

AN ABSTRACT OF THE THESIS OF

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Title: CARBOHYDRATE AND PHOTOSYNTHATE DISTRIBUTION AND NITROGEN

FIXATION IN BEANS (*Phaseolus vulgaris* L.)

Abstract approved: _____

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A series of four studies were conducted in Colombia, South America, in the field and the greenhouse to determine the effect of plant competition and mulching on carbohydrate and photosynthate distribution and nitrogen fixation in beans (*Phaseolus vulgaris* L.).

Mulching beans (determinant growth habit) with rice hulls in the lowland tropics reduced 2:30 p.m. soil temperature at 10 cm 1.3°C prior to flowering and 0.8°C after flowering compared to no mulch. Mulching improved the soil moisture in the top 10 cm 2.18 percentage units prior to flowering and only 0.57 units after flowering. The daily fluctuation of soil temperature was 1.3°C lower under the mulch. Nitrogen fixation (C_2H_2 reduction) showed the typically low rates of the lowland tropics but were three times higher (0.6 vs $0.2\text{ }\mu\text{moles/plt/hr}$) in mulched plants and was positively correlated with nodule fresh weight. Nodule specific activity was not affected by mulching. The roots and leaves of mulched plants contained 37% and 42%, respectively, higher quantities of total carbohydrates.

A determinant and indeterminant bean cultivar were used in two tropical locations (one cool, high altitude, high rainfall and the other hot, low altitude, low rainfall) to determine the effect of

plant population on carbohydrate distribution and nitrogen fixation. Plant development characteristics were similar in both locations. Increasing plant population resulted in lower plant weights and lower shoot/root ratios. The indeterminant cultivar had higher root weights and lower shoot/root ratios than the determinant cultivar. Nitrogen fixation rates and nodule fresh weight were positively correlated ($r = 0.88$ and $r = 0.70$) and the fixation rate was over 10-fold higher (0.5 vs $10 \mu\text{moles/plt/hr}$) in the cooler location. The concentrations of both soluble and insoluble carbohydrates were higher in all plant parts in the cooler location but were not markedly affected by plant population density.

The distribution of photosynthate to the roots and nodules by the leaves at nodes 4 and 8 of a determinant bean cultivar were determined 35, 48 (flowering), 63 and 70 days after planting. Roots retained 45% of the ^{14}C -photosynthate translocated from node 4 throughout crop development but accumulated almost none of the ^{14}C translocated from node 8. From day 35 to 48, activity detected in the lower stem decreased 16% with an equal increase in the nodules. The ^{14}C -photosynthate from node 8 went initially (day 48) to mid and upper stems and leaves (90%) but subsequently 85% went to mid and upper pods. Nitrogen fixation peaked after flowering and was positively related to the quantity of nodule soluble carbohydrate.

In a field study with an indeterminant cultivar, light penetration of the canopy was increased to different depths by defoliating alternate plants and sampling nondefoliated plants. Defoliations to nodes 12, 8, 4 or ground level were made at either 17 days prior to or 3 days after flowering. Nodule dry weight and nitrogen fixation tended to be reduced (not significantly) by all treatments both before and after flowering. Nitrogen content of nodules was reduced by postflowering treatments but no other changes in nitrogen content were observed. The dry weight and carbohydrate content of stems and leaves changed little until one week after flowering when both were increased by preflowering and postflowering treatments; however, only defoliation to ground level resulted in significant increases.

Carbohydrate and Photosynthate Distribution and
Nitrogen Fixation in Beans (Phaseolus vulgaris L.)

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CARBOHYDRATE AND PHOTOSYNTHATE DISTRIBUTION AND NITROGEN FIXATION IN BEANS (Phaseolus vulgaris L.)

INTRODUCTION

It has been estimated that as many as 50% of the people in the world suffer from hunger or malnutrition as a result of inadequate caloric intake or deficiencies in proteins, vitamins or minerals (70). Of these nutritional problems, increasing the supply of dietary proteins has recently received the greatest attention.

Approximately 70% of the protein in the human diet comes from vegetable sources, of which, cereals account for 70%. The food legumes offer significant potential for increasing the dietary proteins since the amino acid composition of legume protein complements that of cereal protein. Furthermore, food legumes are generally accepted and widely consumed even in areas where animal proteins are available. In developing countries, food legume consumption is as high as 72 grams per day (26).

In the synthesis of proteins, nitrogen is required by plants either through absorption from the soil by the root system, generally in the form of NO_3^- , or by reduction of N_2 by the nitrogenase enzyme present in nodules of legumes and several non-legume species. The fixation of N_2 by nodules offers an obvious economic advantage in comparison to chemical fertilizers and, in addition, the process is environmentally more desirable. Nitrogen fertilizers are costly to produce and handle and considerable quantities are lost through leaching, runoff, or erosion. Furthermore, the natural resources required to chemically produce nitrogen fertilizers become more expensive and less available each year.

In many developing countries most of the farmers are poor and work only small holdings and, because of costs, synthetic nitrogen fertilizer is unavailable. Food legumes which can be effectively inoculated for natural fixation of atmospheric nitrogen offer hope in supplying dietary protein requirements.

Nitrogen fixed within nodules is incorporated directly into plant metabolic processes without appreciable loss to the environment. Fixation, however, requires high energy inputs to obtain functioning nodules, reduce N_2 , and then incorporate it into organic compounds that can be utilized in plant growth and development. The nitrogen fixation capacity of a plant is limited by the compatibility of the plant and bacteria in forming a symbiotic relationship, and the efficiency with which the nitrogen fixation takes place, i.e., the energy inputs versus nitrogen reduced. The fixation of N_2 is also limited by the availability of carbohydrates from the leaves.

The purpose of these studies conducted at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, was to examine the effect of soil temperature, plant population, and competition for light on nodulation, nitrogen fixation and carbohydrate distribution in beans (Phaseolus vulgaris L.). The data collected will provide information for developing cultural practices which maximize unit area production.

REVIEW OF THE LITERATURE

Photosynthate Distribution

General Distribution Patterns

Nitrogen fixation in legumes is described as a photosynthate limited process. Nodules store very few reserves (79) and are dependent on the current photosynthate (all the products of photosynthesis which are translocated) both as an energy source and as a supply of carbon for the synthesis of nitrogenous compounds (6, 53). The capacity of the plant to supply the nodules with adequate amounts of assimilate is limited by environmental factors which alter photosynthate production and/or distribution.

In general, lower leaves of beans (Phaseolus vulgaris L.) distribute photosynthate to the lower stem, roots and nodules, while upper leaves supply the upper stem, young leaves and reproductive

parts. The mid-plant leaves are a source of photosynthate for middle plant parts as well as more distant regions (21, 110, 111, 123). When the assimilatory or distributive capacity of portions of the soybean (Glycine max) canopy becomes restricted, other nearby leaves may partially assume the supply responsibilities of the incapacitated leaves (115, 116). In the later developmental stages of soybeans, leaves whose photosynthate had previously gone to vegetative parts, is redirected to supply reproductive structures (57). It has been shown that photosynthate produced after anthesis in peas (Pisum sativum L.) is primarily responsible for pod and seed development (66), although evidence from other crops indicates that there is mobilization of carbohydrate pools during pod fill (30, 35, 88, 94). Nodules receive a much greater share of the carbon from net photosynthesis during vegetative growth (11, 44, 125).

Sucrose accounts for 90% of the photosynthate translocated from the leaves of field peas (Pisum arvense L.) to the roots, nodules, and other plant parts (87). While sucrose is also the major translocated compound in beans and soybeans, small quantities of sugar phosphates, hexoses and some organic acids are found in the translocation stream.

In beans photosynthate moves downward from the node of the leaf attachment to the anastomosis at the next lower node. Presumably at the anastomosis there is the potential for bidirectional movement in separate phloem bundles (21).

Photosynthate Distribution to Roots and Nodules

The movement of photosynthate throughout the plant following assimilation is rapid. In wheat, velocities of 80 - 100 cm/hr were found for upward moving assimilate while downward velocity was approximately half that rate (120). In beans, ^{14}C -assimilate was detected in the leaf petiole in 6 - 10 minutes after exposing the blade to $^{14}\text{CO}_2$ (125). Pate and Greig (86) were able to detect ^{14}C compounds in the bleeding sap of decapitated field peas immediately after exposing the shoot to $^{14}\text{CO}_2$. Lawrie and Wheeler (67) found that only

90 minutes was required for maximum accumulation of ^{14}C in nodules upon exposure of broad bean (*Vicia faba* L.) leaves to $^{14}\text{CO}_2$. They also found low levels of ^{14}C -sucrose in the nodules which was rapidly metabolized into amino compounds.

The detection of amino compounds in the bleeding sap (xylar fluid) of field peas shows a diurnal variation with the maximum at about noon and the minimum at mid-night, presumably reflecting a diurnal nitrogen fixation pattern, since little nitrogen is stored in nodules (86). The diurnal amino acid composition of field pea nodules corresponds to the day and night pattern of accumulation of free amino acids and organic acids in soybean nodules (11). Free amino acids accumulated in nodules in light and then decreased in the dark, suggesting that they were being incorporated into proteins but not being replaced because of a lowered rate of nitrogen fixation. The reverse relationship existed for organic acids which are precursors for amino acids. Lawrie and Wheeler (65) found that nitrogen fixation and accumulation of photosynthates in nodules was reduced when pea plants were placed in the dark and restored when they were placed in the light. They concluded that current photosynthate was critical for nitrogen fixation and that, at night, translocation of photosynthate was unable to meet nodule demand.

Both water and light (shading) stress have been shown to affect the distribution of ^{14}C to roots and nodules of soybeans. Finn (43) found that both roots and nodules contained a greater portion of the recovered ^{14}C when plants were subjected to water stress or shaded. Silvius (101) found that only the roots contain a greater portion of ^{14}C under water stress conditions while nodules contained a smaller portion.

Russel and Johnson (96) found that there was little difference in nodular ^{14}C content upon exposing soybean leaves to $^{14}\text{CO}_2$ any time between 9:00 a.m. (over 4 hours after sunrise) and 3:00 p.m., and concluded that the ^{14}C from photosynthate was rapidly moved out of nodules during the day. Measurements of diurnal changes in soybean photosynthesis showed that maximum net photosynthesis occurred 4 - 10

hours from the beginning of illumination but declined to 85% of maximum by hour 16 because of increased stomatal and mesophyll resistance (117). The decline was concomitant with reduced starch accumulation and a rise in the levels of soluble carbohydrate in the leaves. According to Fraser and Bidwell (45), both photosynthesis and photorespiration in beans are affected by photosynthate demand in the plant but they are controlled by different parameters. They found that photosynthesis is regulated by mesophyll resistance and to a lesser extent by stomatal resistance, but that photorespiration is not controlled by either.

The movement of photosynthate to the roots and nodules is affected by inorganic nitrogen application. When soybeans were supplied with inorganic nitrogen, both the distribution of photosynthates to the nodules and nitrogen fixation were reduced with the NO_3^- form having a more depressing effect than the NH_4^+ form. A direct relationship was found between nitrogen fixation and photosynthate distribution to the nodules (69). Ham *et al.*, (52) noted that inorganic nitrogen application to soybeans reduced nodule weight up to 76% for some cultivars and reduced nodule numbers as much as 55%; however, the nitrogen fertilizer produced yields 3 - 23% greater than nodulation alone. This implies that nitrogen fixation is not always able to fully meet the nitrogen demands of developing seeds, so that full reproductive potential is realized.

Reproductive Development

After flowering, the developing reproductive structures of legumes rapidly become the major sinks for photosynthate (106, 123). A bean pod may accrue over 50% of the photoassimilate from the subtending leaf (123). Szynekier (108) found that pea pods on the same reproductive structure compete with each other for the available energy with the lower pods favored.

Pate and Flinn (89) and Stephenson and Wilson (106), using field peas and soybeans, respectively, demonstrated that mobilization of

stored carbon and nitrogen takes place to a limited extent in legumes during pod development. Pate and Flinn (89) determined that 74 - 76% of the carbon assimilated during flowering and pod development was transferred to the seed, but seeds received only 2% of the carbon assimilated before flowering. In contrast, 51% of the nitrogen taken up by the plant prior to flowering was found in the seed. In cowpeas (Vigna unguiculata L.), differences were found between cultivars in the efficiency of accumulation in pods (4). Pods of lupin are also capable of limited net photosynthetic gains in the early development stages but presumably only as a reassimilation of respired CO_2 (91).

Legume pods compete strongly with nodules for the available photosynthate. Lawrie and Wheeler (66) found that in peas nitrogenase activity and accumulation of photosynthates in the nodules declined by 60% after flowering even though the photosynthetic rate doubled. Continuous removal of pods and flowers increased nitrogenase activity three-fold and photosynthate accumulation ten-fold in the nodules. Short term experiments with vegetative and flowering plants, however, produced different results. Nitrogenase activity declined 8 hours after flower removal, while in vegetative plants activity increased 8 hours after apex removal and then declined after 24 hours. It was concluded that increased nitrogenase activity does not necessarily result from increased photosynthate supply.

Photosynthesis and Nitrogen Fixation

The relationship of photosynthesis, H_2 evolution and nitrogen fixation as measured by C_2H_2 reduction has recently received much attention by Bethlenfalvay et al., (17, 18, 19, 20). They found that the net photosynthetic rate of the lower bean leaves, the major source for roots and nodules, parallels H_2 evolution of root nodules. A decline in H_2 evolution occurred at flowering but the nitrogen fixation activity reached a maximum near the end of flowering at which time the photosynthetic rate of lower leaves began to fall. The decline in nitrogen fixation resulted from reduced specific nodule activity (17).

In peas, Bethlenfalvay et al., (20) found that there was a positive association between nitrogen fixation rate and photosynthesis and carboxylation efficiency. When inoculated peas were grown with low levels of combined nitrogen ($<8 \text{ mM NH}_4^+$), the rate of photosynthesis was enhanced by the presence and activity of nodules (19). Above this nitrogen level, uninoculated plants had higher photosynthetic rates, nitrogen content and dry weight than those inoculated.

Supplemental light and depodding of soybeans (52, 63) and CO_2 enrichment of beans (18) maintained high nodule activity well past the point of normal decline probably as a result of greater photosynthate supply to the nodules. Shading and defoliation caused nitrogen fixation to decline earlier than normal (52, 63). Conversely, Sprent and Bradford (104) found that whole plant shading of broad beans maintained nodule activity for a longer period and delayed senescence. They also found that increased plant population caused a lower nodule activity per plant and made the maximum activity peak during flowering much less distinct.

Studies involving source-sink manipulations in relation to photosynthesis and nitrogen fixation are complicated by the differences that exist between natural changes in net photosynthesis and the changes which result from experimental manipulation. In beans, it has been found that 2 - 3 days were required for an increase in photosynthesis after removal of shoot apices or leaves while more rapid increase takes place with normal changes in plant morphology and metabolism (47). Similar findings with soybeans revealed that, after shading all but one leaf, 2 days elapsed before the net photosynthetic rate increased and it continued to increase until 8 days after shading when a 50% increase was observed (114).

Nitrogen fixation in beans has been shown to vary with genotype (49). Using reciprocal intervarietal grafts, the effect of soybean shoot genotype on nitrogen fixation was through the genotype effect on photosynthesis and nodule fresh weight, not specific nodule activity (64). The root genotype influenced nitrogen fixation through its effect on specific nodule activity and the susceptibility of roots to bacterial infection.

Carbohydrates

Root and Stem Carbohydrates

Root and stem carbohydrate (soluble sugar and starch) levels have been examined in several legumes. Significant differences between cultivars of beans were found in starch concentration of root hypocotyl and stem tissue at flower initiation, mid pod-fill, and physiological maturity (3, 97). Martinez and Adams (75) obtained similar results indicating that some cultivars tend to accumulate appreciable levels of starch while others do not, but this was not correlated with plant habit. Tanaka (110), however, found that determinant cultivars accumulate more stem carbohydrate during pod-fill than semi-determinant cultivars and he concluded that the determinant bean cultivars had excess photosynthetic capacity. This could mean that determinant cultivars provided insufficient sink capacity or they do not use the products of photosynthesis efficiently. An examination of several cultivars showed that nitrogen fixation rates were highest in indeterminate plants (John Halliday, CIAT, unpublished; (49). These plants stored appreciable levels of starch in the stems, apportioned a greater share of their total carbohydrate to nodules, and retained more photosynthate in soluble form.

Reproductive Development

Positive, significant correlations were found in alfalfa (Medicago sativa L.) between root carbohydrate concentration and seed production, number of pods per stem and seed per pod, percent pod set, and average seed weight (37). Other data from alfalfa indicated a positive relationship between total available carbohydrate in the root and seed yield, number of racemes per stem and pods per raceme (38). Male sterile soybeans retained green leaves until killed by frost and accumulated 1.7 - 7.6 times as much total carbohydrate in the roots as the male fertile plants, but no difference was found in nitrogen fixation

between the two genotypes (126). These results show that while pod and seed development can reduce the flow of photosynthate to the roots a lower supply of photosynthate need not affect the nitrogen fixation rate. It is possible that, in this case, the carbohydrate levels in the roots were high enough that altering the content did not affect the nodule carbohydrate levels or nitrogen fixation.

Carbohydrate supply to developing pods has been associated with bean seed size while seed number was thought to be a function of nitrogen supply (111). The concentration of water soluble carbohydrate in soybean stem and petiole tissue increased until seed began to develop and then decreased (39). It was concluded that during seed development the soluble carbohydrate is a limiting factor for seed production.

Nodulation and Nitrogen Fixation

The process of nitrogen fixation requires a large input of energy, and nodules develop on those portions of roots where the greatest content of total and reducing sugars is available (59). Silsbury (100) compared the energy requirement for assimilation of combined nitrogen and fixation of atmospheric nitrogen and found, with subterranean clover, that almost 60% more energy was required for the fixation process. Diurnal changes in the nodules of peas were studied and it was found that during the day, nodule sugar level and respiration increased while the soluble nitrogen level decreased and, at night, the reverse occurred (80). It was estimated that 2.3 times as much carbohydrate was required by nodules during the day than at night. Mederski and Streeter (77) concluded that soybean nodules in the dark maintained at least 40% of the nitrogen fixation rate of nodules in the light. They measured the CO_2 assimilation and nitrogen fixation and speculated that since nitrogen fixation rates were relatively high in the dark, nodule activity depended on both root-shoot carbohydrate reserves and current photosynthate. Peas subjected to long-term CO_2 enrichment had greater nodule deposits of starch but nitrogen

content of the plant was proportional to nodule mass. Short-term CO_2 enrichment has been shown to increase nitrogen fixation rates because of a temporary increase of carbohydrate to nodules while long-term enrichment increased plant development and nodule mass (92).

The portion of the net photosynthate sequestered by the nodules changes during the ontogeny of the legume. In cowpeas, 9% of the carbon from net photosynthesis is translocated to the nodules during fruiting. Of this, 43% is respired, 6% incorporated into dry matter, and 51% incorporated into nitrogenous compounds (54). Immediately prior to flowering, the nodules of peas received 32% of the net carbon assimilated with 16% used for growth, 38% respired, and 46% incorporated into amino compounds (79). The mg of carbon consumed per mg of nitrogen fixed was 6.8 and 8.8 for cowpeas and peas, respectively. These determinations have not been made for beans.

Carbohydrates are transported to the nodules of peas symplastically, primarily in the form of sucrose (36). Bach *et al.*, (11) examined the relationship of various sugars to nitrogen fixation activity of soybean nodules and found that adding sucrose, glucose or fructose to nodule slices produced only partial recovery of the level of fixation found in intact nodules. Slicing nodules reduced nitrogen fixation 2 - 5 fold and a maximum recovery of 60% of the sliced nodule fixation rate was obtained by adding fructose to the sliced nodules. Adding glucose or sucrose was less effective. They concluded that in addition to these carbohydrates, other unknown factors were also involved in limiting nitrogen fixation.

Studies with soybeans have attempted to characterize the carbohydrates in nodules as well as other plant parts. Chromatographic analysis of nodule carbohydrates (107) revealed that the primary carbohydrates found, in order of decreasing quantity, were myo-inositol, (+)-chiro-inositol, sucrose, α -trehalose, glucose, and (+)-pinitol (5-O-methyl-D-inositol) with traces of fructose. The nitrogen fixation rate was positively correlated to the concentration of sucrose and (+)-pinitol.

