7.8	34.2	41.0	3.4
		3.34	10.1	9.1	10.6	28.0	41.3	10.9
		2.90	5.9	18.7	11.1	34.5	29.2	6.4
		1.77	10.1	10.7	5.1	33.1	50.0	1.1
		2.03	9.9	21.8	7.9	45.0	22.3	3.0
		1.74	8.8	5.9	7.2	26.1	52.9	7.8
	Mean	2.15	9.70	12.51	8.74	33.91	39.03	5.76
	S.D.	0.70	5.09	5.88	2.38	6.15	10.84	3.41

Table A3.2

Treatment	Fresh Weight (g)							Number of Nodules	Mean Nodule Weight (mg)
	Total	Above	Leaf	Petiole	Below	Roots	Nodules		
-N-N	5.6010	0.3049	0.7030	0.0290	1.7391	2.4068	0.4182	--	--
	4.1707	0.2661	0.6472	0.0489	1.0366	1.8236	0.3483	72	4.8375
	4.7380	0.4411	0.8040	0.0434	1.1744	1.9635	0.3166	62	5.0258
	4.3863	0.2919	0.7580	0.0317	1.0990	1.9490	0.2567	60	4.2783
	6.1384	0.6400	1.0311	0.0451	1.4980	2.4652	0.4590	59	7.7797
	6.8437	0.6763	1.1746	0.0594	1.8993	2.5742	0.4599	83	5.5410
	5.7679	0.4509	0.8078	0.0396	1.5843	2.4843	0.4010	64	6.2656
	4.2240	0.2385	0.6363	0.0338	1.2153	1.8249	0.2752	73	3.7699
	6.0785	0.6912	0.9673	0.0539	1.6404	2.4024	0.3233	69	4.6855
	4.1574	0.6572	0.8281	0.0391	1.2824	1.0032	0.3474	83	4.1855
	5.0549	0.1775	0.5314	0.0237	1.5885	2.4411	0.2927	66	4.4348
	5.8715	0.3131	0.5696	0.0324	1.9277	2.7198	0.3089	61	5.0639
Mean	5.2527	0.4291	0.7882	0.0400	1.4738	2.1715	0.3502	68.36	5.0789
S.D.	0.9170	0.1909	0.1925	0.0106	0.3069	0.4793	0.0692	8.61	1.1269
-N+N	8.1244	0.9667	1.1429	0.0697	1.9184	3.8469	0.1798	44	4.0864
	7.8228	1.3729	1.1654	0.0632	1.8242	3.1320	0.2651	97	2.7330
	9.0018	1.5233	1.3613	0.0761	2.2102	3.5770	0.2539	76	3.3408
	4.5660	0.2665	0.9648	0.0446	1.2234	1.9559	0.1108	53	2.0906
	5.5710	0.4669	0.9893	0.0404	1.4431	2.4387	0.1926	73	2.6384
	7.7912	0.6544	1.2543	0.0626	1.7754	3.7941	0.2504	76	3.2947
	5.4034	0.3204	0.9758	0.0680	1.4059	2.5271	0.1062	55	1.9309
	5.8766	0.4241	0.9556	0.0462	1.6979	2.5821	0.1707	78	2.1885
	6.0758	0.6211	1.0510	0.0462	1.5375	2.5657	0.2543	82	3.1012
	6.9328	0.6867	1.2243	0.0631	1.6337	3.1022	0.2228	100	2.2280
	7.3948	1.1980	1.2616	0.0607	1.7287	2.9427	0.2031	84	2.4179
	6.2097	0.8760	1.2233	0.0619	1.2776	2.6385	0.1324	50	2.6480
Mean	6.7309	0.7814	1.1308	0.0586	1.6397	2.9252	0.1952	72.33	2.7249
S.D.	1.3185	0.4125	0.1391	0.0114	0.2821	0.5852	0.0568	18.19	0.6306
+N-N	7.1373	0.5514	0.9454	0.0650	1.9516	3.2748	0.3491	55	6.3473
