NITROGEN FIXATION AND CARBOHYDRATE PARTITIONING IN PHASEOLUS VULGARIS L.

Dissertation for the Degree of Ph. D.

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ABSTRACT

NITROGEN FIXATION AND CARBOHYDRATE PARTITIONING IN PHASEOLUS VULGARIS L.

By

Romeo Martinez Rodas

The ontogenetic relationships that may exist between symbiotic nitrogen fixation and carbohydrate partitioning were studied in four dry bean cultivars. Total soluble carbohydrate and starch contents of the primary nodule population were found to be closely correlated in time with nitrogen fixation activity. The decline in nitrogen fixation activity was found to be correlated with loss of nodule dry weight, decreased total soluble carbohydrate and starch of nodules and an increase in soluble carbohydrate and starch in leaves and stems during the early stages of reproductive growth. Starch concentration was found to decline one week prior to the occurrence of nitrogen fixation maxima suggesting that hydrolysis of this polysaccharide may contribute energy to the fixation of molecular nitrogen. Temporary sites of starch accumulation were found to be restricted primarily to parenchyma cells of secondary xylem and pith and to uninfected cells in nodules. Reduction of carbohydrate movement to the nodule, due to "competition" by reproductive sinks, may not be the cause of the observed decline in nitrogen fixation. It is suggested that a

developmentally compensated genetic program predisposes the nodules to the observed decline in N_2 fixation. Thus, loss of nodule competency in N_2 fixation may not be triggered by a reduction in carbohydrate movement to such structures but by an activating signal(s) affecting both nodule and lower leaf senescence, probably hormonal in nature.

Dry bean plants exposed to 1200 ppm carbon dioxide at various developmental stages exhibited higher nitrogen fixation rates, higher nodule fresh weights, higher total soluble carbohydrate and slightly higher organic nitrogen contents. Carbon dioxide treatment during the four weeks prior to reproductive growth was found not to extend the duration nor prevent the decline of nitrogen fixation. The view that the rate of nitrogen fixation per se is limited by photosynthate available to the entire symbiotic system is supported.

NITROGEN FIXATION AND CARBOHYDRATE PARTITIONING IN *PHASEOLUS VULGARIS* L.

by

Romeo Martinez Rodas

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INTRODUCTION

From the viewpoint of the agro-biologist, a bean plant can be defined as a biological system for the reduction and subsequent processing of nitrogen and carbon into forms useful not only to the plant in assuring its survival but to man as consumer of the bean seed. It is deemed important to man that the reduction and subsequent processing of nitrogen and carbon, as key elements in seed formation and seed quality, be conducted in the plant in as efficient a manner as possible. This requires understanding of the system. Great strides have been made in understanding soil nitrogen uptake, transport, and reduction, in symbiotic fixation of nitrogen, and in the synthesis and regulation of synthesis of nitrogen - containing compounds such as protein. Similarly, great strides have been made in understanding the photosynthetic process and in the transport and storage of carbon-containing compounds, for example, proteins, sugars, starch.

In grain legumes, these processes are proceeding simultaneously and there is increasing evidence of interdependence among them. In particular, there is a hypothesis that the decline in nitrogen fixation in legume plants shortly after pod-filling commences is a result of competition by the developing seeds for photosynthate (energy) needed in the nodules to maintain a high rate of nitrogen fixation. There is also evidence that the form and/or ratio of carbohydrate forms stored in root nodules may influence the rate of fixation. Furthermore, different genotypes of legumes appear capable of differential fixation

rates, both of nitrogen and of carbon. The amount of starch storage in root and stem of bean genotypes appears to vary significantly among genotypes.

These disclosures raise numerous questions and suggest several avenues of research, some of which form the bases of this thesis.

It seems essential that the ontogenetic relationships that may exist between nitrogen fixation and carbohydrate partioning be documented in bean genotypes that differ in plant type and agronomic adaptation. The hypothesis of competition between nodule and developing pod should be looked at anew. The experiements conducted and reported in this thesis bear upon these problems.

CHAPTER 1

SYMBIOTIC NITROGEN FIXATION

INTRODUCTION

Biological fixation of molecular nitrogen is considered a fundamental phenomen in the maintenance of life. From the beginning of agriculture, symbiotic nitrogen fixation has been unwittingly utilized in the cultivation of legumes. In the latter part of the nineteenth century, the ability of legumes to grow on poor soils and to improve their fertility was finally explained after many contradictory and negative results. In 1886, Hellriegel and Wilfarth (cited by Virtanen and Miettinen, 1963) demonstrated experimentally that legumes utilize molecular nitrogen if their roots bear nodules induced and formed by bacteria. Two years later Beijerink (cited by Virtanen and Miettinen, 1963), isolated a bacterium in pure culture which formed nodules on the roots of the host plant.

Our understanding of N_2 fixation has advanced most rapidly during the past decade. This rapid advance in knowledge of the processes involved was due to major advances that include: a) recognition of ATP and electron requirements in in vitro assays (Hardy and D'Eustachio, 1964; McNary and Burris, 1962; and Mortenson, 1964); b) separation of the protein components of nitrogenase (Bulen and LeComte, 1966), and, c) use of the acetylene-reduction assay, an indicator of nitrogenase activity (Dilworth, 1966; Schollhorn and Burris, 1966).

To date, a concensus of opinion exists that essential biochemical requirements for N_2 fixation are: a) nitrogenase; b) a source of reductant and electron carriers (reduced flavodoxin or ferredoxin and

NADPH); c) an energy source: ATP, derived from fermentation, substrate level phosphorylation, oxidative phosphorylation or photophosphorylation, oxidative phosphorylation or photophosphorylation; d) a carbon backbone for amino acid and amide synthesis; e) a specialized pathway for assimilation of NH_3; f) a regulatory system for nif genes; g) a mechanism of oxygen protection for nitrogenase; and, h) hydrogen evolution.