Antoniw and Sprent (9) examined bean nodules and found that both the bacteroid and host contained the same compounds. These were sucrose, fructose, glucose, an unknown carbohydrate, malic acid, and an unknown organic acid co-chromatographing with 6-phosphogluconate. These two studies point out differences between soybean and bean nodule contents; however some of the differences could be due to the methods of analysis used.

Phillips and Smith (93) found substantial quantities of (+)-pinitol, glucose, fructose and sucrose with smaller quantities of myo-inositol and 2 unidentified compounds in soybean shoot tissue. In leaf and petiole tissue of 6-week old soybeans, 50% of the soluble carbohydrate was (+)-pinitol.

Plant Population Density

General

Increasing plant population density has been an important way of raising unit area production in crops even though the competition effects on individual plant development are severe. The intense competition for light under high plant density resulted in longer internodes and reduced branching of broad beans (55), and decreased relative growth rate of several species (22). Lodging in soybeans is a problem when densities become excessive and this varies with cultivar (31, 112). Brandes et al., (24, 25) found that leaf area and number of leaves per plant in beans is inversely related to plant density but a higher number of plants more than compensated for reduced leaf area of individual plants. Leaf thickness decreased with density and the maximum plant height was attained 10 days sooner. Bean plants in high densities were reported to be more upright with the pods held higher off the ground (10) and set higher on both bean (32, 41) and broad bean (56) plants.

Reduced light, as would be found in high plant densities, has been shown to increase the shoot/root ratio in beans (27, 28). The overall

growth rate was reduced but the root growth was more severely affected than shoot growth. Increased temperatures have been found to have the same effect on beans (27) and many other species (118).

Components of Yield

As early as 1939 it was shown that higher plant populations of legumes increased unit area yields (124). Yield improvement has been produced by manipulating plant arrangement (73) and plant density, with yields reported up to 40% greater for snapbeans (10, 23, 73).

The components of yield in legumes have been extensively studied. Generally it has been found that the number of pods per plant is the most sensitive yield component and responds negatively to density (16, 24, 48, 58, 71, 73, 110, 122). Other components with significant response to density include nodes per plant (113), racemes per node (16), pods per node (113), branches per plant (16), seeds per pod (24, 40, 122), and seed weight (24, 41, 122). As plant population increased, all of the components cited tended to be reduced but the higher plant numbers per area generally more than compensated for the reductions.

Plant growth habit has shown a differential response to plant density. Crothers and Westermann (32) reported that determinant beans, but not more indeterminant types show a positive response in seed yield and harvest index to increasing density. The indeterminant beans were capable of compensating for reduced plant population with improved plant growth and yield. Kueneman et al., (61) used a wider range of plant habits and found that all types had positive yield response to reduced between-row spacing, but the determinant type showed the greatest gain. None of the plant types responded to within-row spacing. Westermann and Crothers (122) concluded that determinant habits were less subject to competitive stress and had the greatest potential for positive yield response to density. Within any particular growth habit of beans there are also cultivar differences in yield response (58, 73, 98), as well as seasonal responses (24). Cooper (31) found that early maturing soybean cultivars generally produced greater yield improvement

in higher densities than later cultivars probably as a result of a shorter period of interplant competition and less detrimental effect on individual plant development.

Reports concerning the effect of plant density on pod abscission show conflicting results with both increased (71) and decreased abscission effects (24). The data concerning maturity also shows differing effects of density. Crothers and Westermann (32) and Sprent et al., (105) found, with beans and broad beans respectively, that high density advanced maturity while Cutcliffe (33) reported no effect on bean maturity. Part of the differences found might be due to the use of different cultivars.

Nodulation and Nitrogen Fixation

Graham and Rosas (50) studied cultivars of beans with different plant habits and found that variation in nitrogen fixation was more highly correlated to nodule fresh weight than any other parameter. The indeterminant plants showed the most rapid decline in nitrogen fixation with increasing plant density. Increasing plant density reduced fresh weight and tended to increase carbohydrate content of roots, stems and upper leaves while reducing it in the lower leaves.

Sprent and Bradford (104) found a peak in nitrogen fixation activity just after flowering in broad beans with a low plant population. A higher population obscured this peak, generally reduced the activity per plant and caused environmental factors such as water supply to be more important than plant density. Weil and Ohlrogge (121) found that thinning soybeans at the end of flowering increased nodule volume per plant, maintained a greater percentage of red (active) nodules and reduced the percentage of green ones (inactive), and postponed the decline in C_2H_2 reduction by several weeks. They concluded that thinning increased the supply of photosynthates available to the nodules which delayed their senescence.

Carbohydrates

Little information is available concerning the effect of plant population on carbohydrate levels in plants. Graham and Rosas (50) found that bean nodules from higher plant populations contained over 50% more total carbohydrate and as much as 100% higher concentration of soluble carbohydrates than nodules from plants grown at low density. The nodule:root ratio was also higher in the higher densities and it is possible that nodule development was less affected by high plant density than the growth of plant parts and they continued to be a strong sink for photosynthates. In alfalfa, plant population had little effect on the total available carbohydrate content of roots (38).

Varying the light available to leaves has been shown to affect both plant development and leaf function. Reduced light increases the stem internode lengths, shoot:root ratio, and decreases dry weight accumulation in beans (7). Shaded soybean leaves tend to accumulate soluble carbohydrates with reduced levels of starch (114). In rice, shaded leaves degrade proteins and accumulate amino compounds, import higher than normal levels of photosynthate from more illuminated leaves, and reduce the amount of photosynthate going to the roots (82). Shading of pea plants during pod-fill reduced yields in a manner similar to increasing plant density while shading during the vegetative stage had little effect on yields (76). This might be partly explained by the fact that photosynthate produced after flowering is more important in pod development than that produced prior to flowering.

Increasing the plant population progressively reduces the light available from the top of the canopy to the ground. In broad beans, it has been shown that the level of light at the soil surface can vary from 14% to 3% of full sunlight as plant density is increased from 12 to 65 plants/m² with the proportion of the leaves receiving 10% or less of full sun ranging from less than 3% to 38% (56). Tamaki et al., (109) found that in broad beans the photosynthetic rate of upper leaves decreased as plant populations increased. The higher densities

resulted in more rapid leaf senescence and seed maturity as well as higher night respiration rates of leaves and pods.

Mulch

The use of straw mulches has altered soil temperature at varying depths. Waggoner et al., (119) demonstrated that the maximum soil temperature was lowered and the diurnal fluctuation decreased using a hay mulch. Davies (34) noted that air temperatures above a straw mulch are also lowered. Almost every trial with an organic mulch showed a reduction of maximum soil temperatures at 5 or 10 cm depths within the range of 2.5 to 9.0°C (12, 13, 29, 42, 51, 60, 62, 84, 99).

Reynolds (95) found that high soil temperatures in beans were associated with poor germination, restricted nodulation and reduced yields. Applying a mulch lowered soil temperature and doubled yields. Research has established that the processes of infection, nodulation, and nitrogen fixation all have separate temperature optimums and specific temperature sensitivities (14, 46, 102). In forage crops, high soil temperatures reduced nitrogen fixation as well as total nonstructural carbohydrates and root and plant dry weight (15).

Other alterations of the soil microclimate by mulch are reduced loss of soil moisture (12, 13, 29, 42, 62, 99), enhanced water intake (1), and reduced soil evaporation losses (1, 2, 5). Except where soil moisture is excluded as a limiting factor, it is very difficult to separate the moisture and temperature effects on parameters of plant growth (12).

Sprent et al., (105) concluded that water supply may be more important in controlling yield of broad beans than either light or plant competition. In beans, as water stress is increased, net photosynthesis and ribulose-1,5 diphosphate carboxylase activity decrease and stomatal and mesophyll resistance increase (85). Water stress alters assimilate distribution in soybeans (101). More ¹⁴C-photosynthate was translocated to the nodules at all stages. Boyer (23) noted that in cereals sufficient tissue dessication could occur in the

absence of visible symptoms to reduce gross photosynthesis, transpiration, impair chloroplast function, and cause stomatal closure. It appeared that the major effects were on the capacity of the source to produce photosynthate and the capacity of the sink to accept the photosynthate. The ability of the plant to translocate the photosynthate was less impaired.

A high degree of correlation was found between nitrogen fixation in broad beans and soil water content (103). Nodules on plants which had been subjected to water stress were able to recover from stress in one hour after relief of stress; however, if all nitrogenase activity had stopped prior to the relief of stress, the nodule activity never recovered.

Nogueria et al., (83) increased the yield of beans over 50% using rice-hull mulch in years when soil water was limited to production. Miller (78) found that mulching beans more than doubled plant fresh weight but increased pod weight less than 25%.

Mulching corn resulted in higher yields (12, 60, 62), reduced vertical distribution of roots and increased lateral distribution of roots (29, 84), and improved nutrient uptake (60, 84). In alfalfa, mulching increased plant height and delayed maturity (42). In barley, plant height, dry matter production, number of tillers, and number of panicles were increased (5). Grass seed germinated better (99) and became established quicker with a straw mulch (13).

THE EFFECT OF RICE-HULL MULCH ON NITROGEN FIXATION
AND CARBOHYDRATE PARTITIONING IN Phaseolus vulgaris L. IN THE TROPICS¹

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Additional index words. beans, soil temperature, soil moisture.

Abstract. Mulching beans (cv. P498) lowered daily 2:30 PM soil temperature, reduced fluctuation in soil temperature, and slowed moisture loss in a hot tropical lowland valley. Fresh weights of nodules, roots, stem, leaves and total plant increased 50, 38, 49, 24, and 38 percent, respectively, with mulch but mulching did not affect pod or final seed weight. Although the rate of nitrogen fixation (C_2H_2 reduction) was relatively low (never above 0.6 μ moles/plt/hr), it was as much as three times higher in mulched plants than unmulched controls and rates were positively correlated with nodule fresh weight. Mulching did not affect specific nodule activity. The concentration of soluble, insoluble, and total carbohydrate was little affected by mulching. Leaves of mulched plants, however, had a 27% higher concentration of soluble sugar than controls. The total quantity and concentration of carbohydrates in the roots and stems of both mulched and unmulched plants increased significantly during flowering and pod-fill due to increased concentration of insoluble carbohydrates and to greater weight of plant parts.

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Introduction

Satisfactory nodulation and nitrogen fixation of Phaseolus vulgaris L. are rarely obtained in the hot lowland tropics where the high soil temperatures can exceed those suitable for nodulation and nitrogen fixation, each having separate and specific temperature requirements (1, 13, and Graham, P. H. - in press). High temperatures also enhance respirational losses and reduce the availability of carbohydrate (5). Low carbohydrate availability is known to reduce both nodulation and nitrogen fixation (6, 7, 11).

A study using rice hulls as mulch was conducted at the Centro Internacional de Agricultura Tropical (CIAT) in Palmira, Colombia, in an attempt to lower soil temperature, increase nitrogen fixation and determine the effects on carbohydrate distribution, and plant development.

Materials and Methods

The bean cultivar used, 'Puebla 152' (CIAT designation P498), has been shown to nodulate abundantly and fix nitrogen at relatively high rates when grown at a high elevation in the tropics (3). It has a determinant mainstem and indeterminant branches (Type III growth habit) but does not require support.

The experiment was conducted at Palmira, Colombia, in a heavy clay soil. Based on soil mineral analyses, supplemental broadcast fertilization rates were (kg/ha): blended fertilizer 0:2.99:5.66 (using triple superphosphate and KCl), 200; ZnSO_4 , 25; Borax, 5; and Rayplex-Fe, 5. No nitrogen was applied. Seeds were surface sterilized with a calcium hypochlorite solution (1 gm $\text{Ca}(\text{ClO})_2$ + 5 ml concd HCl + 500 ml distilled H_2O), rinsed and dried. Seeds were inoculated and lime pelleted immediately before planting with CIAT strain 1057 Rhizobium phasioli.

Seeding was in rows 50 cm apart with plants thinned to 10 cm within rows. Mulched plots received a 4 cm layer of rice hulls

applied uniformly when primary leaves were fully expanded (10 days after planting). Mulched and unmulched plots were arranged in a randomized complete block design with five replications. Sprinkler irrigation was applied immediately after planting, following mulch application, and when soil moisture content dropped below 15% in the mulched plot. Insects and diseases were successfully controlled with routine applications of CIAT-recommended materials.

Soil temperature and moisture at a 10 cm depth below the soil surface were monitored at 2:30 PM three times weekly for nine weeks. Three in-row soil samples were taken at random in each plot and combined. Percent soil moisture was determined by drying a 50 gm subsample at 55°C for 24 hours. Soil pH remained at 6.3 to 6.4 throughout crop development. Beginning 57 days after planting (mid pod-fill) and daily for five days thereafter, soil temperature fluctuation was measured at 6:30 AM and 2:30 PM at 5 and 10 cm depths in the plots and in an adjacent, unplanted, weed-free, experimental area.

Plants were harvested 21, 35, 51, and 61 days after planting and at maturity on day 89. Twenty plants were selected per replicate but only the 10 plants most representative of the plot were used. After carefully removing adhering soil, plants were divided into nodulated root system, stem, leaves, and pods when present. Nitrogen fixation was estimated by acetylene reduction with the 10 nodulated root systems using the technique described by Graham and Rosas (3). Roots and nodules were then separated.

Fresh weights were taken for all plant parts and 15 gm of fresh tissue (or entire sample if less than 15 gm) immersed in 70% ethanol. Ethanol soluble and insoluble (perchloric acid extractable) carbohydrates were determined as described by Graham and Rosas (3).

Results and Discussion

In mulched plots, soil temperature at 10 cm were lower and soil moisture higher than in unmulched plots (Fig. 1) with the differences being greatest prior to canopy closure and flowering. Thus, mulching

reduced soil temperature at 2:30 PM 1.3°C and increased soil moisture 2.2% prior to flowering, 0.8°C and 0.6% after flowering, and 1.0°C and 1.2% over the entire growing season. At mid pod-fill, the measured fluctuation in soil temperature was 1.3°C lower at 10 cm depths and 1.5°C lower at 5 cm in mulched than unmulched plots (Tables 1 and 2). In bare, weed-free soil, temperature fluctuation was 6.0°C higher at 10 cm and 7.5°C higher at 5 cm than the soil with bean plants and no mulch.

Though levels of nitrogen (C_2H_2) fixation and nodulation were low (Fig. 2), significant increases were obtained in response to mulch. Since rice hulls are decomposed relatively slowly, this is unlikely due to nitrogen immobilization or solubilization of limiting elements. Nitrogen fixation rates in mulched plots were near zero 21 days after planting, peaked near blooming (35 days), declined slightly by day 51 (early pod-fill) and were near zero by 61 (mid pod-fill). The nitrogen (C_2H_2) fixation rate of mulched plants was three times that of unmulched plants at full bloom and, at early pod-fill, was still more than double the unmulched rate. Nodule development followed a pattern similar to nitrogen fixation (Fig. 2), and the two were positively correlated ($r = 0.80$). Nodule weights were over 50% higher for mulched plants at bloom compared to controls and over five times greater at early pod-fill.

In this study, the 2:30 PM soil temperatures in unmulched plots consistently exceeded 25°C and could have been inhibitory to nodulation and nitrogen fixation (1, 13). Although soil temperatures in mulched plots were only $0.8 - 1.2^{\circ}\text{C}$ lower, this difference could be a major factor in the better nodulation and N_2 fixation of the mulched treatments (2). Highest soil temperatures were obtained before canopy closure when nodule development should have been most active. In other research with this cultivar (4) nodule distribution was altered by plant population density and this may also be related to soil temperature.

Soil moisture levels were different in the mulched and unmulched plots during the first 2.5 weeks after mulch application. The higher

soil moisture levels in the mulched plots could have had some beneficial effect on both nodulation and nitrogen fixation but these were probably small in comparison to temperature effects.

Mulching increased root, stem, leaf, and total plant fresh weights by 38, 49, 24, and 38 percent, respectively, but did not influence pod or final seed weight (Fig. 2). Root and leaf fresh weights for all treatments increased significantly until bloom when root weight declined and leaf weight remained unchanged. Stem and total plant weight, for both treatments, continued to increase until late pod-fill and declined as senescence became more evident. Yields are usually not increased by mulching to the extent that vegetative parts are increased (9, 10, 12).

The total amounts of nonstructural carbohydrates of all plant parts were higher in the mulch treatment but the increases in roots (37%) and leaves (42%) were significant (Fig. 4). Total carbohydrates in the roots and stems of all treatments increased with the ontogeny of the plant because of increased weight of the plant parts and higher concentration of total carbohydrate (Table 3). Mulching had no effect on the level of total carbohydrates and the data were combined for stage of development.

The increase in the concentration of total carbohydrates resulted from a rise in the concentration of the ethanol insoluble carbohydrates in both the root and stem, whereas, the concentration of soluble carbohydrates from both treatments remained unchanged throughout plant development (Table 3). The accumulation of the insoluble fraction during pod-fill is consistent with other findings (3, 4, 14) indicating either excess photosynthetic capacity or inefficiency in mobilizing reserves.

The concentration of insoluble carbohydrates in leaves did not change with plant development but the soluble carbohydrate decreased significantly (30%) after bloom (Table 3), indicating a more rapid utilization of carbohydrates by the increasing reproductive sinks and fewer young leaves. Mulching increased the concentration of leaf soluble carbohydrates 27% (data not presented) with a concomitant 24%

increase in leaf weight. There was no additional carbohydrate demand from reproductive structures since neither pod nor seed weight were significantly affected by mulching. The increased concentration of soluble carbohydrates is consistent with the concept that a lower sink capacity for photosynthate results in reduced leaf export (8). In addition, the lower mid-day temperatures in mulched plots probably resulted in lower respirational losses from roots during the day.

The results indicate that the use of a rice-hull mulch is effective in reducing the mid-day and daily fluctuation of soil temperature, increasing the level of nodulation, nitrogen fixation rate, and plant fresh weight. Yields in this study were not increased but the application of greater quantities of mulch may be more beneficial as shown by Nogueira et al., (10). The major effect of the mulch on soil temperature and moisture occurred prior to bloom and this may account for the vegetative response to mulching. The failure of mulch to influence yield may be related to its lack of influence on environmental parameters during the reproductive stage.