	8.4455	0.7810	1.0295	0.0691	2.2011	4.0059	0.3589	92	3.9011
	9.8548	0.9405	1.3857	0.0705	2.1522	4.8718	0.4341	71	6.1141
	10.2035	1.3290	1.3594	0.0938	2.1349	4.7826	0.5028	73	6.8877
	7.3183	0.6243	1.0950	0.0672	1.6796	3.5212	0.3310	44	7.5227
	8.5625	0.9003	1.1668	0.0590	1.8846	4.0997	0.4521	66	6.8500
	10.2946	0.9784	1.5141	0.0950	2.1842	5.0569	0.4660	70	6.6571
	6.6317	0.4382	0.8937	0.0561	2.3236	2.6273	0.2928	40	7.3200
	8.4894	0.5152	1.1143	0.0804	2.6233	3.8400	0.3162	78	4.0538
	7.5968	0.3949	1.0884	0.0738	2.1312	3.7312	0.1773	54	3.2833
	10.0144	1.3407	1.3477	0.1091	2.3196	4.4559	0.4414	62	7.1194
	9.0250	1.1209	1.3310	0.1080	2.2279	3.8426	0.3946	89	4.4337
Mean	8.6312	0.8262	1.1892	0.0789	2.1512	4.0092	0.3764	66.17	5.8742
S.D.	1.2752	0.3300	0.1944	0.0183	0.2383	0.7052	0.0909	16.18	1.5144
+N+N	9.4375	1.2082	1.2923	0.0967	2.3758	4.3199	0.1446	57	2.5368
	8.8363	0.6346	1.4729	0.1105	2.5821	3.9252	0.1110	74	1.5000
	10.7075	1.2009	1.5982	0.1820	2.6558	4.8060	0.2646	56	4.7250
	11.7694	1.4077	2.0963	0.1574	2.4321	5.5113	0.1646	39	4.2205
	10.7490	1.4712	1.8629	0.0907	2.2716	4.9395	0.1131	49	2.3082
	7.7889	0.4676	1.6349	0.0768	1.7626	3.7420	0.1050	52	2.0192
	9.4450	0.6103	1.7416	0.0910	2.1161	4.7639	0.1221	50	2.4420
	9.9303	0.5623	1.3689	0.1034	2.8281	4.9264	0.1412	50	2.8240
	8.6955	0.5451	1.3665	0.0668	2.4110	4.1933	0.1128	47	2.4000
	8.0383	0.5125	1.6803	0.1003	1.8931	3.7779	0.0742	55	1.3491
	7.5401	0.8045	1.4928	0.1222	2.0623	3.0213	0.0370	17	2.1765
	7.7682	0.8604	1.5018	0.1419	1.9703	3.2547	0.0391	19	2.0579
Mean	9.2255	0.8571	1.5924	0.1116	2.2801	4.2651	0.1191	47.08	2.5466
S.D.	1.3634	0.3680	0.2301	0.0338	0.3261	0.7501	0.0602	15.87	0.9953

Table A3.3 Photosynthetic CO_2 uptake of the mature first trifoliate leaf of 21 day old nodulated soybeans grown with varying patterns of nitrate fertilization

-N-N	-N+N	+N-N	+N+N
4.75	3.63	6.76	4.74
6.69	3.26	7.46	2.67
6.06	3.53	5.84	3.52
5.52	6.37	6.07	4.40
9.00	6.28	6.38	3.89
7.20	5.77	5.12	2.67
5.06	6.30	7.12	4.50
7.67	6.99	7.21	
	3.61		
Mean			
6.49	5.08	6.50	3.77
S.D.			
1.44	1.53	0.79	0.85

Units are mg CO_2 removed$\cdot h^{-1} \cdot$g leaf fresh wt^{-1}

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