Among the above requirements three of them in particular, ATP, NADPH, and a carbon backbone for amino acids at the level of carbohydrate, have received considerable attention as possible limiting factors of the fixation process in legumes.

ONTOGENETIC NITROGEN FIXATION

Nitrogen fixation as a function of time has been found, for major cultivated legumes, to follow a typical sigmoid curve. A peculiar characteristic to all profiles of fixation reported relates to a loss of activity during the ontogeny of legumes. The decline in some legumes coincides with the onset of flowering and pod-filling, whereas in other species (or different lines), the highest levels of N_2 fixation appear to occur during the seed-filling stage. Legume crops such as dry beans (Types I and II), (Day, 1972; Graham, 1975; and Dart et al., 1976), peas (Minchin and Pate, 1973, LaRue and Kurz, 1973; Lawrie and Wheeler, 1973, 1974), and broad bean (Pate, 1958, Lawrie and Wheeler, 1974), have been reported to show a decline early in the pod-filling stage. Peanuts (Hardy et al., 1971) and cowpeas (Dart et al., 1976), in contrast, show a rapid post-flowering increase in N_2 fixation, continuing throughout a considerable period of time into the pod-filling stage. Indeterminate cultivars of dry beans (Graham, 1975), show a pattern of sustained maximum fixation rate during flowering and a large part of

the pod-filling stage. The presumed relationship between flowering and nitrogen fixation in soybeans appears to vary with the cultivar and location (Hardy et. al., 1971; Mague and Burris, 1972; Lawn and Brun, 1974; and Thiobodeau and Jaworsky, 1975).

It is conceivable that any one of the seven essential requirements mentioned previously for nitrogen fixation to occur, could theoretically be considered as a potential limiting factor of this process. Several causes have been advanced as possibly limiting N_2 fixation. These have been concisely summarized by Hardy and Havelka (1975). They propose that fixation may be limited by: a) concentration of nitrogenase; b) percentage saturation of nitrogenase by substrates of the reaction, such as ATP, reductant or N_2 and, c) unidentified regulatory molecules. Inefficiency due to hydrogen evolution may also play an important role. The concensus of opinion in the literature, as shown below, favors the viewpoint that the amount of photosynthate available to the nodule may be the most significant factor limiting fixation and that a reduction in availability of photosynthate to these structures is a consequence of "intense competition for photosynthate from reproductive structures". An important modification of this viewpoint appears to exist in dry beans and is expressed further in the context of this thesis.

The hypothesis of carbohydrate supply as a primary factor in legume symbiosis has been entertained since the early 1930's. An example of the stage of knowledge at this time is the work of Allison (1930) and Allison and Ludwig (1939) which can be quoted as follows: "studies to date have shown that the available carbohydrate supply (chiefly sugars and starch) is a primary factor in determining nodule location, growth and size, quantity of nitrogen fixed by good strains,

disintegration of nodules, and similar phenomena" and, "the necessity for a supply of carbohydrate in legume symbiosis has long been recognized". Some years later, with the observations that inorganic nitrogen usually has a negative effect on nodulation and the fixation process, Wilson et. al., (1940) attempted to combine into a single hypothesis (carbohydrate-nitrogen hypothesis) considerations of both the nitrogen and carbohydrate supply and their effects on legume growth.

In the early studies no conclusive evidence was found that would relate carbohydrate supply to the decline in fixation rate, but they have undoubtedly served as the basis for current research. Evidence concerning this prevailing hypothesis can be analyzed primarily from two complementary points of view: first, evidence related to factors that increase photosynthetic output and; second, those which decrease the amount of available carbohydrate to the nodulated system. Consideration of the evidence demands that one should bear in mind that a subtle difference exists between factors that may be "nitrogen fixation limiting" per se and factor(s) that are direct or indirect causes of the observed decline in the activity of the system. A limited amount of information is available in the latter instance for the species under consideration in this thesis.

As previously indicated, photosynthesis and carbohydrate levels in plants were recognized early on as important in symbiotic fixation of nitrogen. The demonstration of enhanced N_2 fixation by CO_2 fertilization in beans by Riedels in 1922 (cited by Allison, 1935), and in red clover by Wilson, et. al., (1933) was neglected until the recent dramatic responses achieved with soybeans by Hardy and Havelka (1973, 1975). Riedels reported that the use of additional CO_2 increased the number of nodules on beans 5-fold and increased plant growth. Wilson

et. al., obtained increases of 100 to 200 percent in the dry weights and in nitrogen fixation when the pCO_2 was increased from that in normal air to 0.2 or 0.4 percent. The plants had two or three times as many nodules as the controls in air, and their size was greatly increased. Similarly, nitrogen fixation and nitrate reductase activities were measured by Hardy and Havelka (1973) at weekly intervals on field grown soybeans. Plants were exposed to air and CO_2-enriched air (800-1200 ppm CO_2) during the day, from preflowering to maturity. Carbon dioxide enrichment according to these authors resulted in a) doubled specific activity of nodules, b) doubled nodule fresh weight and, c) a longer exponential phase and delayed loss of activity. In contrast, nitrate-reductase activity decreased by 65%. Nitrogen fixation accounted for 85% of the nitrogen in mature plants under CO_2-enrichment but only 26% in air. No data were provided in this short communication in 1973. In 1975, data provided relates to various parameters at maturity and supported their conclusions only in part for: a) increased specific nodule activity (.228 mg N/g nodule dry weight to 1.187 mg N/g nodule dry weight); and, b) a longer exponential phase of fixation. Nodule mass reported was not doubled. Evaluation of the evidence is hampered since two different plant populations were used for air and CO_2 treated plants (455,000 plants/ha. for air-controls and 505,000 plants/ha. for CO_2 - treated plants). They suggested that the elevated pCO_2 increased net photosynthesis, due mainly to a decrease in photorespiration.