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Table 1. The daily fluctuation of soil temperature ($^{\circ}\text{C}$) at 5 cm in beans with rice-hull mulch, no mulch, and in weed-free bare soil beginning 57 days after planting (mid pod-fill).

| Day | Time ^z | Mulch | | No Mulch | | Bare Soil | |
|------|-------------------|----------------------------|--------------------------|---------------|--------------------------|---------------|--------------------------|
| | | Temp | $^{\circ}\text{C}\Delta$ | Temp | $^{\circ}\text{C}\Delta$ | Temp | $^{\circ}\text{C}\Delta$ |
| 57 | PM | 26.7 \pm .2 ^y | | 27.3 \pm .4 | | 36.7 \pm .2 | |
| | | | 5.1 \pm .4 | | 6.2 \pm .6 | | 15.5 \pm .2 |
| 58 | AM | 21.6 \pm .2 | | 21.1 \pm .2 | | 21.2 \pm .1 | |
| | | | 4.0 \pm .3 | | 4.8 \pm .4 | | 12.1 \pm .1 |
| | PM | 25.6 \pm .2 | | 25.9 \pm .3 | | 33.3 \pm .2 | |
| | | | 4.0 \pm .2 | | 4.9 \pm .8 | | 11.6 \pm .4 |
| 59 | AM | 21.6 \pm .2 | | 21.0 \pm .5 | | 21.7 \pm .4 | |
| | | | 4.8 \pm .5 | | 6.9 \pm .8 | | 15.7 \pm .4 |
| | PM | 26.4 \pm .3 | | 27.9 \pm .5 | | 37.4 \pm .2 | |
| | | | 3.2 \pm .4 | | 5.0 \pm .4 | | 13.9 \pm .3 |
| 60 | AM | 23.2 \pm .1 | | 22.9 \pm .3 | | 23.5 \pm .3 | |
| | | | 3.0 \pm .3 | | 4.0 \pm .2 | | 10.7 \pm .5 |
| | PM | 26.2 \pm .3 | | 26.9 \pm .3 | | 34.2 \pm .3 | |
| | | | 3.2 \pm .3 | | 4.9 \pm .4 | | 11.3 \pm .3 |
| 61 | AM | 23.0 \pm .1 | | 22.0 \pm .1 | | 22.9 \pm .1 | |
| | | | 3.1 \pm .3 | | 5.3 \pm .4 | | 11.2 \pm .3 |
| | PM | 26.1 \pm .3 | | 27.3 \pm .3 | | 34.1 \pm .3 | |
| Mean | | | 3.8 \pm .4 | | 5.3 \pm .4 | | 12.8 \pm .9 |

^z 6:30 AM, 2:30 PM

^y Mean \pm SE

Table 2. The daily fluctuation of soil temperatures ($^{\circ}\text{C}$) at 10 cm in beans with rice-hull mulch, no mulch, and in weed-free bare soil beginning 57 days after planting (mid pod-fill).

| Day | Time ^z | Mulch | | No Mulch | | Bare Soil | |
|------|-------------------|-----------------------|-----------------|----------|-----------------|-----------|-----------------|
| | | Temp | ^o CA | Temp | ^o CA | Temp | ^o CA |
| 57 | PM | 25.8 ±.1 ^y | | 26.2 ±.2 | | 34.8 ±.3 | |
| | | | 3.0 ±.3 | | 4.2 ±.3 | | 11.7 ±.4 |
| 58 | AM | 22.8 ±.2 | | 22.0 ±.1 | | 23.1 ±.1 | |
| | | | 2.2 ±.2 | | 3.2 ±.2 | | 7.9 ±.2 |
| | PM | 25.0 ±.2 | | 25.2 ±.3 | | 31.0 ±.1 | |
| | | | 2.9 ±.2 | | 3.3 ±.5 | | 9.9 ±.2 |
| 59 | AM | 22.1 ±.3 | | 21.9 ±.2 | | 21.1 ±.2 | |
| | | | 3.2 ±.6 | | 4.7 ±.5 | | 13.6 ±.4 |
| | PM | 25.3 ±.3 | | 26.6 ±.4 | | 34.7 ±.3 | |
| | | | 1.5 ±.2 | | 3.5 ±.4 | | 10.2 ±.3 |
| 60 | AM | 23.8 ±.2 | | 23.1 ±.1 | | 24.5 ±.1 | |
| | | | 1.8 ±.2 | | 3.1 ±.1 | | 7.2 ±.5 |
| | PM | 25.6 ±.2 | | 26.2 ±.2 | | 31.7 ±.4 | |
| | | | 1.9 ±.1 | | 3.2 ±.1 | | 7.5 ±.2 |
| 61 | AM | 23.7 ±.2 | | 23.0 ±.1 | | 24.2 ±.1 | |
| | | | 2.0 ±.1 | | 3.5 ±.2 | | 9.0 ±.2 |
| | PM | 25.7 ±.1 | | 26.5 ±.3 | | 33.2 ±.3 | |
| | | | | | | | |
| Mean | | | 2.3 ±.3 | | 3.6 ±.3 | | 9.6 ±1.0 |

^z 6:30 AM, 2:30 PM

^y Mean \pm SE

Table 3. The effect of stage of plant development (days after planting) on the concentration of soluble, insoluble, and total nonstructural carbohydrates in roots, stems, and leaves of beans.

| Plant Part | Carbohydrate Fraction | <u>Days After Planting</u> | | | <u>LSD 5%</u> |
|------------|-----------------------|----------------------------|-----------|-----------|---------------|
| | | <u>35</u> | <u>51</u> | <u>61</u> | |
| | | % of residue dry wt | | | |
| Root | Soluble | 2.38 | 2.08 | 2.04 | ns |
| | Insoluble | 3.91 | 6.94 | 9.72 | 1.56 |
| | Total | 6.29 | 9.02 | 11.76 | 1.28 |
| Stem | Soluble | 4.41 | 3.28 | 3.60 | ns |
| | Insoluble | 5.55 | 7.87 | 13.68 | 3.10 |
| | Total | 9.96 | 11.15 | 17.28 | 2.85 |
| Leaf | Soluble | 3.40 | 2.51 | 2.38 | 0.75 |
| | Insoluble | 4.47 | 3.81 | 4.89 | ns |
| | Total | 7.87 | 6.32 | 7.26 | ns |

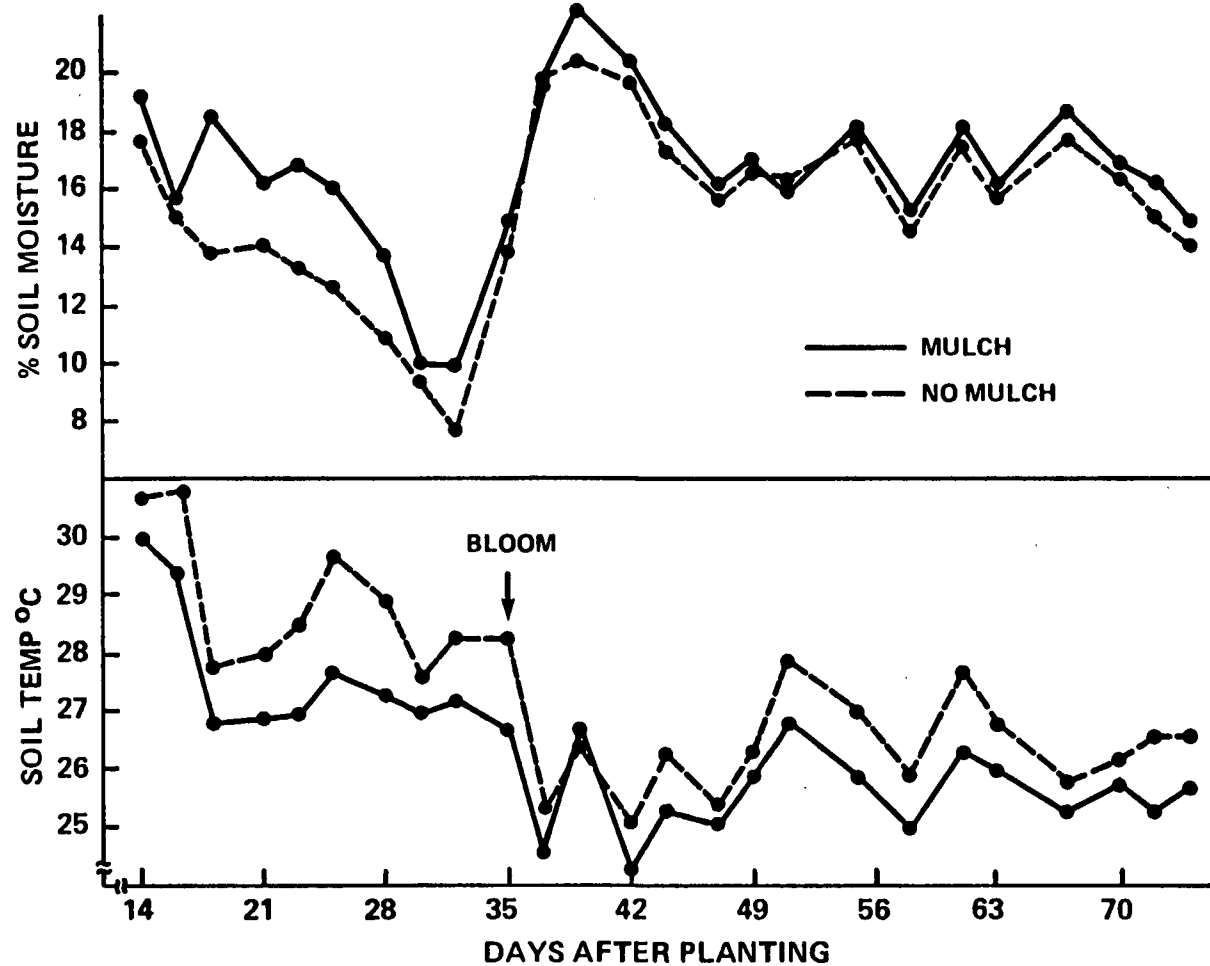


FIGURE 1
THE EFFECT OF RICE HULL MULCH ON SOIL TEMPERATURE AT
10 cm DEPTH AND SOIL MOISTURE CONTENT IN
THE TOP 10 cm SOIL IN BEANS

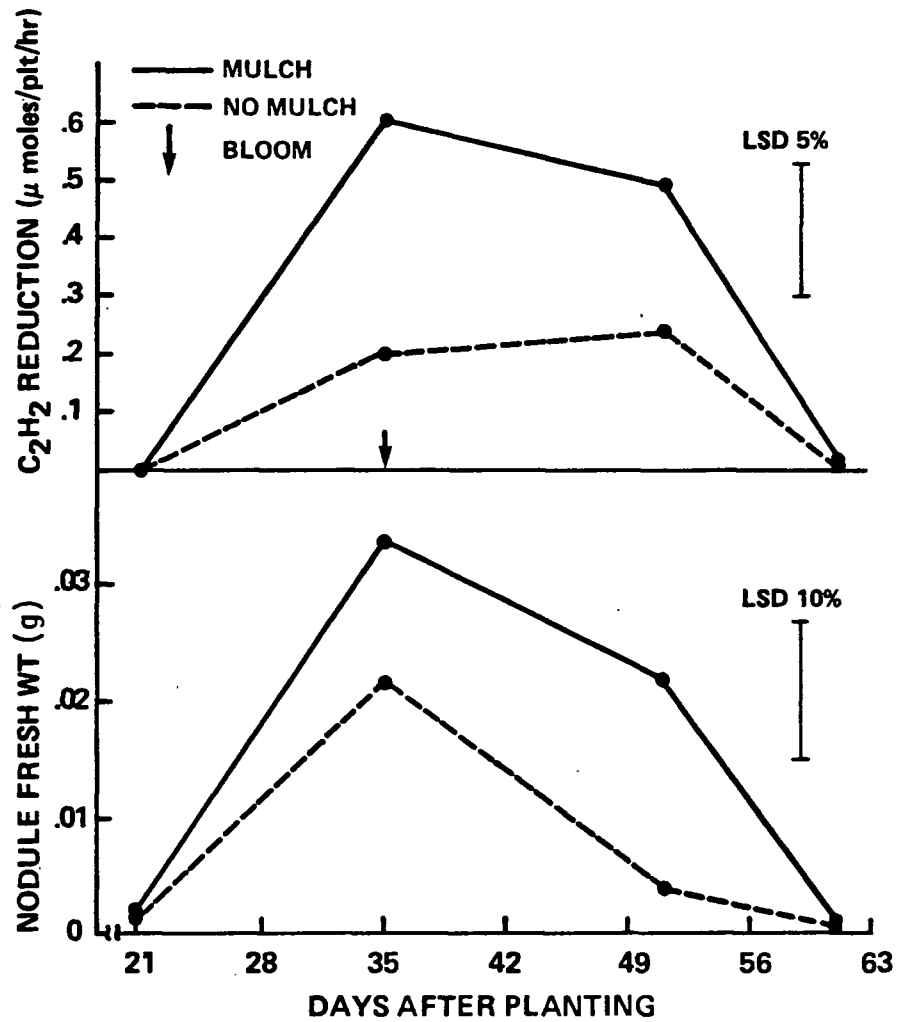


FIGURE 2
THE EFFECT OF RICE HULL MULCH ON NODULE FRESH WEIGHT
AND NITROGEN FIXATION IN BEANS

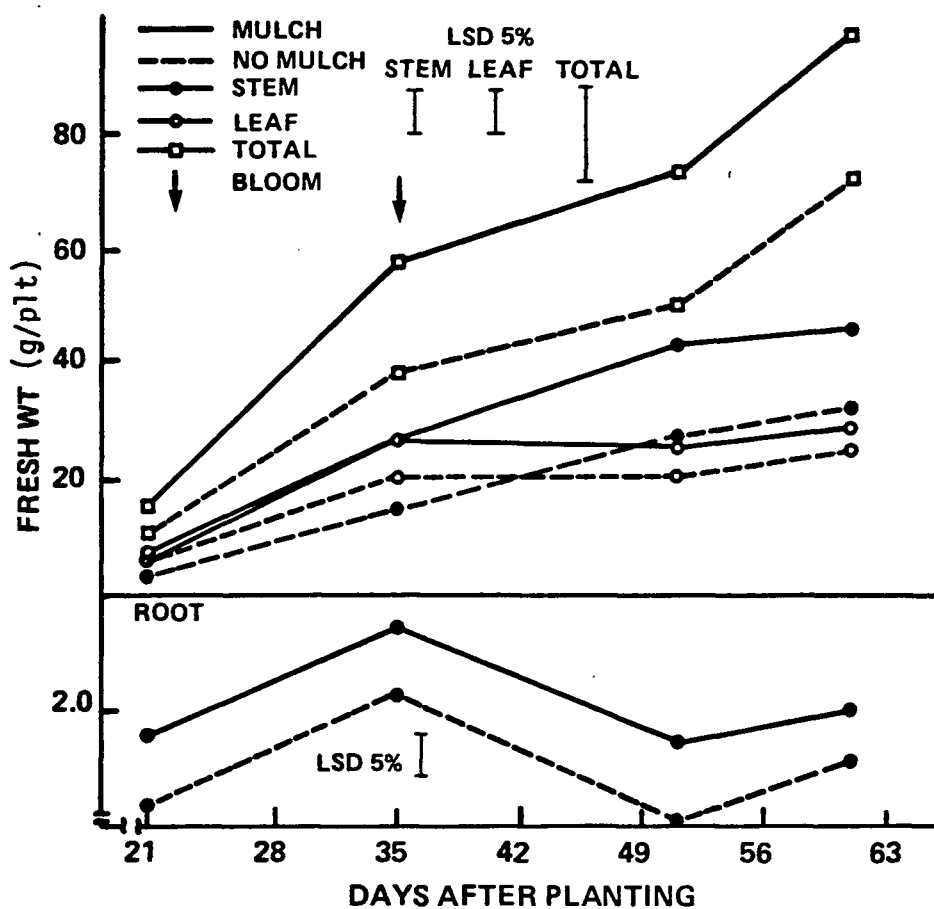


FIGURE 3
THE EFFECT OF RICE HULL MULCH
ON FRESH WEIGHT OF BEAN PLANTS

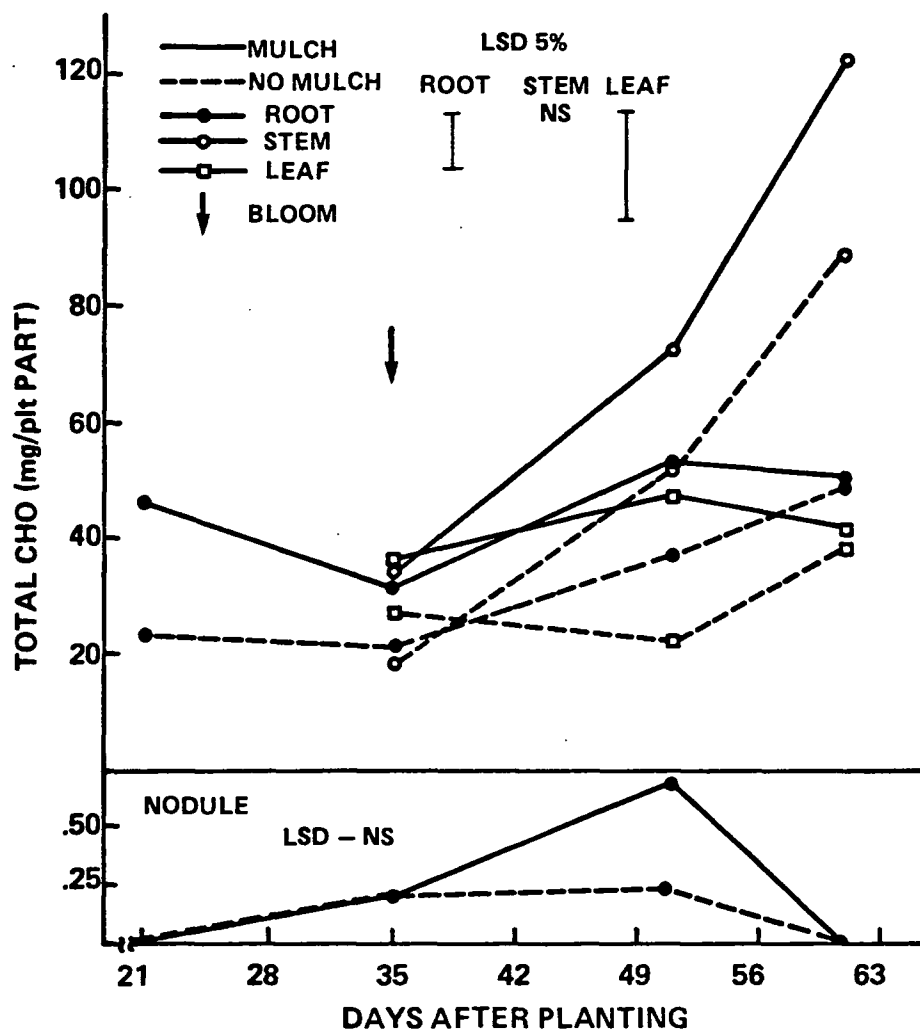


FIGURE 4
THE EFFECT OF RICE HULL MULCH ON THE TOTAL (QUANTITY)
CARBOHYDRATE CONTENT OF BEANS

THE EFFECT OF TROPICAL LOCATION AND PLANT POPULATION
ON CARBOHYDRATE PARTITIONING AND NITROGEN FIXATION
OF TWO BEAN (*Phaseolus vulgaris* L.) CULTIVARS¹

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Additional index words. shoot/root ratio, crop development

Abstract. Two tropical locations were used representing a cool, high rainfall mountainous area with a loose structured volcanic soil (Popayan) and a hot, medium rainfall tropical valley with a heavy clay soil (Palmira). At both locations, increasing plant population reduced fresh weights of all plant parts; however, shoots were more severely affected than roots. Cultivar P590 (indeterminant) had higher root weights and a lower shoot/root ratio than P498 (determinant). Nitrogen fixation rates and nodule fresh weight were over 10-fold higher, and mid-day soil temperatures at 10 cm averaged 7.8°C lower at Popayan than at Palmira. Nitrogen fixation rate was correlated to nodule fresh weight at Popayan. The concentrations of soluble and insoluble carbohydrates were higher in all plant parts at Popayan. The roots and shoot parts of P498 had higher concentrations of insoluble carbohydrates than P590, but nodule concentration was lower. Population had little effect on carbohydrate concentrations or nitrogen fixation.

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Introduction

Increasing plant population has increased unit area yields in beans (5, 14, 26). With increasing plant density, an individual plant intercepts a decreasing portion of the available radiation. Penetration of light to lower leaves, the major source of carbohydrates for lower stem, roots and nodules (10, 23) is especially reduced. Reducing light intensity to beans has reduced plant growth (including nodules), nitrogen fixation, and nodule carbohydrate levels (2, 3, 4). Research with other leguminous crops (13, 17, 19) has shown a close relationship between photosynthate supply, nodulation and nitrogen fixation. Thus, at a high plant density a bean plant may have a reduced capacity to produce nodules and fix nitrogen. This has been found in soybeans (12), peas (22), and to a limited extent in a study with beans at CIAT (Centro Internacional de Agricultura Tropical) in Colombia (11). The degree to which beans are able to provide carbohydrate (photosynthate) to the lower parts of plants grown at high density is not well understood.

The purpose of these studies was to determine the effect of plant population on carbohydrate distribution and nitrogen fixation during the development of beans with different growth habits. They were grown at two locations in Colombia, Popayan (alt. 1,850 m) and Palmira (alt. 1,000 m), to determine if the same responses could be obtained in different environments. Popayan has a mean annual temperature of 17.5°C with 1,922 mm rainfall and Palmira, 23.8°C with 989 mm rainfall (9).

Materials and Methods

Two cultivars, 'Puebla 152' and 'Cargamanto' (CIAT designated P498 and P590, respectively), which have demonstrated potential for abundant nodulation and high nitrogen fixation rates were used. P498 has a determinant mainstem and indeterminant branches, while P590 has an aggressive indeterminant climbing growth habit. These respective growth habits are classified at CIAT as Types III and IV. P498 and P590 were

seeded at two plant densities (200,000 and 600,000 plants/ha) in a randomized complete block design with five replications. At the low density, rows were spaced 50 cm apart and 10 cm between plants, at the high density, 35 cm apart and 4.75 cm between plants. Transplants were used to replace missing plants but not used for data collection.

Popayan. Soil was analyzed and fertilizer was broadcast at the following rates (kg/ha): lime, 1,000 (for pH 6.5); superphosphate, 300; KCl, 50; MgSO_4 , 100; ZnSO_4 , 7; Borax, 2; and NaMoO_4 , 1. Seeds were surface sterilized with a calcium hypochlorite solution (1 gm $\text{Ca}(\text{ClO})_2$ + 500 ml concd HCl + 500 ml distilled H_2O), rinsed and dried. Immediately prior to planting, seeds were lime pelleted with Rhizobium phaseoli (CIAT strain 1057). Insects and diseases were controlled routinely.

Plants were harvested at 21 days after planting and thereafter for five two-week intervals plus a final (maturity) harvest on day 118. All samples were harvested between 10:00 AM and noon, and soil temperatures were measured simultaneously within rows at a 10 cm depth in the center of each plot. At each harvest 10 plants each from two adjacent 1 m sections of row were removed with a shovel and 10 plants most representative of the plot selected. Soil was shaken from the roots without washing and plants were divided into nodule root systems, stems, leaves, and pods when present. The nitrogen fixation rate was estimated using acetylene reduction as described by Graham and Rosas (10). The root systems were then washed to remove clinging soil and divided into nodules and roots.

After weighing each plant part, 15 gm of fresh tissue was emersed in 70% ethanol. Ethanol soluble and insoluble (perchloric acid extractable) carbohydrates were isolated and assayed as described by Graham and Rosas (10).

Palmira. This study was conducted in essentially the same manner as that at Popayan except for fertilizer application and irrigation. The soil was analyzed and supplemental fertilizers were broadcast (kg/ha): mixed fertilizer (0:15:15 using triple superphosphate and KCl), 200; ZnSO_4 , 25; Borax, 5; and Rayplex-Fe 5. Irrigation was applied

immediately after planting and throughout crop development as required. Irrigation was not available at the Popayan site but generally is not required.

Plants were harvested at 21 days after planting and at two-week intervals for four samplings plus a final harvest at day 89.

Results and Discussion

Plant Development. At the higher plant population, individual plant weight was reduced but unit area yields were greater than at the low population as previously shown with beans (5, 14, 26). At both locations, the fresh weight of individual plant parts (Tables 1 and 2) was significantly reduced at 60 plants/m². At Palmira the reduction in nodule and pod weight was not significant due to interreplicate variability. These differences in the fresh weight of roots, stems, leaves and pods between populations were evident throughout plant development in both locations (Figs. 1 and 2). Seed weight per plant (Table 3) was reduced 50% and 41% (6.1 and 2.2 g) by higher populations at Popayan and Palmira (only P498 flowered) but unit area weights increased 51% and 77% (1253 and 805 Kg/ha), respectively. Seed yield/plant and yield/ha of P498 at Palmira were 62% and 60% (6.7 g and 2140 kg) lower than at the cooler Popayan site.

Increasing the plant population caused a relatively greater depression of shoot than root weight at Popayan as indicated by an overall 34% reduction in the shoot/root ratio (Fig. 3). At Palmira, this effect was evident only for the indeterminant cultivar P590 and the shoot/root ratio was only reduced 11%. The shoot/root ratio of beans is dependent on the environments (8) and higher temperatures tend to increase the shoot/root ratio (7, 18, 24). The mid-day soil temperatures at Palmira averaged 7.8°C higher than at Popayan. The shoot/root ratios observed at Palmira, averaged over the entire experiment, were 127% higher than at Popayan although the maximum ratio attained was not greatly different between the locations. The differences in

shoot/root ratios were due to higher stem (37.5%) and leaf (16.9%) weight and lower root weight (55.5%) at Palmira compared to Popayan.

Nitrogen Fixation. The nodule fresh weights and nitrogen fixation rates at Popayan were over 10-fold higher than at Palmira (Fig. 4). Consistent with other data (11), the nitrogen fixation rate and fresh weight of nodules were correlated at both Popayan ($r = .88$) and Palmira ($r = .70$). Specific nodule activity was not correlated with any plant weight or carbohydrate concentration. Plant population and cultivar had no significant effect on nitrogen fixation unlike other reports (10, 22).

It is possible that the plants subjected to higher temperatures at Palmira translocated their photosynthate preferentially to shoot parts, as suggested by the higher shoot/root ratio (24), resulting in less photosynthate available to roots and nodules and reduced nodule weight and nitrogen fixation. The concentration of ethanol insoluble (Tables 4 and 5, Fig. 5) and soluble (Tables 6 and 7) carbohydrates was 35 and 2.3 times higher, respectively, in nodules at Popayan than Palmira. The difference in nodule carbohydrate concentration between locations was higher than in any other plant part. Nitrogen fixation is limited both by nodule development, as indicated in this study and others (11, 25), and by the photosynthate available to the nodules (14). Reduced transport of photosynthate to the nodules could lower fixation rates. Clearly, the lower fixation rates at Palmira are due to poor nodule development, possibly partially attributed to low carbohydrate availability (2, 4, 12, 13). The fixation levels obtained in this study were comparable to those obtained in some previous studies (4, 11) and lower than the rates from other studies from Phaseolus vulgaris (10) and Vicia faba (22).

The optimum soil temperature for nitrogen fixation is reported to be approximately 17 - 21°C which is the same as observed at Popayan (6, 21). The optimum temperature for nodulation is less clear. Barrios (6) and Small (21) reported optimum nodulation temperatures of 25°C.

The mean, mid-day soil temperature, measured at Palmira was 27.5°C which is clearly above the optimum for nodulation and nitrogen fixation and was likely a major factor in the low N₂ fixation recorded at this site.

Carbohydrates. Plant population had no effect on the concentration of ethanol insoluble or soluble carbohydrates in vegetative plant parts at Palmira (Tables 5 and 7) and had only limited effects at Popayan (Tables 4 and 6). Cultivar, however, had a significant influence on the soluble concentrations of all plant parts in both locations, except for nodules at Palmira (Tables 4 and 5). It has been previously observed that the concentration of carbohydrate reserves in bean stems and roots varies with cultivar and plant habit (1, 11, 15, 20). P590 (indeterminant) had higher insoluble carbohydrate concentrations in the nodules and lower concentrations in all other plant parts than the determinant P498. This relationship was the same at both locations except that the nodule differences at Palmira, although still higher in P590, were not statistically significant. Unlike other data (11, 23), the insoluble carbohydrates did not accumulate continuously throughout crop development. Insoluble carbohydrate accumulated to a high concentration in the stems and roots of P498 after flowering but then declined during late pod-fill suggesting their remobilization and utilization in seed development. The highest seed yields were obtained from treatments having the higher accumulation of insoluble carbohydrates in the roots and stem immediately after flowering (Fig. 5).

The most striking differences in carbohydrate concentrations in plants were between locations. The concentrations of both ethanol insoluble and soluble fractions were lower at Palmira than Popayan but the reduction of the insoluble concentrations was as much as 10-fold greater than the soluble fraction. The higher temperature at Palmira also resulted in accelerated shoot growth as described above and indicated by the higher shoot/root ratio. This probably resulted in greater utilization of carbohydrates in the shoot and reduced their

availability to roots and nodules. Presumably respirational losses would also be greater because of the higher temperatures at Palmira (9).

Given the evidence from other studies (7, 8, 24), the increased shoot/root ratio may be related to the higher temperature at Palmira (Fig. 3); however, most striking is the substantial reduction in the concentration of carbohydrates, particularly the insoluble fraction, in all parts of plants grown in the higher temperature. This probably reflects large respirational losses of carbohydrates since the weight of plant parts was much less affected by temperature. A portion of these losses could also have resulted from the greater water stress inherent in the hotter climate.

Increased unit area yields and reduced individual plant weights are associated with increased populations (5, 14, 26), but changes in plant population did not influence nitrogen fixation and carbohydrate concentration in this study, unlike other studies (11, 23). It would be expected that a population lower than 20 plants/m², would have substantially reduced interplant competition and resulted in greater treatment differences.

The higher concentrations of insoluble carbohydrates in the stem and root tissue is consistent with other studies in which this tendency for determinant beans to accumulate stem carbohydrates (23). In the present study, the higher yield associated with the cultivar having greater carbohydrate accumulation is probably due to the cultivar used.

This work implies that soil N₂ fixation in beans in the tropics will be restricted to the cooler high altitudes unless cultivars are developed which show a less negative response to higher temperatures in carbohydrate partitioning and effective nodulation.

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Table 1. The effect of cultivar, density, and stage of development on the fresh weight of bean plant parts at Popayan, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|--------------------------------|------------------|--------------|--------------|---------------|-------------|
| | (g fresh wt/plt) | | | | |
| <u>Cultivar</u> | | | | | |
| P498 | 0.14 | 2.04 | 12.77 | 13.78 | 24.84 |
| P590 | 0.19 | 2.90 | 12.67 | 18.56 | 9.29 |
| LSD 5% | ns | 0.34 | ns | 3.08 | 4.36 |
| <u>Density</u> | | | | | |
| Low | 0.20 | 2.78 | 17.03 | 22.46 | 24.40 |
| High | 0.13 | 2.16 | 8.41 | 9.88 | 9.73 |
| LSD 5% | 0.06 | 0.34 | 1.71 | 3.08 | 4.36 |
| <u>Days After Planting</u> | | | | | |
| 21 | 0.07 | 2.72 | - | - | - |
| 34 | 0.18 | 2.78 | 4.51 | 6.84 | - |
| 46 | 0.11 | 3.34 | 9.25 | 12.98 | - |
| 60 | 0.40 | 3.30 | 15.32 | 16.53 | 0.96 |
| 75 | 0.13 | 2.42 | 23.02 | 25.65 | 17.39 |
| 90 | 0.09 | 2.49 | 18.71 | 18.84 | 32.85 |
| 116 | - | 0.23 | 5.53 | - | - |
| LSD 5% | 0.09 | 0.33 | 3.69 | 3.09 | 4.45 |
| <u>Interaction^z</u> | | | | | |
| Cv x Den | * | ** | ns | ns | * |
| Cv x Har | ** | ** | ** | ** | ** |
| Cv x Den x Har | ** | ** | * | ** | ** |

^z Significance at 5% (*), 1% (**), or nonsignificant (ns).

Table 2. The effect of cultivar, density, and stage of development on the fresh weight of bean plant parts at Palmira, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|--------------------------------|------------------|--------------|--------------|---------------|----------------|
| | (g fresh wt/plt) | | | | |
| <u>Cultivar</u> | | | | | |
| P498 | .0102 | 0.99 | 19.13 | 17.87 | 14.62 |
| P590 | .0199 | 1.21 | 21.37 | 19.95 | - ^z |
| LSD 5% | .0060 | 0.10 | ns | ns | - |
| <u>Density</u> | | | | | |
| Low | .0165 | 1.29 | 25.33 | 23.79 | 14.62 |
| High | .0136 | 0.91 | 15.04 | 14.02 | 5.12 |
| LSD 5% | ns | 0.10 | 3.23 | 2.69 | ns |
| <u>Days After Planting</u> | | | | | |
| 21 | .0025 | 0.85 | 4.56 | 9.11 | - |
| 35 | .0230 | 1.37 | 14.24 | 19.61 | - |
| 51 | .0270 | 1.43 | 29.18 | 24.57 | 0.99 |
| 63 | .0076 | 1.59 | 34.27 | 22.34 | 18.75 |
| 91 | - | 0.22 | 19.04 | - | - |
| LSD 5% | .0090 | 0.18 | 3.20 | 3.47 | 13.30 |
| <u>Interaction^y</u> | | | | | |
| Cv x Den | ns | ns | ns | * | - |
| Cv x Har | ns | ns | ns | ** | - |
| Cv x Den x Har | ns | ns | * | ns | - |

^z Only cultivar P498 flowered, Den and Har data for P498 only.

^y Significance at 5% (*), 1% (**), or nonsignificant (ns).

Table 3. The effect of cultivar and plant population on the seed yield of beans at two locations.

| <u>Variables</u> | <u>Popayan</u> | | <u>Palmira</u> | |
|-----------------------------|----------------|--------------|----------------|----------------|
| <u>Cultivar</u> | <u>g/plt</u> | <u>Kg/ha</u> | <u>g/plt</u> | <u>Kg/ha</u> |
| P498 | 10.88 | 3588.0 | 4.16 | 1448.0 |
| P590 | 7.59 | 2581.8 | - ^z | - ^z |
| LSD 5% | 2.55 | 357.0 | - ^z | - ^z |
| <u>Density</u> ^y | | | | |
| Low | 12.29 | 2458.2 | 5.23 | 1045.6 |
| High | 6.19 | 3711.6 | 3.08 | 1850.4 |
| LSD 5% | 2.55 | 357.0 | 1.58 | 752.2 |

^z P590 did not flower.

^y Palmira density data for P498 only.

Table 4. The effect of cultivar, density, and stage of development on the concentration of ethanol insoluble (perchloric acid extractable) carbohydrates in bean plant parts during plant development at Popayan, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|-----------------------------------|----------------|--------------|--------------|---------------|-------------|
| (% ETOH extracted residue dry wt) | | | | | |
| <u>Cultivar</u> | | | | | |
| P498 | 8.88 | 8.71 | 19.03 | 13.15 | 27.39 |
| P590 | 11.43 | 7.64 | 14.91 | 11.42 | 14.72 |
| LSD 5% | 1.41 | 0.92 | 2.17 | 0.90 | 2.83 |
| <u>Density</u> | | | | | |
| Low | 9.87 | 8.07 | 15.36 | 11.97 | 21.05 |
| High | 10.45 | 8.28 | 18.58 | 12.60 | 21.06 |
| LSD 5% | ns | ns | 2.17 | ns | ns |
| <u>Days After Planting</u> | | | | | |
| 21 | 7.79 | 5.86 | - | - | - |
| 34 | 9.87 | 6.81 | 12.21 | 11.61 | - |
| 46 | 9.74 | 4.94 | 8.43 | 10.98 | - |
| 60 | 13.83 | 10.70 | 19.28 | 15.22 | 5.25 |
| 75 | 12.04 | 13.88 | 23.33 | 12.15 | 21.15 |
| 90 | 7.69 | 10.64 | 20.59 | 11.47 | 36.77 |
| 116 | - | 4.40 | - | - | - |
| LSD 5% | 2.23 | 1.98 | 2.92 | 2.04 | 2.93 |
| <u>Interaction^z</u> | | | | | |
| Cv x Den | ns | ns | ns | ns | ns |
| Cv x Har | ** | ** | ** | ** | ** |
| Den x Har | ns | * | ns | ns | ns |
| Cv x Den x Har | ns | ns | ns | ns | ns |

^z Significance at 5% (*), 1% (**), or nonsignificant (ns).

Table 5. The effect of cultivar, density, and stage of development on the concentration of ethanol insoluble (perchloric acid extractable) carbohydrates in bean plant parts during plant development at Palmira, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|--------------------------------|-----------------------------------|--------------|--------------|---------------|-------------|
| | (% ETOH extracted residue dry wt) | | | | |
| <u>Cultivar</u> | | | | | |
| P498 | 0.11 | 4.57 | 7.98 | 2.89 | 14.83 |
| P590 | 0.47 | 3.06 | 4.39 | 1.97 | 0 |
| LSD 5% | ns | 0.68 | 0.98 | 0.76 | 2.25 |
| <u>Days After Planting</u> | | | | | |
| 21 | - | - | - | - | - |
| 35 | 0 | 4.22 | 5.79 | 3.01 | - |
| 51 | 0.69 | 3.68 | 5.11 | 2.23 | 3.55 |
| 63 | 0.18 | 5.09 | 7.66 | 2.06 | 26.12 |
| 91 | - | 2.26 | - | - | - |
| LSD 5% | 0.50 | 0.90 | 1.46 | 0.74 | 2.08 |
| <u>Interaction^z</u> | | | | | |
| Cv x Den | ns | ns | ns | ns | ns |
| Cv x Har | ns | ** | ** | ns | ** |
| Den x Har | ns | ns | ns | ns | ns |
| Cv x Den x Har | * | ns | ns | ns | ns |

^z Significance at 5% (*), 1% (**), or nonsignificant (ns).

Table 6. The effect of cultivar, density, and stage of development on the concentration of ethanol soluble carbohydrates in bean plant parts during plant development at Popayan, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|--------------------------------|-----------------------------------|--------------|--------------|---------------|-------------|
| | (% ETOH extracted residue dry wt) | | | | |
| <u>Density</u> | | | | | |
| Low | 2.25 | 2.47 | 6.05 | 5.40 | 10.71 |
| High | 2.20 | 2.12 | 5.82 | 5.88 | 7.52 |
| LSD 5% | ns | 0.25 | ns | 0.47 | ns |
| <u>Days After Planting</u> | | | | | |
| 34 | 1.62 | 2.12 | 4.62 | 5.83 | - |
| 46 | 0.73 | 2.84 | 6.06 | 4.77 | - |
| 60 | 2.49 | 2.69 | 6.54 | 6.34 | 5.36 |
| 75 | 2.51 | 2.13 | 6.02 | 6.27 | 13.18 |
| 90 | 3.76 | 2.90 | 6.43 | 4.98 | 8.80 |
| 116 | - | 1.09 | - | - | - |
| LSD 5% | 0.84 | 0.41 | 0.89 | 0.93 | 3.59 |
| <u>Interaction²</u> | | | | | |
| Cv x Den | ns | ns | ns | ns | ns |
| Cv x Har | ** | ** | ** | ns | ** |
| Den x Har | ns | ns | ** | ns | ns |
| Cv x Den x Har | ns | ns | ns | ns | ns |

² Significance at 5% (*), 1% (**), or nonsignificant (ns).

Table 7. The effect of cultivar, density, and stage of development on the concentration of ethanol soluble carbohydrates in bean plant parts during plant development in Palmira, Colombia.

| <u>Variables</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | <u>Pods</u> |
|--------------------------------|-----------------------------------|--------------|--------------|---------------|-------------|
| | (% ETOH extracted residue dry wt) | | | | |
| <u>Cultivar</u> | | | | | |
| P498 | 1.10 | 1.68 | 2.95 | 2.69 | 8.48 |
| P590 | 0.86 | 1.56 | 2.25 | 2.88 | 0 |
| LSD 5% | ns | ns | 0.59 | ns | ns |
| <u>Density</u> | | | | | |
| Low | 0.86 | 1.65 | 2.40 | 2.91 | 4.88 |
| High | 1.09 | 1.58 | 2.79 | 2.67 | 12.08 |
| LSD 5% | ns | ns | ns | ns | 6.87 |
| <u>Days After Planting</u> | | | | | |
| 21 | - | - | - | - | - |
| 35 | 1.44 | 1.65 | 2.60 | 1.85 | - |
| 51 | 1.47 | 2.25 | 2.33 | 3.24 | 1.85 |
| 63 | 0 | 1.91 | 2.86 | 3.27 | 6.63 |
| 91 | - | 0.66 | - | - | - |
| LSD 5% | 0.46 | ns | ns | 0.64 | 5.40 |
| <u>Interaction^z</u> | | | | | |
| Cv x Den | ns | ns | ns | ns | ns |
| Cv x Har | ns | ns | ns | ns | ns |
| Den x Har | ns | ns | ns | ns | ** |
| Cv x Den x Har | ns | ns | ns | * | ns |

^z Significance at 5% (*), 1% (**), nonsignificant (ns).