Quebedeau, et. al., (1975) reported similar results concerning pCO_2 effects on nitrogen fixation and dry matter production. They found that N_2 fixed was increased by CO_2/O_2 ratios greater than those

of air and was decreased by ratios samaller than those of air. Treatment combinations of 300 ul CO_2/l with 5 and 10% O_2 resulted in relative increases to air of 125% and 50% respectively. A treatment combination of 1200 ul CO_2/l and 21% O_2 yielded a 339% increase over the air-control soybean plants. They proposed that such an increase in total nitrogen fixed and total growth by subambient O_2 and CO_2-enrichment can be attributed to a reduction of photorespiration, and a direct O_2 inhibition of photosynthesis. Data reported at maturity shows that nodules were still present at that time.

Other treatments such as low planting density, increasing source size by grafting, supplemental light and pod removal have, in general, been found to increase N_2 fixation rates and other associated parameters.

Streeter (1974) reported increases of 75% over grafted controls in nitrogen fixation due to doubling the shoot:root ratio, while dry weight of nodules increased by 30% over control plants. In contrast, Lawn and Brun (1974) reported no increase in nodule fresh weight due to grafting or in the specific activity of nodules. The reported values for total activities of root genotypes appear to be significant. Whereas, Streeter utilized soybean plants grown in a sand-culture, nitrogen-free medium, Lawn and Brun utilized field-grown soybean plants.

Lawn and Brun (1974) reported a positive response of nodule activity and protein yield for two soybean varieties to supplemental light during the day. Partial depodding (removal of all pods from alternate nodes), resulted in 3.3% and 1.9% increases in seed protein and 18.9% and 12.1% in nodule activity over control plants (Chippewa 64 and Clay varieties, respectively). They suggested that the responses

indicated previously are consistent with the hypothesis of a limitation to symbiotic nitrogen fixation by competition for photosynthates from developing pods.

Bach et. al., (1958) before the advent of the acetylene-reduction assay, reported that specific radioactivity in nodules as compared to roots (after exposure to "several hours" to $^{14}CO_2$), was higher by a factor of 1.34 and 2.08 for day and night samples. Exposure of excised and/or crushed nodules to $^{15}N_2$ to determine the possibility of restoring their activity upon the addition of sucrose, fructose and glucose, resulted, according to them, in increases in fixation. Their data supports their conclusion only for the addition of sucrose in one of the experiments reported. Two additional experiments, one in which the three sugars were tested, and another in which sucrose alone was tested, do not support their contentions. Wong and Evans (1971) also working with soybean plants, reported that the addition of sucrose, fructose, glucose, pyruvate, succinate or malate to nodules exhibiting low nitrogenase activity failed to restore their activity.

More recent literature in which $^{14}CO_2$ labelling has been used provides, in some instances, more direct evidence for the hypothesis under consideration.

In 1974, Lawrie and Wheeler, working with pea plants, reported data on experiments concerning the extent to which "photosynthesis of the plant can satisfy the competition for assimilates between different metabolic sinks". They studied changes in nitrogenase activity and accumulation of photosynthates in the nodules during flowering and fruit formation. They reported that maximum nitrogenase activity and specific activity of nodules occurred 3 weeks after planting at flowering, and subsequently declined during fruit development.

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Senescence of nodules was evident from the presence of green nodules in the fourth week. Total radioactivity of plants doubled between weeks 3 and 4 to reach a maximum in week 4. Radioactivity levels in nodules, roots, leaves (1 through 7), and flower apices was determined for 2, 3 and 4-week old plants. Stems were not analyzed. In their discussion section they claim to have obtained a 60% decrease in specific activity of the nodules as the plants entered the reproductive phase, following a 6-hour cold-chase period with carbon dioxide. But, in the figures depicting the distribution of C^{14}-labelled photosynthates in relation to plant age, no differences in the radioactivity of the nodules after 6 and 25 hour cold-chase periods in any one of the 2, 3, and 4 week-old plants can be detected.

The alternative approach in the literature to evaluating the hypothesis under consideration has been to assess the effect of those treatments that reduce the supply of "energy" to the nodulated system. Those that seem to be more relevant are the effect of removal of "competitive sinks", reduction of source size and low light effects.

Hardy et. al., (1968) reported on the effect of leaf removal and exposure to toal darkness of soybean plants. Acetylene-reducing activity decreased 12% relative to the control after 1 day and was 14% of the control plants even ten days after leaf removal. Exposure to 17 hours of darkness resulted in a 70% decline in nitrogenase activity with respect to control plants. Similar results of the effect of leaf removal were reported by Lawn and Brun (1974) for soybeans. Lawrie and Wheeler (1973) working with Pisum reported that a marked correlation was found between the accumulation of photosynthates in nodules and the rates of acetylene reduction during the growth cycle of pea

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plants. When plants were maintained in the dark for periods of 38, 62, and 72 hours and subsequently returned to the light, nitrogenase activity recovered in plants exposed to 38 hours darkness almost to the level of plants maintained in continuous light. Plants darkened for periods of 62 and 72 hours did not fully recover. Radioactivity levels in nodules were similar to those of plants maintained in continuous light for the 38 hour dark-treated plants, but not for the 62 and 72 hour dark-treated plants. It is unfortunate that an inappropriate statistical treatment of the data does not allow one to determine the validity of the results reported. Also, it would appear that the appropriate control to use for comparison is not plants subjected to continuous light but plants grown in a normal light regime. In another experiment conducted on the effect of darkening on the rate of acetylene reduction, nitrogenase activity was found to decline, upon exposure to darkness, to near zero, while radioactivity level in the nodule appeared to decrease only after 72 hours exposure to darkness. But, the radioactivity level in control plants kept in continuous light was lower than that of darkened plants! They proposed that the continued increase of radioactivity in the nodules of darkened plants up to 48 hours suggests that "a substantial proportion of nodule photosynthate must also be used to support other activities such as growth and multiplication of the endophyte". It is interesting that no positive correlation between radioactivity level and the loss of acetylene reduction activity was found until 72 hours darkness which also could be interpreted as an indication that photosynthate per se is not "limiting".

The most direct kind of evidence found to support the idea that nitrogen fixation might be reduced as a consequence of reduced energy

supply to the nodules has been recently published by Ching, et. al., (1975). Working with detatched soybean nodules, they reported that exposure of soybean plants to a 1-day dark treatment resulted in reductions of 50% in nitrogenase activity in the nodule tissue, 15% of the energy charge, 60% of the sucrose content, 70% of the ATP content, 60% of total adenosine phosphate content and 55% of the ratio of ATP/ADP. Longer periods of darkness resulted in further decreases.

The effect of removal of "competing sinks" on N_2 fixation has received considerable attention in recent literature; the results reported showing discrepancies but in general supporting the view that removal of pods and/or flowers usually results in maintenance of reducing activity of nodules. Hardy et. al., (1968) reported no positive effect due to pod removal, in fact, they report decreases in nitrogenase activity and nodule weight due to pod removal. In contrast, Lawrie and Wheeler (1974), found in peas that the continuous removal of flowers "prevented decrease in acetylene-reduction activity". Nodule weight of intact plants was shown to be three times larger in 5-week old plants. Lawn and Brun (1974) found similar positive effects on N_2 fixation due to partial pod removal in soybeans. They also reported that a stage is reached in soybean plants when the growth rate of pods equals that of the tops and at this stage all of the dry matter increase in the tops is accounted for by the increase in pod dry matter (Note: vegetative organ competition is not mentioned). Subsequent to this stage, pod growth was found to exceed the growth rate of tops, indicating mobilization and translocation of previously stored assimilates from other plant parts into pods. Decline of nodule activity occurred immediately prior to the stage at which pod growth equalled the growth rate of the tops. They also proposed that this

can be taken as evidence that reduction of symbiotic activity is associated with development of the pod as a strong assimilate sink. Their suggestion is compatible with the observation of Hume and Crisswell (1972) that only small amounts of label were recovered from the roots and nodules after pod filling commences. Similarly, with peas, Minchin and Pate (1973) reported that nodule senescence coincides with the initiation of flower primordia. They speculated that it is possible that nodules "fare badly in competition with the root for assimilates" during the reproductive period. With dry beans, Dart (1974) reported that nodules of many dry bean varieties senesce at flowering (in contrast to soybeans and cowpeas) with a new population of nodules forming during the early pod filling period.

Knowledge concerning the possible causes of nodule senescence is limited. No known studies have revealed the internal cause(s) responsible for this phenomenon. Klucas (1974) found no significant changes in dry weight or total nitrogen in tap root nodules of soybeans during the period in which permanent loss of nitrogenase activity was observed. Total leghemoglobin content did not decrease during the initial decline in N_2 fixation. In contrast, poly-B-hydroxybutyrate was found to accumulate as the nodules aged, a significant increase being reported prior to or concomitant with the decrease in nitrogenase activity. The significance of this polymer remains unknown. It has been proposed that such a polymer could be an energy source, but Wong and Evans (1971) have shown that the presence of a supply of poly-B-hydroxbutyrate in soybean nodule bacteriods is not sufficient for maintenance of high nitrogenase activity under conditions of limited carbohydrate supply from the host plant, i.e., a) incubation of plants in the dark (up to 16 days); b) incubation of excissed nodules in dark (up to 60 hours); and c) the onset of senescence of nodules.

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CHAPTER 2

CARBOHYDRATE PARTITIONING AND NITROGEN FIXATION *PHASEOLUS VULGARIS* L.

Abstract

The ontogenetic relationships that may exist between symbiotic nitrogen fixation and carbohydrate partitioning were studied in four dry bean cultivars. Total soluble carbohydrate and starch contents of the primary nodule population were found to be closely correlated in time with nitrogen fixation activity. The decline in nitrogen fixation activity was found to be correlated with loss of nodule dry weight, decreased total soluble carbohydrate and starch of nodules and an increase in soluble carbohydrate and starch in leaves and stems during the early stages of reproductive growth. Starch concentration was found to decline one week prior to the occurrence of nitrogen fixation maximuma suggesting that hydrolysis of this polysaccharide may contribute energy to the fixation of molecular nitrogen. Reduction of carbohydrate movement to the nodule, due to competition by reproductive sinks, may not be the cause of the observed decline in nitrogen fixation. Temporary sites of starch accumulation were found to be restricted primarily to parenchyma cells of secondary xylem and pith in stems and to uninfected cells in nodules.

INTRODUCTION

The ontogenetic decline in the rate of nitrogen fixation in many legumes has been clearly documented (2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 15, 20, 21, 22, 23). A decreased supply of photosynthate to the nodules has been advanced as a possible cause for the drop in fixation during the initial phases of the reproductive period (6, 8, 10, 11, 12, 13, 22, 23). The coincidence of decreased nitrogenase activity with a given stage of development in the legume under consideration has prompted several investigators to deduce that "competition" for photosynthetic assimilates by the developing reproductive structures has a direct negative influence on the energy supply to the nodules.