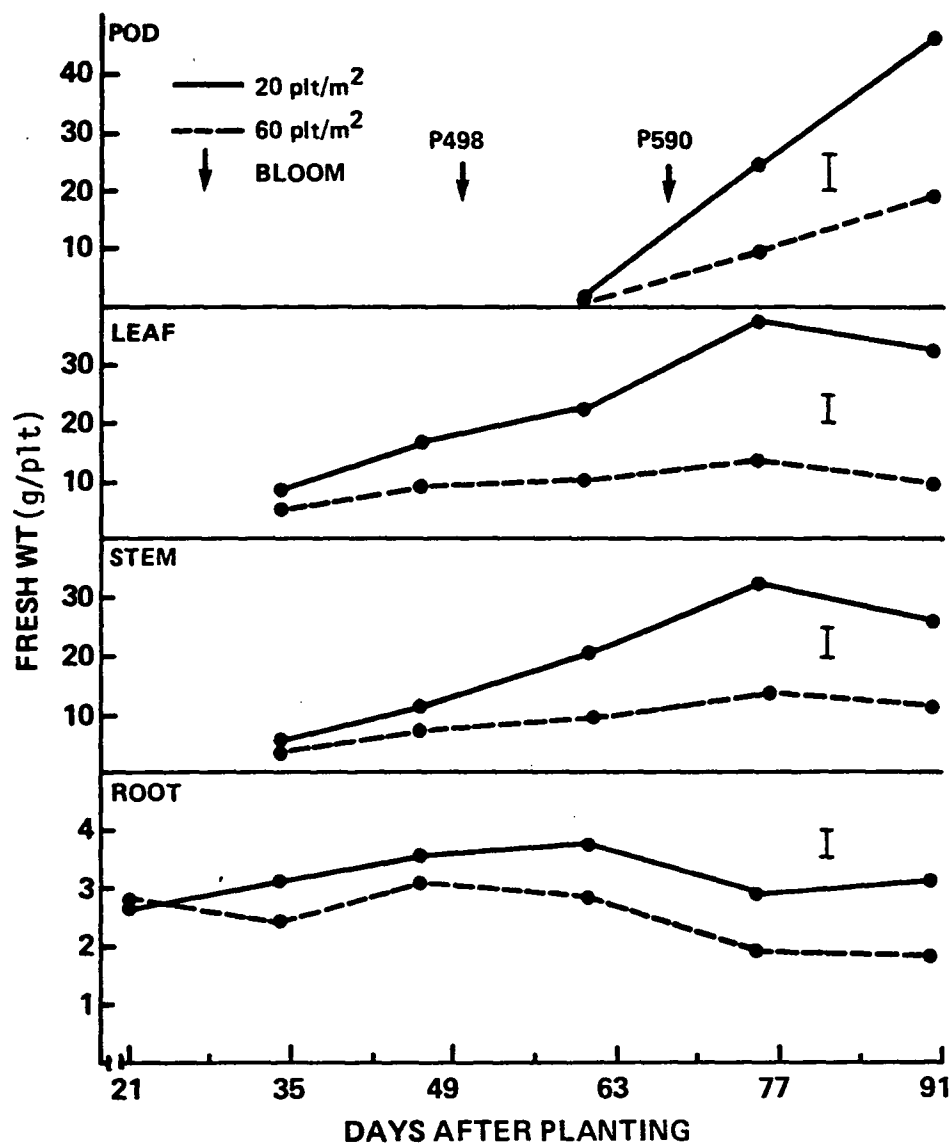


FIGURE 1
THE EFFECT OF PLANT DENSITY AND STAGE OF DEVELOPMENT ON
BEAN PLANT FRESH WT AT POPAYAN, COLOMBIA.
CULTIVAR DATA COMBINED BY DENSITY. VERTICAL BARS LSD 5%

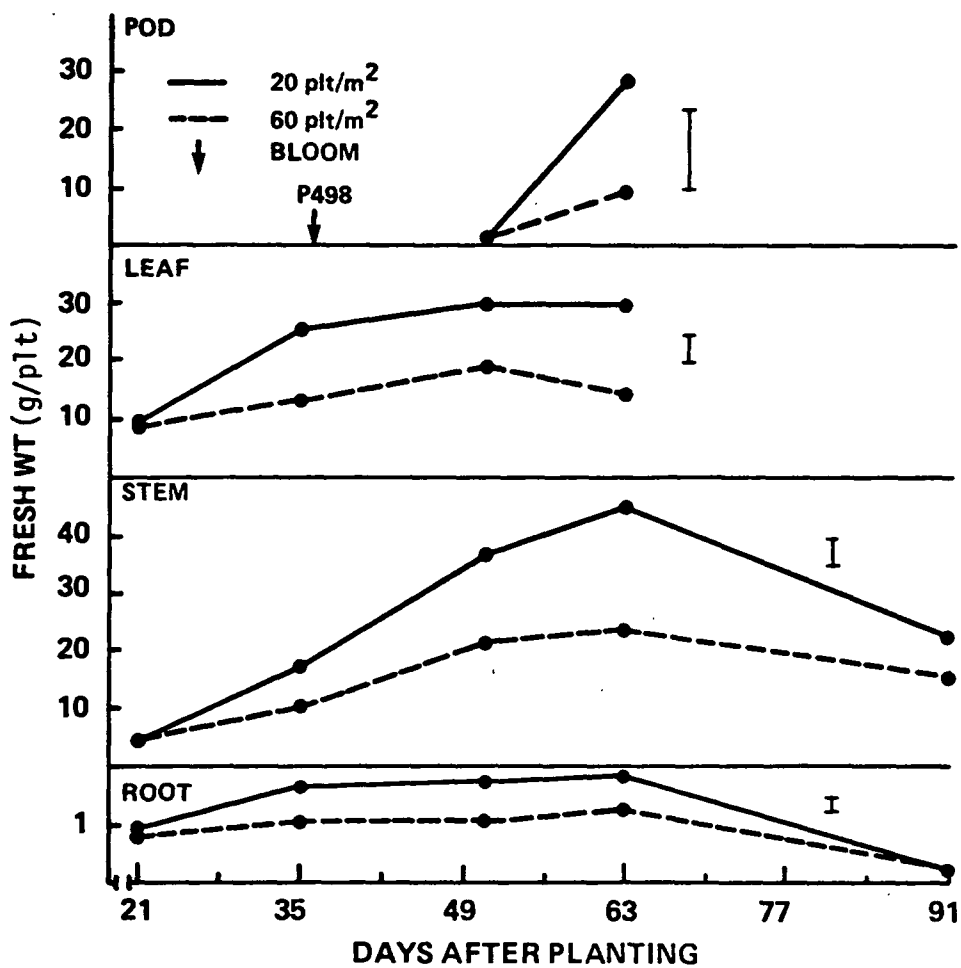


FIGURE 2
THE EFFECT OF PLANT DENSITY AND STAGE OF DEVELOPMENT ON
BEAN PLANT FRESH WT AT PALMIRA, COLOMBIA.
CULTIVAR DATA COMBINED BY DENSITY. VERTICAL BARS — LSD 5%

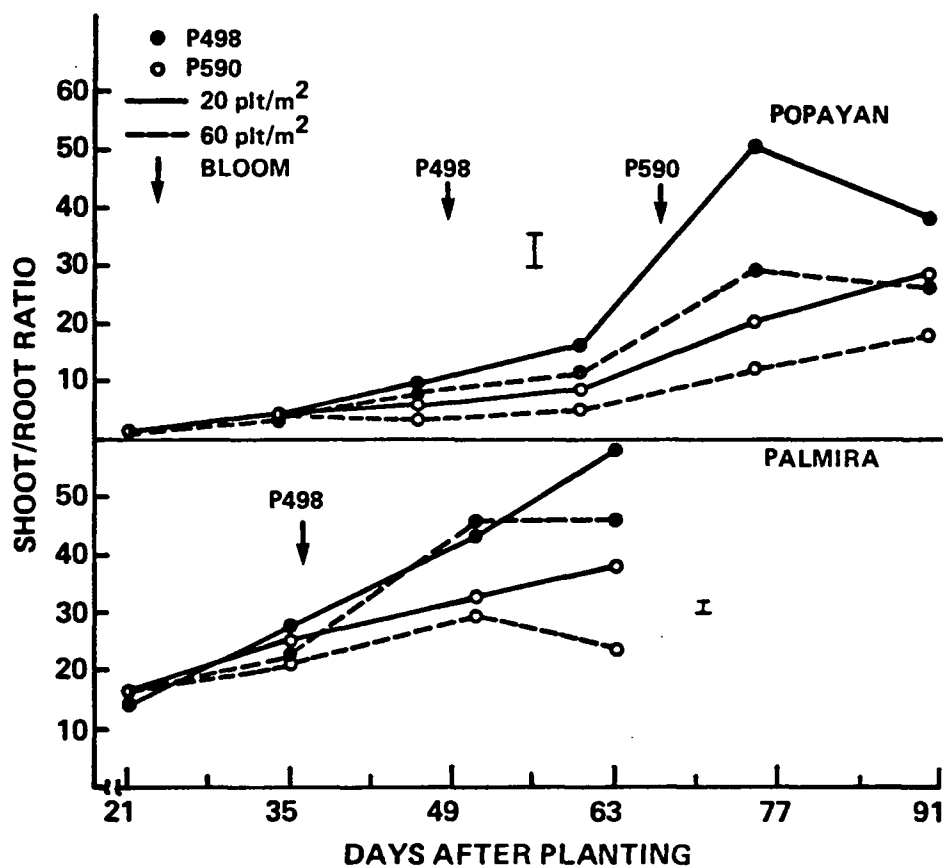


FIGURE 3
 THE EFFECT OF CULTIVAR, DENSITY AND STAGE OF DEVELOPMENT
 ON SHOOT/ROOT RATIO OF BEANS IN TWO LOCATIONS IN COLOMBIA.
 VERTICAL BAR — LSD 5%.

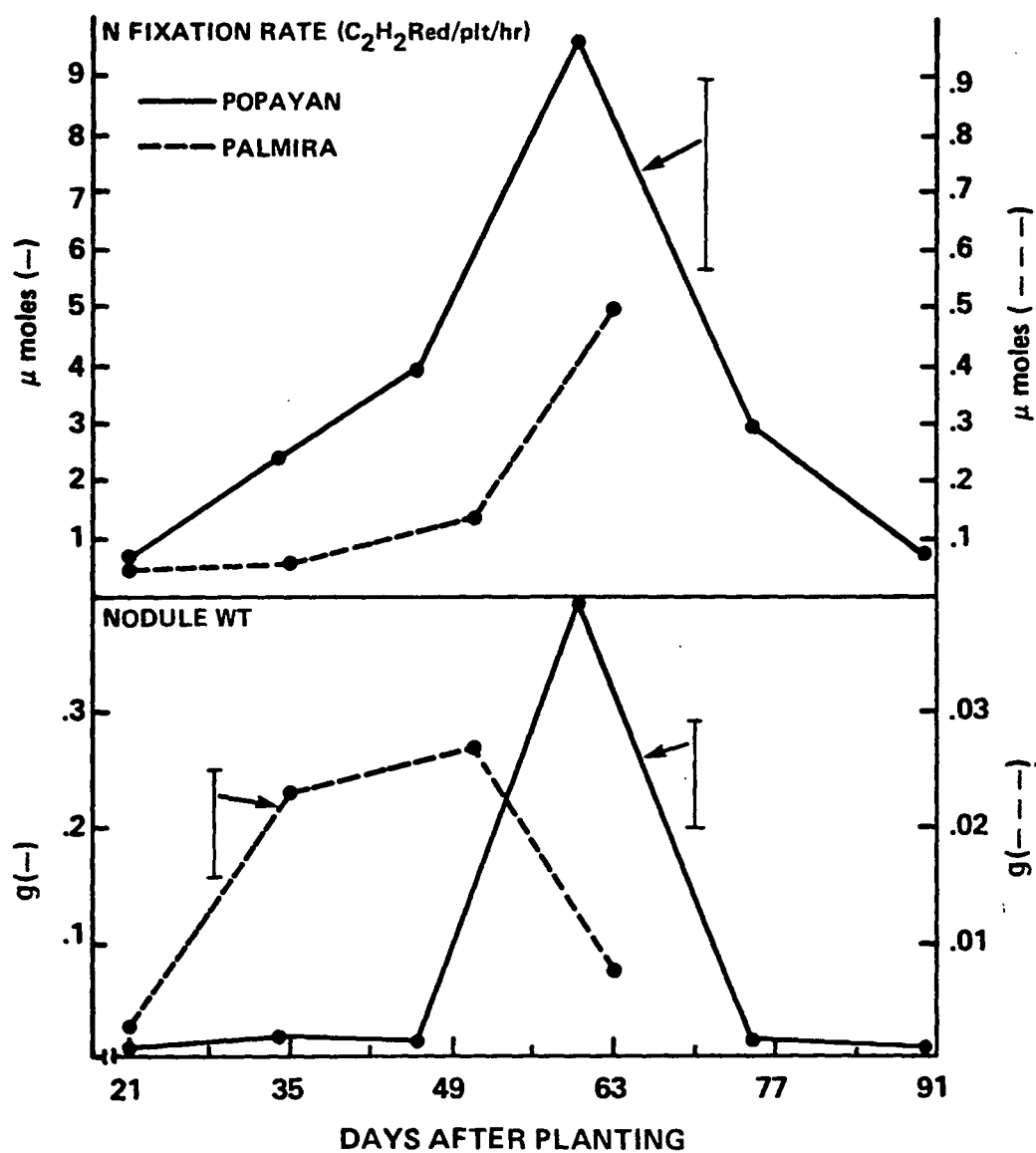


FIGURE 4
THE EFFECT OF STAGE OF DEVELOPMENT ON
NITROGEN FIXATION AND NODULE WT OF BEANS
AT 2 LOCATIONS. CULTIVAR AND DENSITY DATA
COMBINED FOR HARVEST DATES. VERTICAL BARS — LSD 5%

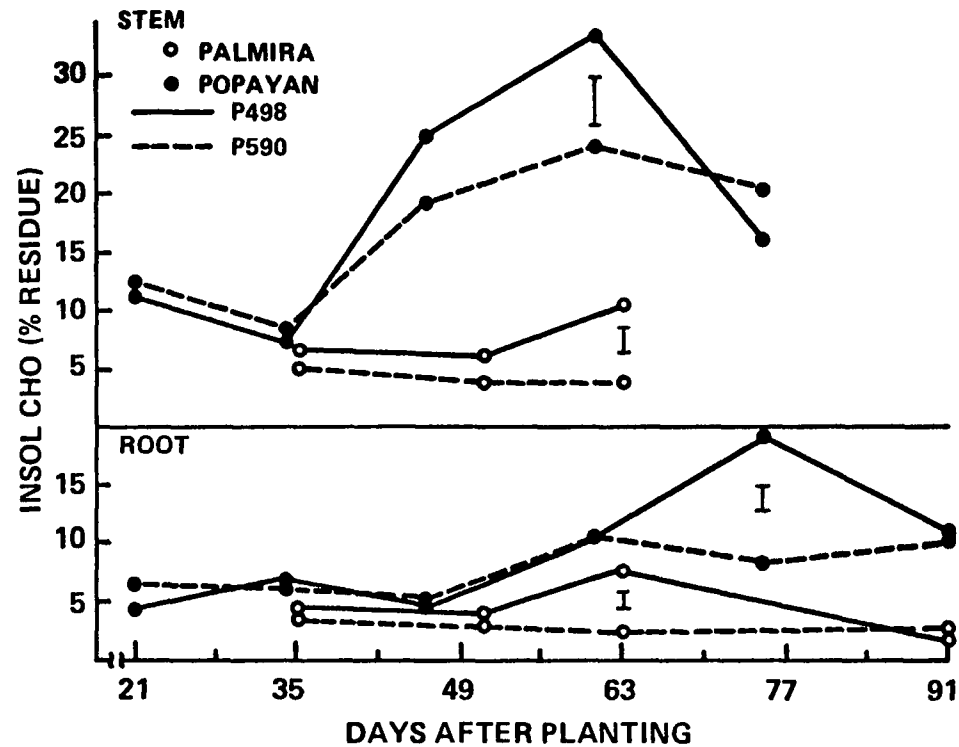


FIGURE 5
THE EFFECT OF CULTIVAR AND STAGE OF DEVELOPMENT ON
THE CONCENTRATION OF ETHANOL INSOLUBLE CARBOHYDRATES IN BEAN PLANTS
AT TWO LOCATIONS IN COLOMBIA. DENSITY DATA COMBINED FOR CULTIVARS.
VERTICAL BARS — LSD 5%.

DISTRIBUTION OF ^{14}C LABELLED PHOTOSYNTHATE AND
NITROGEN FIXATION IN Phaseolus vulgaris L. AT DIFFERENT
STAGES OF PLANT DEVELOPMENT AND PLANT POPULATIONS¹

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Additional index words. starch, sugars, translocation, plant population

Abstract. Leaves at nodes 4 or 8 of greenhouse grown beans (cv. P498) were exposed to $^{14}\text{CO}_2$ for 24 hours, 35, 48, 63, and 70 days after planting. At day 35, over 85% of the ^{14}C -photosynthate translocated from node 4 was recovered in roots, nodules and lower stem. By day 48 (flowering), the percentage of total radioactivity in the lower stem had decreased 16%, with a corresponding increase in the nodule radioactivity. Roots retained 45% of the ^{14}C -photosynthate translocated from node 8 was found in mid and upper stem and leaves at flowering. Two weeks later, over 85% of the radioactivity was found in the mid and upper pods. Nitrogen fixation rates peaked two weeks after flowering and then declined rapidly. N_2 (C_2H_2) fixation per plant and specific nodule activity (SNA) were lower than in other studies using this cultivar but there was a positive relationship between the total quantity of soluble carbohydrate in the nodules and nitrogen fixation. The concentration of starch in the lower stem increased continuously from the time of flowering while in other plant parts there was a decline at mid pod-fill. The concentration of soluble sugars in the

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nodules and roots declined at mid pod-fill while all three stem sections reached a common concentration. Plant population did not influence ^{14}C -photosynthate distribution, nitrogen fixation rate or specific nodule activity.

Introduction

Leaves on the lower nodes are the major contributors of photosynthate to roots and lower stems in beans (20, 21, 22), but data are not available on their contribution to nodules or on changes in distribution with plant age. Since canopy closure reduces light penetration to the lower leaves (10) and decreases their photosynthetic activity (2), the dependence of nodules on photosynthate from lower leaves could be a major factor limiting fixation (11,12). This study compares the contribution of leaves at nodes 4 and 8 to plant and nodule function and attempts to relate this to N_2 fixation and plant development. Data were collected from 13 days preflowering to 20 days postflowering.

Materials and Methods

The cultivar used, 'Puebla 152' (CIAT designation P498), is of a Type III growth habit with determinant mainstem and indeterminant branches. 'Puebla 152' has a demonstrated capacity for abundant nodulation and N fixation (8).

Uniform seeds were inoculated with a peat culture of Rhizobium phaseoli (CIAT strain 1057) and then planted three seeds/pot in 15 cm plastic pots containing a sterilized soil:peat (4:1) medium supplemented with 3 g hydrated lime (for pH 6.5), 500 mg triple superphosphate, and 250 mg K_2SO_4 per pot. No nitrogen fertilizer was added. Three seeds were covered with approximately 1 cm of sterilized sand. Plants were thinned to 1 per pot after primary leaves developed, and they were supported with a stake. Two plant populations were obtained by spacing pots at 45 x 15 cm, and 45 x 45 cm. The plants were grown in a greenhouse from March to June in a randomized-block design with six replications. Branches were removed as they appeared in order to restrict photosynthate movement to the mainstem and associated leaves and flowers.

Plants were selected for uniformity and removed from each density for exposure of the node-4 or 8 leaf (numbering from the cotyledonary node) to $^{14}\text{CO}_2$ at 35 (pre-flowering), 48 (flowering), 63 (early pod-fill), and 70 (mid pod-fill) days after planting. Leaf 8 had not appeared by day 35, and leaf 4 abscised by day 70. At about 11:00 AM, a leaf was sealed in a polyethylene bag along with a gel capsule containing 50 $\mu\text{Ci Na}_2^{14}\text{CO}_2$ (specific activity 20 $\mu\text{Ci}/\mu\text{M}$). While dosing, days were clear and plants were placed in indirect light to prevent leaf-damaging heat buildup in the bags. $^{14}\text{CO}_2$ was generated by injecting perchloric acid into the bag and dissolving the gel capsule. After 1 hour the bag was removed. Plants were returned to the greenhouse and harvested 23 hours later. One plant from each population replication was used for each leaf position being dosed with $^{14}\text{CO}_2$.