The purpose of this study was to evaluate the hypothesis based on reported research that a change in the pattern of partitioning of carbohydrates between vegetative and reproductive sinks is correlated with or is the ultimate cause of an observed decline in the rate of nitrogen fixation in Phaseolus vulgaris L.

MATERIALS AND METHODS

Plants utilized in this study were grown at the experimental site "Las Guacas" of the Secretaria de Agricultura del Valle del Cauca, located in Popayan, Colombia. This site has been used by the International Center for Tropical Agriculture (CIAT) in Cali, Colombia, as its major location for nitrogen fixation studies in dry beans.

The experimental areas were fertilized on September 16, 1975 by broadcasting the following fertilizer equivalents per hectare: 1,000 kg of triple superphosphate ; 50 kg KCL ; 0.5 kg $NaMoO_4$; 7.0 kg $ZnSO_4$; 2.0 kg Borax ; 1,000 kg of agricultural lime ; 100 kg $MgSO_4$; and 2.0 kg $CuSO_4$. The experimental area consisted of a section 30 by 70 m. The

fertilizer was incorporated by disc-harrowing. A split-plot experimental design with randomized blocks was utilized, harvests (10 harvests) were the main plots and cultivars (4 cultivars) were sub-plots. Four replications were used. The experimental unit consisted of 4 rows, 4 m long per harvest per cultivar. Planting distances were 50 cm between rows and 7.5 cm between plants in the row.

Four cultivars were selected for this study according to their previously determined nitrogen fixation capability. The cultivars selected were "72 VUL 26689" , "ICA Pijao", "NEP-2", and "Porrillo Sintetico". All four cultivars are of semideterminate (Type II) growth habit.

Seed of the four cultivars was inoculated with an efficient Rhizobium phaseoli strain, CIAT 57, and pelleted immediately prior to hand planting. The "peat" (turba)-based inoculum was bouned to the seeds with a 40% Gum Arabic aqueous solution and pelleted with technical grade $CaCO_3$. Seeds were sown on September 19, 1975.

Acetylene - Ethylene reduction assays and sample preparation.

Plants of each variety were harvested at weekly intervals beginning 25 days after planting. Assays were conducted between 10 and 12 a.m. Fifteen plants per variety per replicate were carefully uprooted. Five nodulated roots were used per variety for each acetylene assay. The tops of these five plants were placed in paper bags and stored in styrofoam coolers containing dry ice until further processing of the samples. This subsample of five plants was utilized for chemical determinations after separation into component parts and drying. Drying was accomplished in 2 days at 60 C. Subsequently, plant parts were weighed and ground in a Wiley Mill to pass a 60-mesh screen. Ground samples were stored in 25 by 125 mm teflon-lined screw

cap culture tubes until analyzed. The remaining ten plants of each sample were similarly treated (with the exception of grinding) for complete growth analysis. Combining weights of the 5 plants used in the acetylene assays with weights of the 10 plants indicated above allowed expressing values in grams/15 plants.

Assays were performed in dark-brown, 1 l glass jars having a vacutainer stopper in the center of the lid and a gas tight seal. One tenth of an atmosphere of acetylene (welding grade) was injected after removing an equivalent amount of air. Incubation time was 30 minutes. A 10-ml gas subsample was transferred into vacutainer tubes at the end of the incubation period. Ethylene analysis was performed with a Perkin-Elmer Gas Chromatograph with a Flame Ionization Detector. A 1.8 m. stainless steel column packed with Porapak N was used. Calibration of detector response was obtained by using a 98 ppm C_2H_4 standard. Volume of sample injected was 1 ml.

Analysis of total organic nitrogen.

One hundred milligram subsamples of dried and ground plant tissues were analyzed by micro-kjeldhal following the procedure of Yoshida, et. al., (1971).

Analysis of sugars (ethanol-soluble carbohydrates) and starch.

Ethanol-soluble and insoluble (starch) carbohydrates were determined quantitatively by a modification of the antrhone method of Yoshida et. al., (1971). The modified procedure is as follows: a subsample of approximately 100 mg dried tissue was extracted three times for 30 minutes each with 80% analytical grade ethanol in a constant temperature water bath (80 C). After which each sample was centrifuged (2000 X g). Tubes were capped with glass balls during extraction. Ethanol extracts were combined and adjusted to 30 ml volumes. The

residue remaining was used for starch determinations as indicated below. An aliquot (usually 1 ml) of extract was diluted (20X to 50X) with deionized-distilled water. Two ml aliquots of this diluted extract were transferred into 25 by 150 mm pyrex screw capped culture tubes and the tubes were placed in an ice-water bath for 10 minutes. Four ml of anthrone reagent (0.5 g of anthrone in 1000 ml of analytical grade sulfuric acid, 95%, aged in the dark for 1 hour prior to use) were added by running the reagent on the side of the test tubes and kept in ice-water bath for five more minutes. Subsequently, test tubes were swirled with a test tube agitator and chromogen development was performed in a water bath (100 C), for exactly 7 minutes. Absorvance was determined using a Spectronic-20 colorimeter, using a blank containing deionized-distilled water plus the antrhone reagent at 630 nm. A set of five standards (0.0, 0.025, 0.050, 0.075 and 0.100 mg glucose/2 ml) were run every time a set of samples was analyzed.