Harvested plants were divided into nodules, roots, and 4-node shoot sections corresponding to nodes 1-4, 5-8, and over 8. The tissue from these sections were designated S-1, S-2, and S-3 for stems; L-1, L-2, and L-3 for leaves (not including labelled leaves); and P-1, P-2, and P-3 for pods with 1 being nodes 1-4. The parts were dried at 55°C for 48 hours, weighed, and ground to 40 mesh. A 20 mg aliquot of each part was weighed and suspended in 15 ml of a scintillation cocktail (38 g Cab-O-Sil, 250 mg dimethyl POPOP, and 4 g PPO in 1 liter toluene) and ^{14}C -activity determined with a Packard Tri-Carb Scintillation Counter. Quench was monitored using an external standard and corrected for by a quench curve derived from internal standards. The ^{14}C -activity in each plant part (counts adjusted to total dry weight) is expressed as % of the total recovered activity per plant exclusive of the treated leaf.

At each harvest six plants from each density not exposed to $^{14}\text{CO}_2$ were used to estimate N fixation (C_2H_2 reduction), dry weight distribution and CHO (ethanol soluble and starch) content. Nitrogen (C_2H_2) reduction was determined using the sequence described by Graham and Rosas (7, 8) with ethylene production assayed on a gas chromatograph

using a 12-foot Poropak R column operated at 50°C with helium carrier gas. The rate of acetylene reduction ($\mu\text{moles/plt/hr}$) was calculated using peak areas. The root and nodules were then separated.

Plants from these analyses were divided, dried, weighed, and ground in the same manner as plants dosed with $^{14}\text{CO}_2$. Soluble CHO was extracted by suspending a 100 g sample above 20 ml of boiling 80% ethanol for two hours. The residue was then boiled for three minutes in 50 ml distilled H_2O and incubated three hours with 10 ml 0.25% amyloglucosidase and 1 ml of 1.0 M sodium acetate buffer (ph 4.2) in order to hydrolyze starch (16). Soluble CHO and starch were determined, using anthrone, and are expressed as % of dry weight (23). Starch values were multiplied by 0.9.

Results and Discussion

Plant Development. The plants germinated uniformly, developed and flowered normally. During early pod-fill (day 63), the leaves at node 4 and below yellowed, and many senesced and abscised by day 70. The 2:00 PM soil temperatures of the pots ranged from 20.6 to 31.8°C with the temperature in pots in the higher population averaging 1.5°C lower than in low population pots, presumably as a result of less pot surface being exposed to the sun. The highest temperatures (on clear, warm days) were above the optimum for nodulation and N fixation (4, 18).

High population plants were 17% taller, with 12% more leaf area than low population plants, probably because of greater competition for light. In contrast to other reports (3, 6, 14), plant population had no significant effect on the dry weight of any plant part and the data were combined for each stage of development (Table 1); however, the weights of vegetative plant parts except nodules were 4.1 - 15.7% greater in plants from the higher population. Dry weight of roots increased 90% during flowering and pod-fill, unlike other vegetative plant parts which either decreased or ceased to accumulate dry weight after flowering. The late root growth is not in agreement with other

data (7, 8), and may be due in part to the removal of pod-producing branches which would have resulted in less lateral plant development, a smaller shoot sink, and a greater supply of photosynthate available to the roots.

^{14}C -Distribution. Plant population did not influence the distribution of ^{14}C -photosynthate from leaves at either node 4 or 8, and the data were combined and presented for stage of development (Fig. 1a and 1b). The plant parts not shown in Fig. 1a and 1b contain less than 3% of the radioactivity in any individual plant part.

At pre-flowering, over 85% of the ^{14}C -photosynthate from the node-4 leaf was recovered in the nodules, roots and S-1 with less than 15% in all other plant parts (Fig. 1a). The results for lower stem and roots parallel those obtained in other works (5, 17, 21, 22). The roots contained 45% of the translocated radioactivity and this level of distribution continued throughout crop development. Almost 40% of the radioactivity was found in S-1 but only 3.5% in the nodules.

At flowering (day 49), the % of total radioactivity translocated from the node-4 leaf to S-1 declined to 23% while nodule radioactivity increased to 19%. Nodules are strong sinks from photosynthates (8, 9, 15) and, in the present study, the increased radioactivity in nodules was at the expense of S-1. There was no change in the movement of radioactivity from the node-4 leaf to other plant parts. Less than 1% of the ^{14}C -photosynthate from the node-8 leaf was distributed to the nodules throughout the study.

Over 90% of the ^{14}C -photosynthate translocated from the node-8 leaf at flowering was found in the stem sections immediately above and below this node (Fig. 1b). At this time, the upper-stem section (S-3) was still actively elongating while S-2 was rapidly accumulating starch and increasing in dry weight (Tables 1 and 2). Some leaves above node 8 (L-3) were immature and were another important sink for ^{14}C -photosynthate. All reproductive parts contained less than 5% of the total ^{14}C -photosynthate translocated from the node-8 leaf, and no other part including nodules contained more than 1%.

Between flowering and early pod-fill little change occurred in the distribution of radioactivity from the node-4 leaf; however, there was a dramatic shift in the distribution from the node-8 leaf, with the developing fruits (P-2 and P-3) acquiring over 85% of the radioactivity translocated. A similar proportion of ^{14}C -photosynthate was found in both groups of pods, although the dry weight of P-3 was 62% greater than that of P-2.

Lower leaves can supply appreciable amounts of photosynthate to pods developing higher in the bean plant (13, 22). In this study, no such movement occurred possibly due to either varietal differences, branch removal or abscission of the lower leaves during reproductive development. If auxillary branches had been allowed to develop at the lower nodes, their pods might have competed for photosynthate from leaves on the lower mainstem.

During flowering to early pod-fill, S-2 and S-3 experienced a large increase in the total amount of both soluble sugars and starch (Table 3). This was due partly to increased size (Table 1) but tissue concentration also increased (Table 2). After early pod-fill, there was a rapid reduction in the amount of sugars and starch in S-2 and S-3 reflecting the movement to rapidly developing pods as evident in the ^{14}C results (Fig. 1a).

At mid pod-fill there was a decline in P-3 and an increase in P-2 radioactivity (both insignificant) but these two pod sections still contained over 90% of the ^{14}C -photosynthate from the node-8 leaf. This reflects the greater photosynthetic activity of the upper leaves (L-3) during the later stages of pod-fill and their subsequent contribution to P-3.

The results show that ^{14}C -photosynthate from the node-4 leaf moves primarily to the roots (Fig. 1a), and leaves below node 4 should follow the same pattern of photosynthate distribution as well as distribution of substances released during leaf senescence. The nodules, roots and S-1 receive the bulk of their photosynthate from the lower leaves and almost none from leaves as high as node 8. This confirms previous results on translocation to roots and lower stems of beans (20,22).

Similar results have been found with soybeans and field peas (5, 17). It also appears that distribution from lower leaves is restricted to movement downward and that distribution from the node-8 leaf was less restricted with substantial movement above and below the labelled leaf.

Nitrogen Fixation. The nitrogen fixation rate remained relatively unchanged at approximately 1.8 $\mu\text{moles/plt/hr}$ until flowering (day 48) before increasing to 4.3 $\mu\text{moles/plt/hr}$ at day 63 (Fig. 2b). This rate is approximately 30% of the maximum rate reported for this cultivar by Graham and Rosas (8). The rate declined rapidly at day 70. The peak in nitrogen fixation is consistent with other reports which show that the nitrogen fixation rate peaked after the initiation of flowering (7, 19).

Plant population had little effect on nitrogen fixation except at day 70 when the higher population produced a slightly higher rate possibly due to slightly lower soil temperatures in the high population. Specific nodule activity was not affected by plant population and nitrogen fixation appeared to be independent of dry weight of plant parts. This contradicts the findings of Graham and Rosas (8) which indicated a strong positive correlation between nitrogen fixation rate and nodule dry weight.

The quantity of soluble sugar found in the nodules and stem tissue (Table 3) followed a pattern of development similar to that of the nitrogen fixation rate. This change in nodule sugar content is reflective of changes in both the sugar concentration (Table 2) and nodule dry weight (Table 1). These facts tend to substantiate other reports which have shown that nitrogen fixation is associated with nodule weight and photosynthate supply (8, 11, 12).

Carbohydrates. Increased plant population reduced the concentration of soluble sugars in the nodules from 58 to 48 mg/g dry weight but did not affect the concentration of either soluble sugars or starch in any other part. Consequently, the data were combined and presented for stage of development (Table 2). Root and nodule soluble sugar concentration decreased significantly at mid pod-fill but the

concentration reached approximately the same level in all three parts of the stem. It is possible that, after stems cease to elongate, a stable concentration of soluble sugar is reached indicating the completion of structural development. Starch concentration increased significantly in S-1 during flowering and pod-fill but declined in other parts by mid pod-fill.

High plant population significantly increased the total amount of soluble sugars (14%) and starch (37%) in S-1. The amount in other plant parts was not affected by population and, hence, the data were combined (Table 3). The amount of starch in nodules, roots and S-1 increased from flowering to mid pod-fill. This accumulation of starch, particularly in the stem, has been previously observed and may indicate that beans are inefficient in their use of the products of photosynthesis or provide inadequate sink capacity for the source present (1, 7, 21). The soluble sugar (amount/plant part) in all parts declined during reproductive development.

The results of this study imply that cultural practices, such as high plant population, or environmental conditions which would reduce the photosynthetic capacity of the leaves at node 4 and below would also affect the supply of photosynthates available to the nodules, roots and lower stem. This should then affect both the development and function of these plant parts. Reducing photosynthetic rates in upper leaves should be most critical during pod development and have little affect on the photosynthate supply to the roots and nodules.

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Table 1. Dry weight (g/plt) of plant parts at different growth stages.

| <u>Days After Planting</u> | <u>Nodule</u> | <u>Root</u> | <u>Stem Sections</u> | | | <u>Leaf Sections</u> | | | <u>Pod Sections</u> | | | <u>Total</u> |
|--------------------------------|---------------|-------------|----------------------|----------|----------|----------------------|----------|----------|---------------------|----------|----------|--------------|
| | | | <u>1</u> | <u>2</u> | <u>3</u> | <u>1</u> | <u>2</u> | <u>3</u> | <u>1</u> | <u>2</u> | <u>3</u> | |
| 35 | .37 | 1.96 | .86 | .85 | - | 2.91 | 1.80 | - | - | - | - | 8.74 |
| 48 | .43 | 1.94 | 1.05 | 1.16 | .48 | 2.70 | 3.29 | .69 | .00 | .10 | .13 | 11.97 |
| 63 | .85 | 2.78 | 1.50 | 2.35 | 1.79 | 1.65 | 3.44 | 2.82 | .00 | 1.84 | 2.99 | 22.01 |
| 70 | .82 | 3.71 | 1.11 | 1.36 | 1.13 | .58 | 2.81 | 2.85 | .67 | 1.75 | 4.55 | 21.34 |
| LSD 5% | .27 | .72 | .14 | .27 | .32 | .38 | .75 | .78 | ns | 1.06 | 1.73 | 1.94 |

Table 2. The effect of stage of development on the concentration of soluble sugars and starch in bean nodules, roots, and stems.

| <u>Days After Planting</u> | <u>Nodule</u> | <u>Root</u> | <u>Stem 1</u> | <u>Stem 2</u> | <u>Stem 3</u> |
|--------------------------------|---------------|-------------|---------------|---------------|---------------|
| Soluble Sugars (mg/g dry wt) | | | | | |
| 35 | 83.45 | 79.56 | 70.03 | 103.25 | - |
| 48 | 44.01 | 86.39 | 40.15 | 52.18 | 48.04 |
| 63 | 51.76 | 69.93 | 73.02 | 77.26 | 159.89 |
| 70 | 32.90 | 41.68 | 73.49 | 74.23 | 75.47 |
| LSD 5% | 13.37 | 15.04 | 10.65 | 12.83 | 32.31 |
| Starch (mg/g dry wt) | | | | | |
| 35 | 148.27 | 17.53 | 231.33 | 152.93 | - |
| 48 | 87.20 | 35.85 | 203.70 | 683.10 | 70.81 |
| 63 | 57.45 | 50.08 | 305.70 | 628.35 | 475.87 |
| 70 | 64.40 | 32.66 | 403.13 | 363.83 | 315.46 |
| LSD 5% | 23.79 | 9.00 | 98.09 | 82.40 | 159.11 |

Table 3. The effect of stage of development on the total soluble sugars and starch in bean nodules, roots, and stems.

| <u>Days After Planting</u> | <u>Nodule</u> | <u>Root</u> | <u>Stem 1</u> | <u>Stem 2</u> | <u>Stem 3</u> |
|--------------------------------|---------------|-------------|---------------|---------------|---------------|
| Soluble Sugars (mg/plt) | | | | | |
| 35 | 29.31 | 156.11 | 60.21 | 85.91 | - |
| 48 | 18.80 | 169.94 | 41.81 | 60.40 | 49.74 |
| 63 | 44.33 | 183.84 | 110.02 | 182.69 | 160.43 |
| 70 | 24.33 | 149.81 | 77.82 | 96.56 | 78.54 |
| LSD 5% | 12.52 | ns | 11.78 | 25.06 | 49.96 |
| Starch (mg/plt) | | | | | |
| 35 | 54.57 | 34.29 | 200.49 | 130.83 | - |
| 48 | 35.54 | 67.61 | 219.64 | 790.94 | 60.78 |
| 63 | 49.69 | 131.32 | 476.27 | 1479.52 | 481.78 |
| 70 | 52.09 | 124.08 | 463.50 | 518.13 | 304.34 |
| LSD 5% | ns | 34.24 | 131.64 | 180.94 | 221.49 |

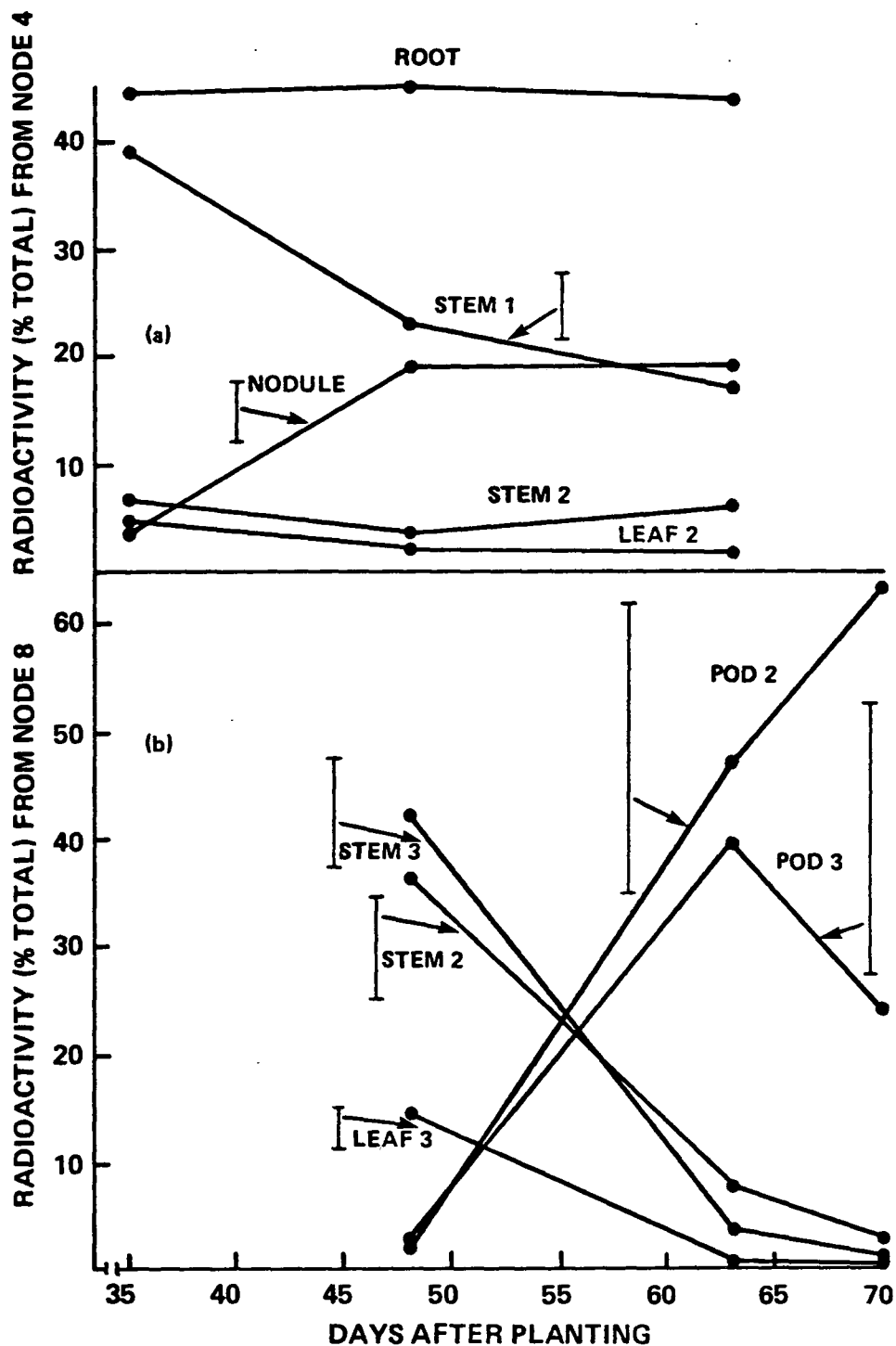


FIGURE 1
THE DISTRIBUTION OF ^{14}C ACTIVITY FROM
NODES 4 (a) AND 8 (b) TO BEAN PLANT PARTS.
VERTICAL BARS - LSD 5% (NO BAR - INSIGNIFICANT)

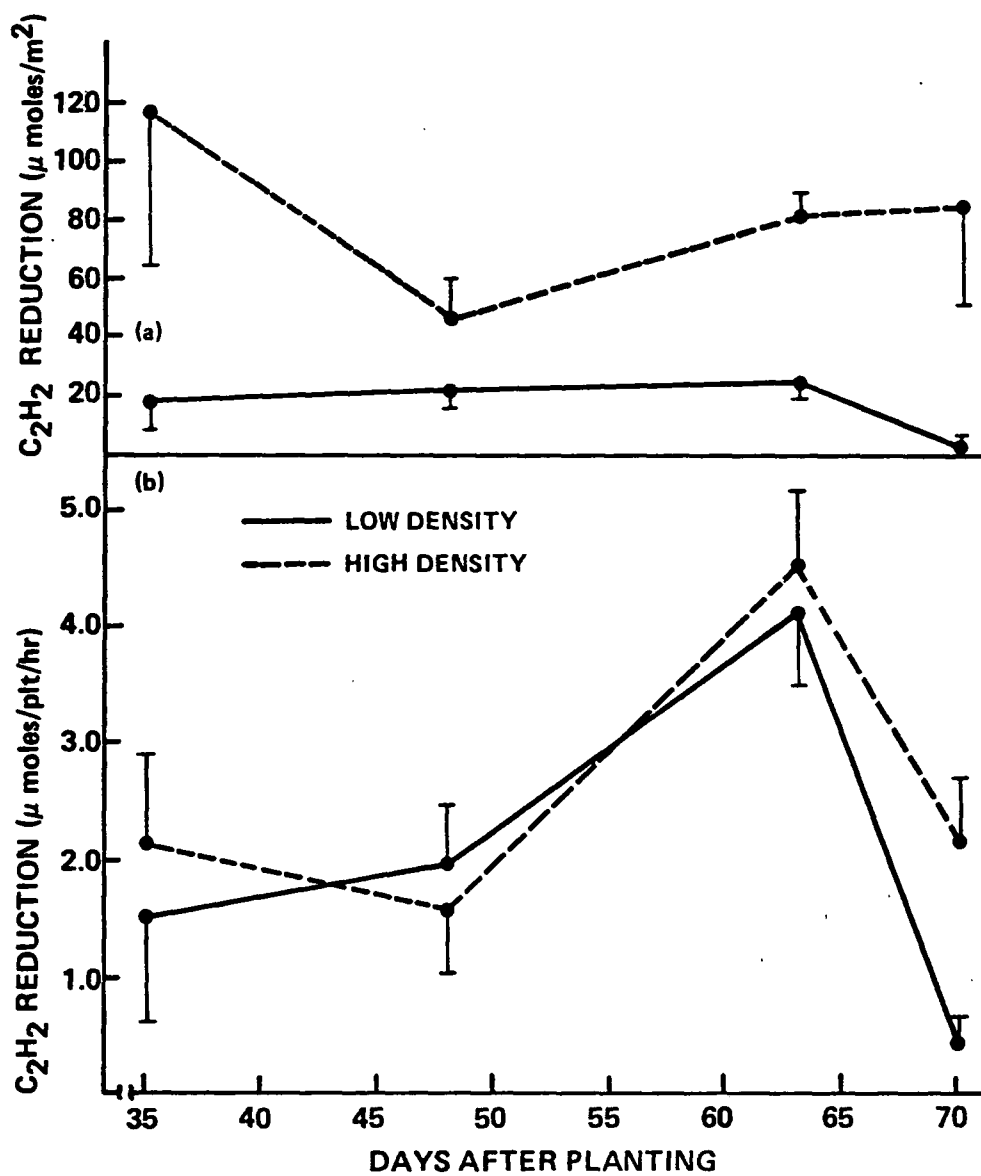


FIGURE 2
THE EFFECT OF PLANT POPULATION DENSITY ON NITROGEN FIXATION,
RATE/UNIT AREA (m^2) (a) AND PER PLANT (b).
VERTICAL BARS — S.E. \bar{X} .