The residue remaining after ethanol extraction and centrifugation was dried overnight at 80 C. Following this, 2 ml of deionized-distilled water were added to each sample, stirred in a test tube agitator and placed in a water bath at 100 C for 15 minutes. Samples were then allowed to cool to room temperature and 2 ml of 9.2 N $HCLO_4$ were added and stirred occassionally for 15 minutes. Volume was adjusted to 10 ml with deionized-distilled water and centrifuged at 2000 x g. A second extraction was performed with 2 ml of 4.6 N $HCLO_4$, adjusted to 10 ml volume and centrifuged. The combined extracts were adjusted to 20 ml volume and chromogen development with anthrone was performed as indicated previously. Chemical analysis of samples strictly adhered to the experimental design utilized in the field.

Histochemical Analysis of Nodules and Stems.

The procedures concerning the fixation of nodule and stem samples, the dehydration, embedding in plastic, and staining with Toluidine Blue and Periodic Acid Schiff Reagent have been carried out after Feder and O'Brien (1968).

According to O'Brien and McCully (1969), Toluidine Blue "binds with carboxylated polysaccharides (pectic acids) to give a pinkish purple color and to molecules with free phosphate groups (for example, nucleic acids), give purplish or blue green. Hydroxylated polysaccharides, such as cellulose and starch are not stained by this dye. DNA stains blue or blue green and RNA purple.

The mercuric-Bromphenol Blue procedure was carried out after Mazia et. al., (1953), with some modifications as follows:

1) 0.1% Bromphenol Blue in saturated aqueous $HgCl_2$ - 20 minutes.
2) 0.5% acetic acid in saturated aqueous $HgCl_2$ - 10 minutes.
3) Sections immersed (not rinsed) in demineralyzed water (pH 7.0), two changes, one minute each.

Mazia et. al., (1953) reported that either an aqueous or alcoholic solution of Bromphenol Blue in saturated $HgCl_2$ can be used in the above procedure. The aqueous solution was selected since an alcoholic solu-solution caused plastic infiltrated sections to slide-off the glass slides. According to Ruthman (1970), proteins are stained intensely blue.

RESULTS AND DISCUSSION

Symbiotic nitrogen fixation during ontogeny

Analysis of the results obtained for the four dry bean cultivars 72 VUL 26689 (C_1), ICA Pijao (C_2), NEP-2(C_3) and Porrillo Sintetico (C_4)

revealed a close similarity in trends. These consistent patterns exhibited for almost all parameters measured has lead to restricting the presentation of results and disucssion to two of the four cultivars studied. The cultivars to be considered in detail are 72 VUL 26689 and Porrillo Sintetico. The results obtained for the four cultivars and their appropriate statistical analyses are presented in the Appendix Tables. Significant departures from the observed general trends are indicated when present.

Seasonal profiles of nitrogen fixation activity are shown in Figure 1. Corresponding values and Analysis of Variance are shown in Appendix Table 10. A peculiar characteristic of the profiles is the sharp decline in nitrogenase activity of the cultivars ICA Pijao (C_2) and NEP-2 (C_3) at 67 days after planting henceforth referred to as (dap), i.e., at harvest 7. In contrast, the cultivars Porrillo Sintetico (C_4) and 72 VUL 26689 (C_1) exhibit this decline seven days later, at 74 (dap) (harvest 8). Macroscopic evidence of the onset of the reproductive period (as indicated by the presence of open flowers) in all four cultivars was present at 53 dap (harvest 5). One can deduce that the decline in fixation occurs during the initial phases of pod development. The cultivar NEP-2 had a growth cycle of 112 days, whereas the other three cultivars had a growth cycle of 116 days.

The cultivars 72 VUL 26689 and Porrillo Sintetico sustain maximum fixation rates for at least a 7-day period. In contrast, NEP-2 and ICA Pijao reached a maximum fixation level at 60 dap (harvest 6) and subsequently declined to approximately the same level present at 39 dap (harvest 3). A small burst in nitrogen fixation activity for the four cultivars was observed at 81 dap (harvest 9). Similar activities, due possibly to "secondary nodule population levels", have