THE EFFECT OF OPENING THE PLANT CANOPY
ON NITROGEN FIXATION, CARBOHYDRATE ACCUMULATION AND
GROWTH OF BEANS (Phaseolus vulgaris L.)¹

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Additional index words. nitrogen concentration, indeterminant, tropics

Abstract. Leaves of alternate plants were removed to different levels in the canopy, before and after flowering, and complete defoliation resulted in larger plants. Dry weight and carbohydrate content of stems and leaves changed little until one week after flowering when both were increased by preflowering and post-flowering treatments. Pod carbohydrate concentrations were also increased. Root dry weight of nondefoliated plants increased when the alternate plants were completely defoliated prior to flowering. Nitrogen content of nodules was significantly reduced by post-flowering treatments. Nitrogen fixation and nodule dry weight of nondefoliated plants tended to be reduced by all treatments but differences were not significant. It is possible that light levels were sufficiently high that opening the canopy did not result in improved photosynthate supply to the nodules and the increased photosynthate was utilized in plant development, principally stems and leaves.

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Introduction

As beans develop in commercial plantings, the radiation intercepted by leaves in the middle and lower parts of the canopy is greatly reduced and results in a reduction in net photosynthesis in these portions of the plant. Since the major source of photosynthate for the lower stem, roots and nodules is the lower leaves (14, 20, 21, 22), growth of these organs might be restricted and nitrogen fixation reduced in dense stands.

Increasing the plant population of Vicia faba (17) reduced nitrogen fixation while plant population effects on beans (8) varied with cultivar and plant habit. CO₂ enrichment of peas (15) or increasing the irradiance on beans (1, 2) increased nitrogen fixation and net photosynthesis. Shading of broad beans reduced the carbohydrate and increased the nitrogen content of plant parts, especially the stem (19). Nitrogen fixation in soybeans (11) and peas (12, 13) is limited by the available photosynthate both as a result of competitive sink development or reduced irradiance.

In Colombia, at the Centro Internacional de Agricultura Tropical (CIAT), a field study was conducted in which interplant shoot competition was reduced, before and after flowering, by defoliating every other plant to different depths in the plant canopy. The purpose of this study was to determine if improving the light environment of non-defoliated plants affected their rate of nitrogen fixation and patterns of dry weight and carbohydrate accumulation.

Materials and Methods

One cultivar, Cargamanto (CIAT designated P590), was used which has a Type IV growth habit (aggressive climbing indeterminant) and a demonstrated capacity for abundant nodulation and nitrogen fixation. The trial was conducted at Popayan, Colombia, at 1850 m altitude with a mean annual temperature of 17.5°C and 1922 mm of rainfall.

Seeds were hand planted 10 cm apart in rows spaced 50 cm (20 plants/m²) with plants supported on a split bamboo trellis. Seven defoliation treatments plus a nondefoliated control were arranged in a randomized complete block design with five replications. Plots were four rows wide and only the two center rows were used for data collection. Treatments consisted of defoliation of alternate plants downward to different levels (mainstem nodes) at 43 and 63 days after planting (17 days before, and 3 days after bloom) with leaves of both defoliated plants competing below those levels. The plants had 10 - 11 and 14 - 15 mainstem nodes at 43 and 63 days after planting, respectively. The treatments were applied as shown below with nodes numbered acropetally.

| <u>Treatment</u> | <u>Time of Defoliation (days after planting)</u> | <u>Level of Defoliation (highest node not defoliated)</u> | <u>Nodes Competing</u> |
|------------------|--|---|----------------------------|
| 1 | none (control) | - | - |
| 2 | 43 | Ground | 0 |
| 3 | 43 | 4 | 1-4 |
| 4 | 43 | 8 | 1-8 |
| 5 | 63 | Ground | 0 |
| 6 | 63 | 4 | 1-4 |
| 7 | 63 | 8 | 1-8 |
| 8 | 63 | 12 | 1-12 |

Soil was analyzed and fertilized prior to planting with (kg/ha): lime, 1,000; superphosphate, 300; KCl, 50; MgSO₄, 100; ZnSO₄, 7; Borax, 2; and NaMoO₄, 1. Seeds were surface sterilized with a calcium hypochlorite solution (1 g Ca(ClO)₂ + 500 ml concd HCl + 500 ml distilled H₂O), rinsed and dried. Seeds were lime pelleted with Rhizobium phaseoli (CIAT strain 1057) immediately before planting. Insects and diseases were controlled with routine application of CIAT recommended materials.

Sampling began 27 days after planting in control plots and continued at an interval of seven days or less until day 101. A maturity harvest was made at day 119. Harvest of treatments 2 - 4 began on day 44 and treatments 5 - 8 on day 64 coinciding with control

harvests. At harvest, five consecutive nondefoliated plants were removed from each of the two center rows with a shovel and divided into nodulated root systems, 4-node stem sections, 2-node leaf sections, and pods when present. The nitrogen fixation rate (C_2H_2 reduction) of the 10 nodulated root systems was estimated as described by Graham and Rosas (7) and then divided into nodules and roots.

Plant parts were dried at $55^{\circ}C$ for 48 hours, weighed and ground. Total carbohydrate concentration (% of dry wt) was determined as described by Graham and Rosas (7). Nitrogen concentration (% of dry wt) of selected samples was determined using the microkjeldahl.

Results and Discussion

Nodulation and Nitrogen Fixation. All treatments reducing the interplant shoot competition tended to reduce both the nitrogen fixation rate and nodule dry weight; however, by late pod-fill both were similar to the control (Tables 1 and 2). Specific nodule activity followed a pattern similar to that of nitrogen fixation (Table 3).

The nitrogen fixation rate and nodule dry weight of plants adjacent to completely defoliated plants were less severely reduced than those adjacent to plants not completely defoliated (Tables 1 and 2). This might be expected since greater illumination of plants should increase the carbohydrate available to nodules for growth and fixation (3, 4, 5, 11). Positive associations between carbohydrate availability and nodule activity have been found (9, 12, 13). The tendency for nodule weight and activity to decline after reducing the interplant shoot competition might be due to photosynthate being utilized in plant development, principally stems and leaves, at the expense of the nodules. Soybean roots (6, 16) and nodules (6) contained higher percentages of ^{14}C -photosynthate when plants were placed under water stress or shaded. Possibly roots and nodules of bean plants relieved of varying degrees of shading by reducing interplant shoot competition received proportionately less photosynthate than the shoot. Shading of

field beans has been shown to prolong nodule activity and delay senescence (17) possibly indicating that more photosynthate was available to the nodules under those conditions.

Antoniw and Sprent (1, 2) found the opposite effect with beans. When plants were transferred from low (7 W/m^2) to high (28 W/m^2) irradiance conditions, nodule weight and activity increased; however, these irradiance levels were obtained with fluorescent lamps and were probably only a small fraction of those occurring in the field study reported here. It is possible that when light levels are sufficiently high, opening the canopy by defoliating alternate plants fails to appreciably improve the supply of photosynthate to the nodules. Opening the canopy did result in greater development of shoot parts (Figs. 1 and 2).

There was a strong correlation ($r = .78$) between nodule dry weight and nitrogen fixation supporting the conclusion of others (2, 8) that the nitrogen fixation changes are due to factors which influence nodule development.

Dry Weight. Root dry weight was significantly increased when alternate plants were completely defoliated prior to flowering (Table 3) presumably as a result of exposure of the leaves at nodes 4 and below to greater light intensity. This is consistent with other data which have shown that the lower leaves are the primary source of photosynthate for the roots (14, 20, 21, 22).

Total defoliation of alternate plants after flowering did not influence root dry weight but other treatments produced small weight reductions. This might be expected since root growth may stop during pod-fill (18). There was a shorter period of time for treatments to affect root development and competitive reproductive sinks for photosynthate had begun to develop (13).

Stem and leaf dry weight did not change in response to any of the treatments used until approximately one week after flowering when there were appreciable increases in those treatments with total defoliation of alternate plants (Table 4, and Figs. 1 and 2). The largest increase occurred at nodes above 12 although this was not significant with stems

from preflowering treatments. The post-flowering increase in stem and leaf dry weight corresponds to the period when the rate of photosynthesis would have increased commensurate with flowering as shown with peas (13). The partitioning of the accumulating dry matter to shoot parts rather than nodules and roots tends to confirm results with soybeans showing that the shoot contained higher percentages of ^{14}C under nonshaded conditions (6, 16).

Pod dry weight (Table 5) was reduced by all the treatments used; however, the final seed weight was not significantly affected by any treatment. This appears to indicate that the reduced interplant shoot competition delayed maturity which is consistent with field bean data (18). Although not statistically significant, complete defoliation of alternate plants resulted in higher final seed weight as would be expected.

Carbohydrate. None of the preflowering treatments appreciably affected plant carbohydrate concentrations of individual plant parts (Tables 6 and 7, Figs. 3 and 4); however, at 87 days after planting, total plant carbohydrate was 24% higher with complete defoliation of alternate plants prior to bloom. Reducing interplant shoot competition after flowering resulted in a marked increase in the carbohydrate concentration in all plant parts, particularly stem and, to a lesser extent, pod tissue. This increased dry weight and carbohydrate content of these plant parts is interpreted as indirect evidence that defoliation of alternate plants resulted in greater light penetration of the canopy with an associated increase in photosynthesis.

Nitrogen Content. The nitrogen concentration of plant parts was little affected by canopy manipulations either before or after flowering. The treatments generally reduced the nitrogen content of all plant parts, particularly the nodes (Table 8) and nitrogen content of plant parts tended to decrease with increasing age as found in field beans (17). This is probably a reflection of the reduced activity of the nodules since the soil used for this study was relatively infertile and received no supplemental nitrogen.

In this study, defoliating alternate plants resulted in higher plant dry weight (Tables 3 and 4) with small reductions in nodulation and nitrogen fixation. This canopy manipulation presumably allowed greater light penetration and the higher carbohydrate concentrations in the stem tissue and higher plant dry weights support this conclusion. This would imply that greater light availability was not beneficial to nodulation or nitrogen fixation.

The failure of canopy-opening defoliations to increase nitrogen fixation and nodulation may be due to changes in the hormonal balance in the plant, as suggested by Sprent et al., (18), with the higher levels of light in the canopy after defoliation. These results may also indicate that there is a threshold above which nodules are no longer able to use additional photosynthate and other sinks in the plant, such as the stems and leaves in this study, compete more effectively for the available photosynthate. This is possibility supported by data from soybean research (23) in which the use of male-sterile plants increased the root carbohydrate levels to 1.7 - 7.6 times those of male fertile plants with no affect on nitrogen fixation.

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Table 1. The effect of reduced interplant shoot competition to different canopy depths (mainstem nodes), before and after flowering, on nodule dry weight (g/plt) of beans at different stages of development.

| <u>Days After Planting</u> | <u>Canopy Depth (node)</u> | | | | | | | |
|--------------------------------|---|----------|----------|---|----------|----------|-----------|----------------|
| | <u>Ground</u> | <u>4</u> | <u>8</u> | <u>Ground</u> | <u>4</u> | <u>8</u> | <u>12</u> | <u>Control</u> |
| | Preflowering ^z (43 days ^y) | | | Post-flowering ^z (63 days ^y) | | | | |
| 44 | .153 | .179 | .147 | - | - | - | - | .189 |
| 46 | .136 | .208 | .098 | - | - | - | - | .089 |
| 49 | .133 | .173 | .141 | - | - | - | - | .129 |
| 55 | .083 | .101 | .078 | - | - | - | - | .112 |
| 64 | .072 | .072 | .058 | .112 | .084 | .062 | .081 | .109 |
| 66 | .110 | .103 | .086 | .131 | .100 | .095 | .069 | .132 |
| 69 | .097 | .068 | .075 | .090 | .084 | .091 | .073 | .105 |
| 76 | .093 | .063 | .104 | .071 | .100 | .073 | .066 | .089 |
| 80 | .091 | .079 | .053 | .064 | .057 | .066 | .071 | .076 |
| 87 | .065 | .096 | .065 | .047 | .053 | .066 | .037 | .065 |

^z Preflowering SE - .195; post-flowering SE - .193.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 2. The effect of reduced interplant shoot competition to different canopy depths (mainstem nodes), before and after flowering, on nitrogen fixation rate ($\mu\text{moles/plt/hr}$) of beans at different stages of development.

| <u>Days After Planting</u> | <u>Canopy Depth (node)</u> | | | | | | | |
|--------------------------------|---|----------|----------|---|----------|----------|-----------|----------------|
| | <u>Ground</u> | <u>4</u> | <u>8</u> | <u>Ground</u> | <u>4</u> | <u>8</u> | <u>12</u> | <u>Control</u> |
| | Preflowering ^z (43 days ^y) | | | Post-flowering ^z (63 days ^y) | | | | |
| 44 | 12.16 | 16.08 | 14.64 | - | - | - | - | 20.36 |
| 46 | 12.48 | 19.18 | 8.88 | - | - | - | - | 9.34 |
| 49 | 3.42 | 6.26 | 5.06 | - | - | - | - | 6.40 |
| 55 | 4.46 | 12.92 | 7.18 | - | - | - | - | 14.68 |
| 64 | 7.44 | 6.68 | 5.76 | 8.40 | 8.98 | 6.60 | 7.32 | 12.76 |
| 66 | 11.34 | 10.26 | 13.10 | 13.30 | 13.20 | 10.38 | 11.12 | 16.74 |
| 69 | 13.58 | 8.82 | 6.58 | 14.24 | 8.74 | 11.32 | 10.52 | 12.30 |
| 76 | 12.06 | 5.92 | 7.84 | 4.16 | 5.56 | 4.28 | 6.84 | 8.22 |
| 80 | 7.94 | 7.16 | 2.98 | 6.04 | 3.82 | 5.22 | 10.06 | 6.20 |
| 87 | 6.54 | 14.22 | 7.04 | 6.62 | 4.38 | 6.38 | 3.96 | 7.80 |

^z Preflowering SE - 2.11; post-flowering SE - 1.78.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 3. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on nodule and root dry weight, nitrogen fixation and specific nodule activity of beans. Values averaged over harvests.

| <u>Canopy Depth^z</u> | <u>Nodule Dry wt (g)</u> | <u>N Fixation (μmoles/ plt/hr)</u> | <u>SNA^y (μmoles/ g dry wt)</u> | <u>Root Dry wt (g)</u> |
|--|------------------------------|---|--|----------------------------|
| Preflowering (43 days ^x) | | | | |
| Ground | .103 | 9.14 | 9.71 | .58 |
| 4 | .114 | 10.75 | 10.24 | .50 |
| 8 | .091 | 7.91 | 9.07 | .50 |
| Control | .109 | 11.45 | 11.26 | .52 |
| LSD 5% | .020 | 1.84 | 1.43 | .04 |
| Post-flowering (63 days ^x) | | | | |
| Ground | .086 | 8.79 | 12.45 | .61 |
| 4 | .080 | 7.45 | 9.16 | .57 |
| 8 | .075 | 7.36 | 12.91 | .55 |
| 12 | .066 | 8.30 | 12.90 | .53 |
| Control | .096 | 10.67 | 12.19 | .59 |
| LSD 5% | ns | 2.03 | ns | .05 |

^z Main stem node.

^y SNA - Specific Nodule Activity.

^x Days after planting (time of competition reduction) with 10 -11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 4. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on the stem and leaf dry weight (g) in 4-node sections of beans. Values averaged over harvests.

| Canopy Depth ^z | Stems | | | | | Leaves | | | | |
|--|------------|------------|-------------|-------------|--------------|------------|------------|-------------|-------------|--------------|
| | <u>1-4</u> | <u>5-8</u> | <u>9-12</u> | <u>12 +</u> | <u>Total</u> | <u>1-4</u> | <u>5-8</u> | <u>9-12</u> | <u>12 +</u> | <u>Total</u> |
| Preflowering (43 days ^y) | | | | | | | | | | |
| Ground | .41 | .67 | .83 | 1.79 | 2.96 | .59 | 1.16 | 1.27 | 1.51 | 4.21 |
| 4 | .39 | .57 | .80 | 1.56 | 2.52 | .56 | 1.01 | 1.11 | 1.11 | 3.50 |
| 8 | .41 | .65 | .88 | 1.69 | 2.59 | .55 | 1.10 | 1.21 | .93 | 3.54 |
| Control | .40 | .57 | .80 | 1.67 | 2.49 | .56 | 1.01 | 1.19 | 1.03 | 3.49 |
| LSD 5% | ns | .08 | ns | ns | .33 | ns | .12 | ns | .30 | .41 |
| Post-flowering (63 days ^y) | | | | | | | | | | |
| Ground | .44 | .84 | 1.40 | 1.43 | 4.10 | .40 | 1.34 | 1.91 | 1.37 | 5.04 |
| 4 | .42 | .77 | 1.33 | .88 | 3.40 | .36 | 1.19 | 1.83 | .87 | 4.25 |
| 8 | .47 | .76 | 1.27 | 1.22 | 3.72 | .41 | 1.32 | 1.83 | 1.17 | 4.73 |
| 12 | .43 | .69 | 1.11 | .93 | 3.14 | .41 | 1.12 | 1.67 | .96 | 4.15 |
| Control | .45 | .70 | 1.18 | 1.18 | 3.51 | .45 | 1.13 | 1.81 | 1.22 | 4.61 |
| LSD 5% | ns | .11 | .20 | .23 | .43 | ns | .14 | ns | .25 | .52 |

^z Main stem node.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 5. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on pod dry weight (g), final seed weight (g) and carbohydrate concentration (% dry wt) in 4-node sections. Values averaged over harvests.

| Canopy Depth ^z | Pod dry wt | | | | | Pod CHO | | | | |
|--|------------|------------|-------------|-------------|-----------------------|------------|------------|-------------|-------------|--------------|
| | <u>1-4</u> | <u>5-8</u> | <u>9-12</u> | <u>12 +</u> | <u>Seed Yield</u> | <u>1-4</u> | <u>5-8</u> | <u>9-12</u> | <u>12 +</u> | <u>Total</u> |
| Control | .01 | .76 | 2.75 | 2.73 | 15.76 | 1.19 | 9.97 | 9.63 | 9.14 | 9.79 |
| Preflowering (43 days ^y) | | | | | | | | | | |
| Ground | .01 | .43 | 1.92 | 2.16 | 21.19 | .65 | 7.21 | 9.44 | 7.32 | 9.20 |
| 4 | .00 | .31 | 1.85 | 1.53 | 16.44 | .00 | 7.41 | 7.76 | 7.45 | 7.81 |
| 8 | .03 | .46 | 2.26 | 1.31 | 14.71 | 2.01 | 8.65 | 8.64 | 7.88 | 8.95 |
| Post-flowering (63 days ^y) | | | | | | | | | | |
| Ground | .02 | .64 | 2.41 | 1.45 | 19.17 | 2.84 | 11.40 | 11.05 | 10.33 | 11.31 |
| 4 | .00 | .36 | 2.04 | .85 | 15.93 | .00 | 10.35 | 11.53 | 9.28 | 10.29 |
| 8 | .02 | .37 | 1.82 | 1.28 | 14.64 | 2.12 | 9.08 | 10.34 | 9.05 | 11.09 |
| 12 | .00 | .61 | 2.34 | 1.28 | 15.19 | .70 | 10.23 | 10.54 | 9.08 | 11.70 |
| LSD 5% | .02 | ns | ns | .63 | ns | 2.52 | 1.91 | 1.46 | 1.58 | ns |