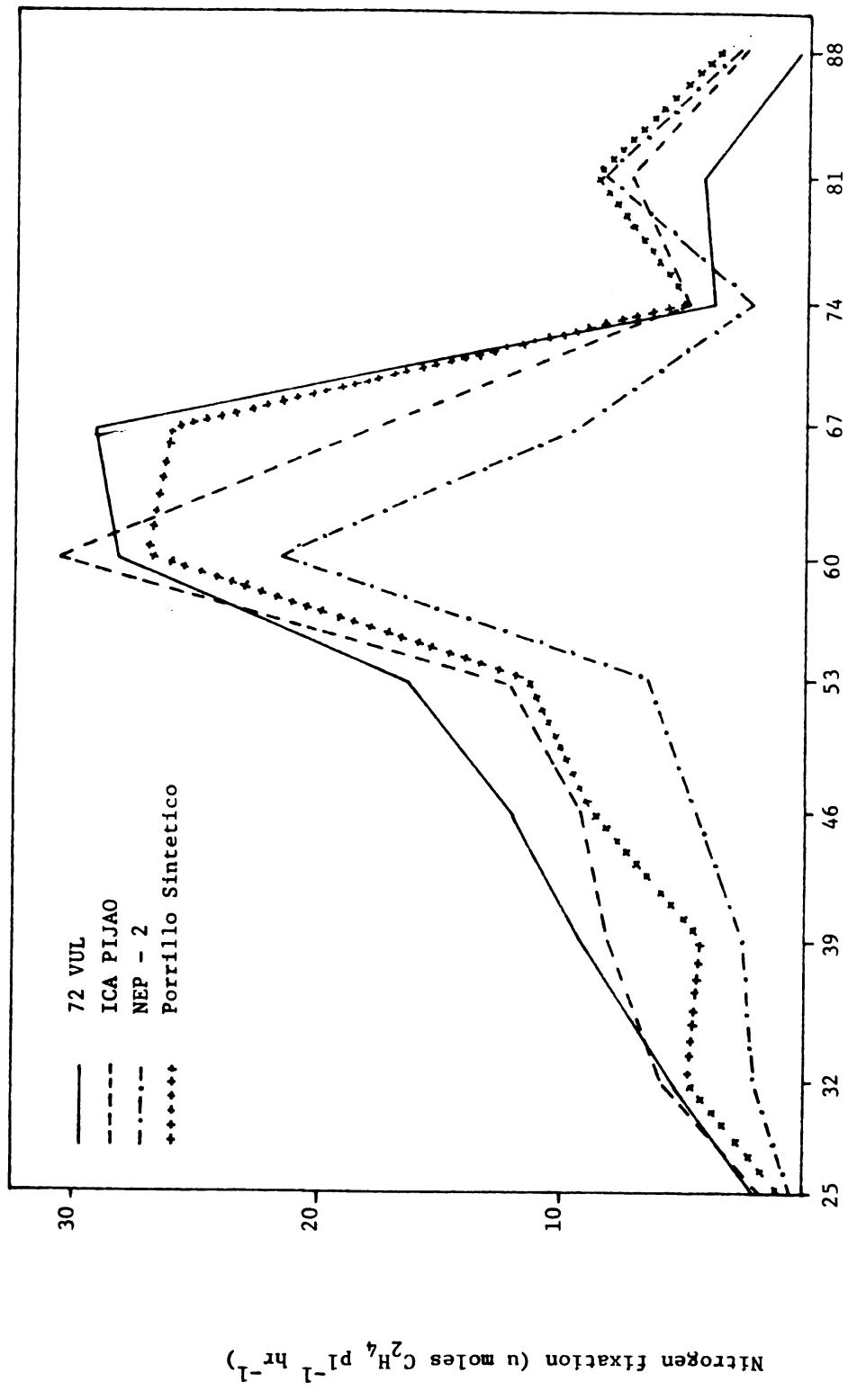

Figure 1. Nitrogen fixation rates per plant of four dry bean cultivars taken at weekly intervals throughout ontogeny.

been previously reported by Dart (1974) and Graham (1975), for dry bean cultivars. Estimates of milligrams of N_2 fixed/plant were computed using the following relationship (see Table 1):

$$\frac{\text{mg } N_2 \text{ fixed}}{\text{pl wk}} = \frac{1}{3}\left(\frac{\text{umoles } C_2H_4}{\text{pl hr}} \times \frac{24 \text{ hr}}{1} \times \frac{7 \text{ days}}{\text{wk}} \right) \quad /5 \text{ plants}$$

where 0.333 = theoretical conversion factor of mg C_2H_4 produced/mg N_2 fixed.

0.81 = diurnal factor for nitrogenase activity.

They indicate that 72 VUL 26689 and Porrillo Sintetico fixed 30.67% and 26.86% of the total symbiotically-fixed N_2 prior to the appearance of open flowers. Similarly, 69.33% was fixed by 72 VUL 26689 and 73.13% by Porrillo Sintetico from 53 dap onwards, i.e., in the reproductive phase of development. Values for ICA Pijao and NEP-2 were 24.86% and 23.17% prior to macroscopic flowering, and 75.13% and 79.82% during the reproductive phase. The highest fixation levels per week occurred at harvest 6 and 7 for the four varieties. During these two weeks, 72 VUL 26689 fixed 50.56% (73.28 mg N_2), ICA Pijao fixed 48.75% (63.63 mg N_2), NEP-2 fixed 49.91% (39.84 mg N_2) and Porrillo Sintetico fixed 47.62% (67.58 mg N_2) of the total N_2 fixed during their ontogenies.

Table 1 shows that 72 VUL 26689 and Porrillo Sintetico were the highest fixers. Better fixation in these cultivars came about primarily from maintaining their fixation activity at their maximum levels for 2 weeks. The cultivar 72 VUL 26689, the best fixer, also shows higher rates of fixation than the other three cultivars prior to the period in which N_2 fixation rates reach maximum levels. Fixation levels beyond the point of decline in maximum activity can be observed

Table 1. Dinitrogen fixed and percentage of total nitrogen fixed of ten sequential weekly intervals of four dry bean cultivars.

Harvest time (dap)[1]	Cultivars							
	72 Vul 26689		ICA Pijao		Nep-2		Porrillo Sintetico	
	N_2 fixed		N_2 fixed		N_2 fixed		N_2 fixed	
	(mg/wk)	(% of total)	(mg/wk)	(% of total)	(mg/wk)	(% of total)	(mg/wk)	(% of total)
25	9.12	6.29	8.36	6.40	2.62	3.09	4.68	3.30
32	8.09	5.58	2.02	1.55	2.59	3.04	6.08	4.28
39	11.81	8.15	10.25	7.85	3.31	3.89	5.49	3.87
46	15.44	10.65	11.82	9.06	11.17	13.15	21.86	15.41
53	20.67	14.26	15.51	11.88	8.33	9.81	14.60	10.29
60	36.09	24.90	39.04	29.91	27.61	35.51	34.37	24.22
67	37.19	25.66	24.59	18.84	12.23	14.40	33.21	23.40
74	4.89	3.37	6.28	4.81	2.88	3.39	5.99	4.22
81	1.27	0.88	9.35	7.16	10.64	12.53	11.07	7.80
88	0.37	0.26	3.31	2.53	3.55	4.18	4.55	3.20
Total	144.96	100.00	130.53	100.00	127.58	100.00	141.92	100.00

1: day after planing.

to remain low, but appear not to reach a zero level, probably indicating that nodules induced in more advanced stages of development of the bean cultivars, could account for this sustained but low activity.