^z Main stem node.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 6. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on the carbohydrate concentration (% dry wt) of nodules, roots and stems in 4-node sections of beans. Values averaged over harvests.

| Canopy Depth ^z | Nodule | Root | Stem | | | | Total |
|--|--------|------|------|-------|-------|-------|-------|
| | | | 1-4 | 5-8 | 9-12 | 12 + | |
| Preflowering (43 days ^y) | | | | | | | |
| Ground | 5.23 | 4.88 | 6.49 | 7.08 | 7.49 | 7.79 | 7.26 |
| 4 | 4.77 | 4.62 | 5.73 | 6.42 | 6.92 | 7.66 | 6.76 |
| 8 | 5.19 | 5.23 | 6.80 | 7.01 | 6.99 | 8.10 | 7.18 |
| Control | 5.50 | 5.09 | 7.59 | 7.44 | 7.80 | 8.59 | 7.83 |
| LSD 5% | 0.50 | ns | 0.62 | 0.62 | ns | ns | 0.53 |
| Post-flowering (63 days ^y) | | | | | | | |
| Ground | 5.25 | 5.08 | 9.54 | 10.86 | 11.21 | 10.75 | 10.78 |
| 4 | 4.97 | 5.37 | 9.24 | 9.84 | 9.80 | 10.33 | 9.95 |
| 8 | 5.65 | 5.21 | 9.20 | 9.60 | 11.13 | 10.01 | 10.38 |
| 12 | 5.67 | 5.74 | 9.54 | 10.41 | 10.71 | 10.34 | 10.44 |
| Control | 4.70 | 4.65 | 7.91 | 8.45 | 9.36 | 8.89 | 8.84 |
| LSD 5% | 0.72 | 0.58 | 1.06 | 1.22 | 1.35 | 1.13 | 0.84 |

^z Main stem node.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 7. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on the carbohydrate concentration (% dry wt) of leaves in 4-node sections of beans. Values averaged over harvests.

| Canopy Depth ^z | Nodes | | | | Total |
|--|-------|------|------|------|-------|
| | 1-4 | 5-8 | 9-12 | 12 + | |
| Preflowering (43 days ^y) | | | | | |
| Ground | 4.56 | 4.42 | 4.39 | 3.09 | 4.78 |
| 4 | 4.10 | 4.19 | 4.00 | 2.98 | 4.24 |
| 8 | 4.19 | 4.28 | 3.68 | 3.31 | 4.35 |
| Control | 4.46 | 4.14 | 3.89 | 4.05 | 4.50 |
| LSD 5% | ns | ns | ns | 0.69 | 0.33 |
| Post-flowering (63 days ^y) | | | | | |
| Ground | 3.97 | 3.96 | 4.64 | 4.37 | 4.39 |
| 4 | 3.11 | 3.48 | 4.00 | 4.26 | 3.93 |
| 8 | 3.35 | 4.01 | 4.74 | 4.13 | 4.29 |
| 12 | 3.57 | 3.77 | 4.51 | 4.36 | 4.23 |
| Control | 3.41 | 3.38 | 4.02 | 3.92 | 3.81 |
| LSD 5% | 0.56 | ns | ns | ns | ns |

^z Main stem node.

^y Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

Table 8. The effect of reduced interplant shoot competition, before and after flowering, to different depths of the plant canopy on the nitrogen concentration (% dry wt) of nodules, roots, stems and leaves of beans. Values averaged over harvests.

| <u>Canopy Depth^z</u> | <u>Nodules</u> | <u>Roots</u> | <u>Stems</u> | <u>Leaves</u> | |
|--|----------------|--------------|--------------|---------------|---------------------------|
| | | | | <u>%N</u> | <u>LSD 5%^y</u> |
| Preflowering (43 days ^x) | | | | | |
| Ground | 1.74 | 0.79 | 0.74 | 1.76 | 0.21 |
| 4 | 2.13 | 1.01 | 0.86 | 2.16 | ns |
| 8 | 1.78 | 0.84 | 0.92 | 1.81 | 0.15 |
| Control | 1.92 | 0.83 | 1.03 | 2.09 | -- |
| LSD 5% | 0.22 | 0.10 | ns | -- | |
| Post-flowering (63 days ^x) | | | | | |
| Ground | 2.20 | 0.84 | 1.05 | 2.63 | 0.20 |
| 4 | 1.77 | 0.72 | 0.94 | 1.99 | ns |
| 8 | 1.85 | 0.68 | 0.81 | 1.95 | ns |
| 12 | 1.76 | 0.74 | 0.80 | 1.94 | ns |
| Control | 2.17 | 0.67 | 0.81 | 2.08 | -- |
| LSD 5% | 0.27 | ns | 0.14 | -- | |

^z Main stem node.

^y LSD comparing the individual treatment with the control.

^x Days after planting (time of competition reduction) with 10 - 11 nodes at preflowering treatments and 14 - 15 nodes at post-flowering treatments.

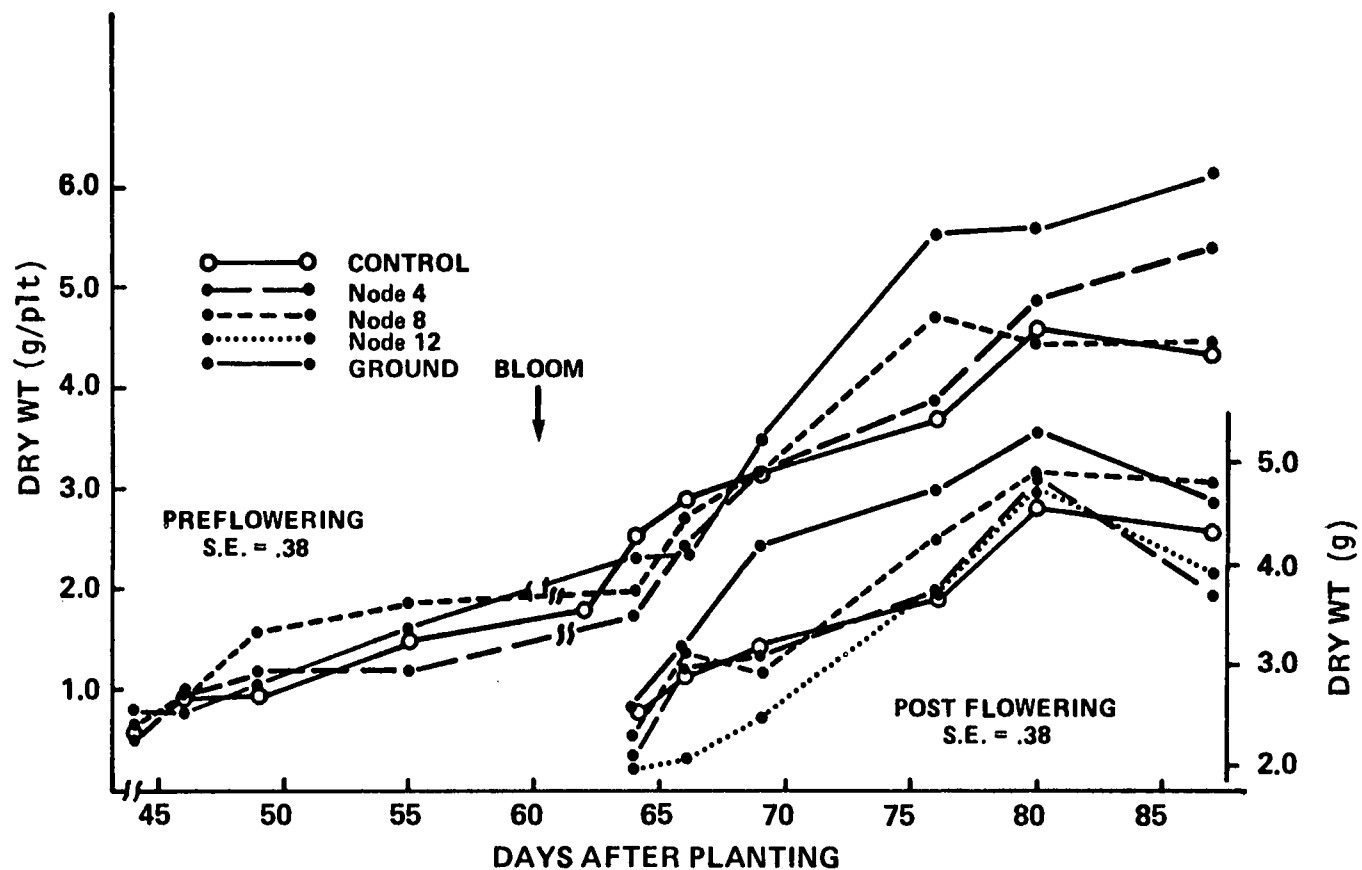


FIGURE 1
THE EFFECT OF REDUCED INTERPLANT SHOOT COMPETITION
TO DIFFERENT CANOPY DEPTHS (MAINSTEM NODES),
BEFORE AND AFTER FLOWERING, ON STEM DRY WEIGHT
IN BEANS

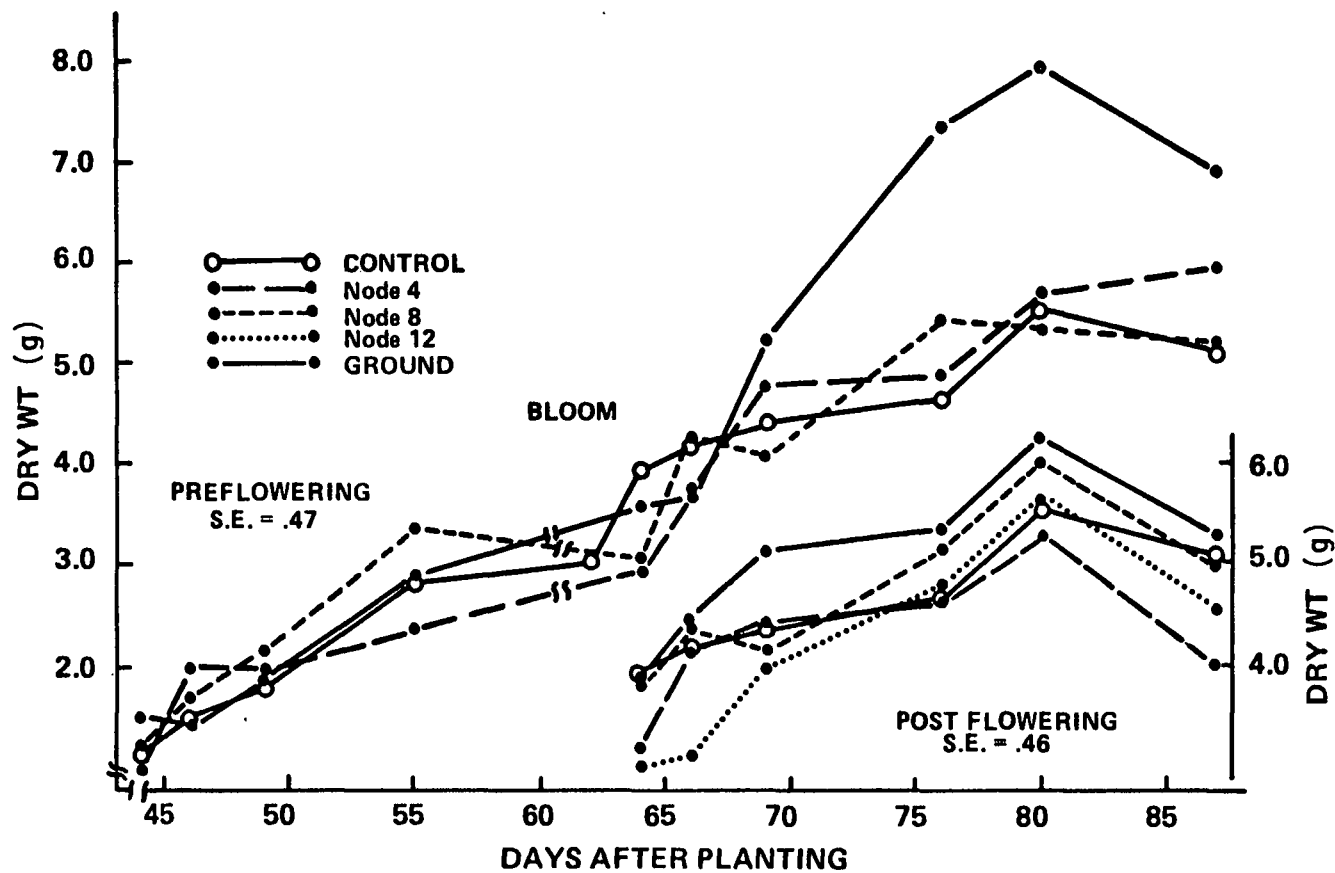


FIGURE 2
THE EFFECT OF REDUCED INTERPLANT SHOOT COMPETITION
TO DIFFERENT CANOPY DEPTHS (MAINSTEM NODES),
BEFORE AND AFTER FLOWERING, ON LEAF DRY WEIGHT
IN BEANS

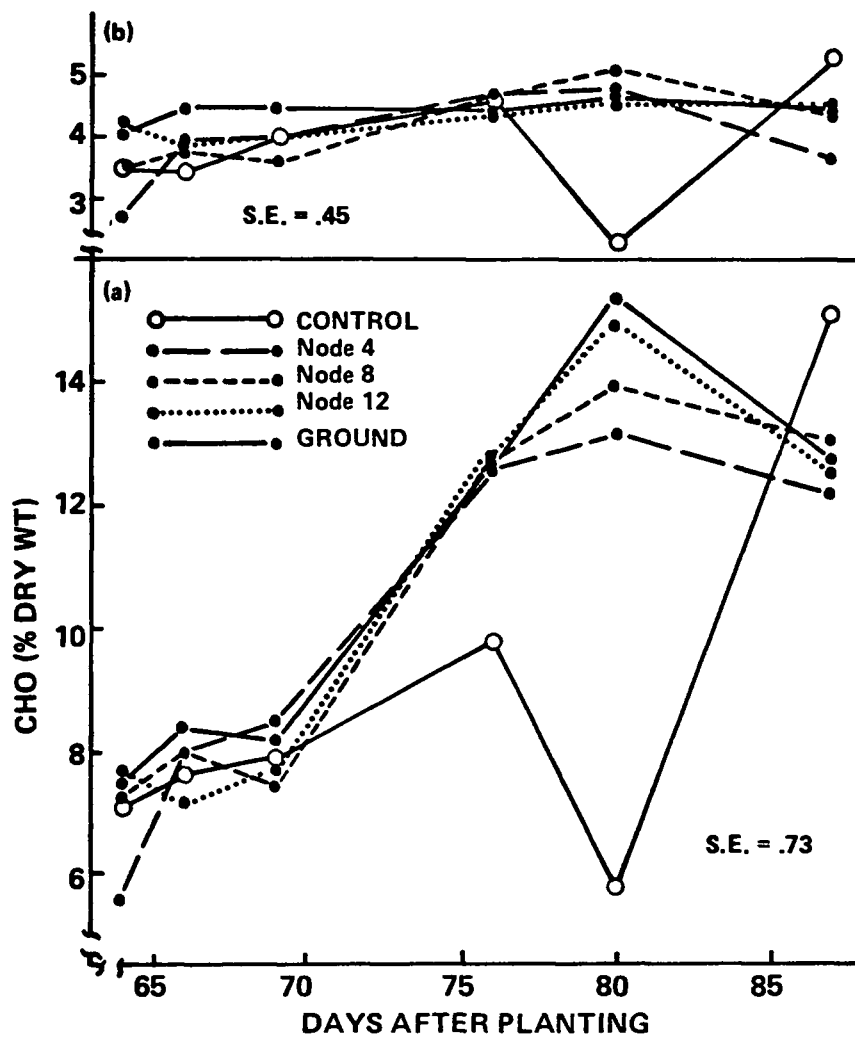


FIGURE 3
THE EFFECT OF REDUCED INTERPLANT SHOOT COMPETITION
TO DIFFERENT CANOPY DEPTHS (MAINSTEM NODES)
AFTER FLOWERING, ON STEM (a) AND LEAF (b) CHO CONTENT
OF BEANS

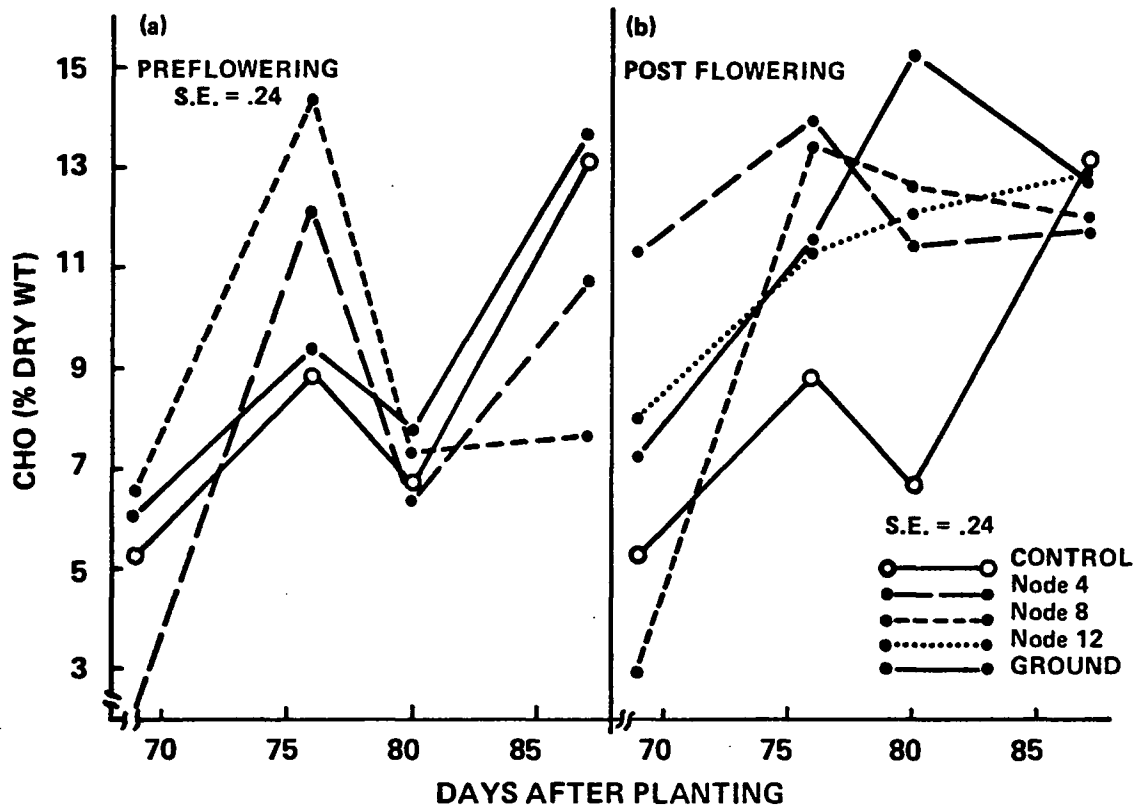


FIGURE 4
THE EFFECT OF REDUCED INTERPLANT SHOOT COMPETITION
TO DIFFERENT CANOPY DEPTHS (MAINSTEM NODES),
BEFORE (a) AND AFTER (b) FLOWERING ON POD
CHO CONTENT OF BEANS

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