Partitioning of dry matter

Figures 2, 3, 4, and 5, show that total plant dry weight increased continuously up to 81 dap (harvest 9) in the cultivars 72 VUL 26689 and Porrillo Sintetico. Nodule dry weight increases occur up to 60 dap (harvest 6) and subsequently decline at 67 dap (harvest 7) and 74 dap (harvest 8) for 72 VUL 26689 and Porrillo Sintetico, respectively. At harvest 9 (81 dap), a small increase in nodule dry weight was again observed in all four cultivars. This increase in nodule mass is consistent with the observed increase in fixation rates observed at harvest 9. Maximum fixation rates can be seen to coincide with maximum dry weights of the nodule population, one week after the appearance of open flowers (53 dap). These trends were exhibited by all four varieties as shown in Appendix Tables 1 through 9 and 11.

Dry weights of leaves increased up to 67 dap (harvest 7). A small decrease in dry weight of leaves was observed at 74 dap (harvest 8) in all cultivars. This decrease in dry weight of leaves appears to be due primarily to leaf fall, a characteristic of dry bean varieties of determinate and semideterminate growth habit. Leaf senescence and abscission appears to be restricted primarily to the lower leaves of bean cultivars. Data from component parts revealed that at the cotyledonary scar positions, new branches and leaves are formed from 53 dap on. This fact may be important in the carbohydrate distribution pattern and its possible relationship to the decline in N_2 fixation activity.

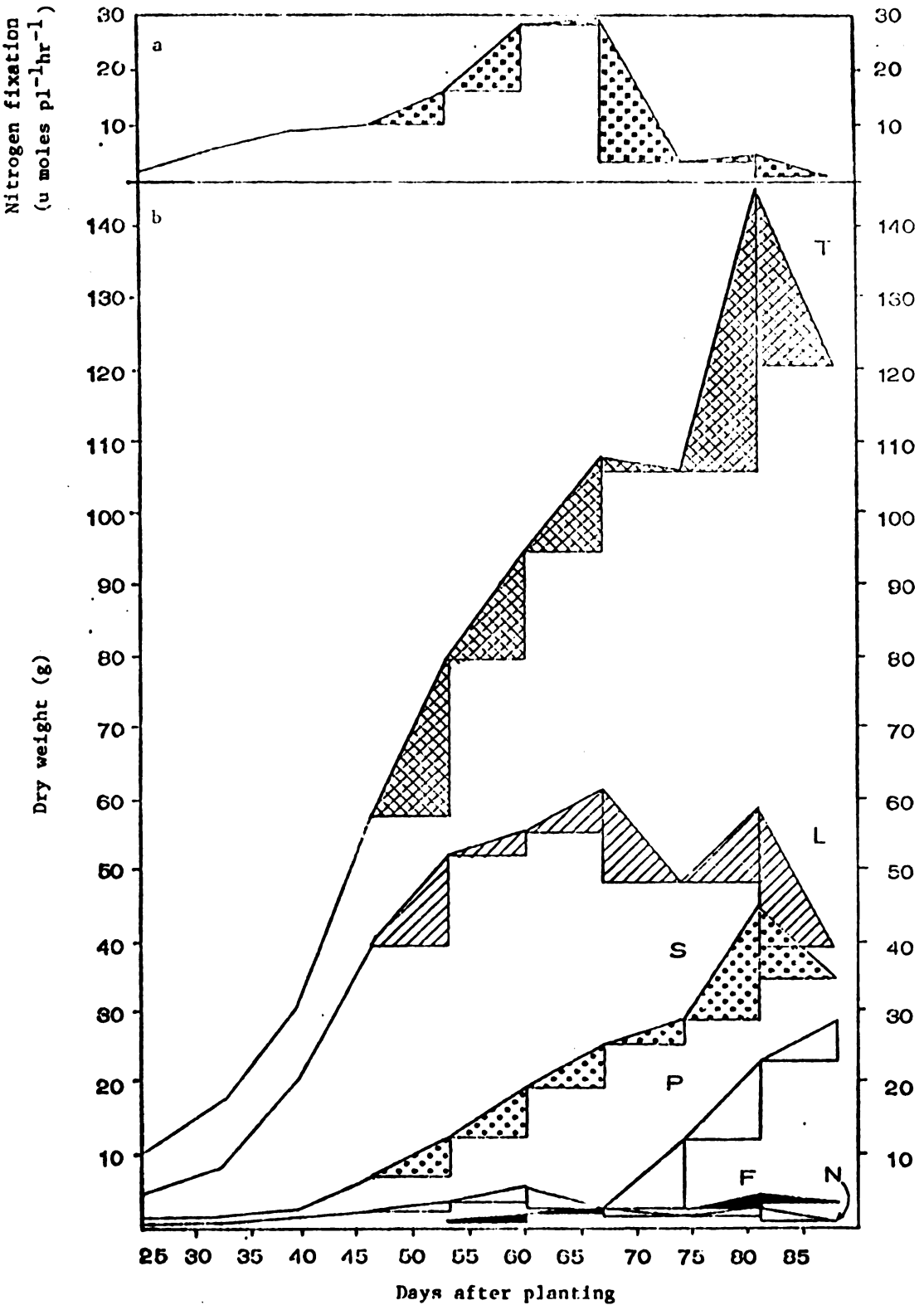

Figure 2. Nitrogen fixation (a) and ontogenetic distribution of dry weight (b): total (T); leaves (L); stems (S); flowers (F); pods (P); and nodules of the cultivar 72 VUL 26689.

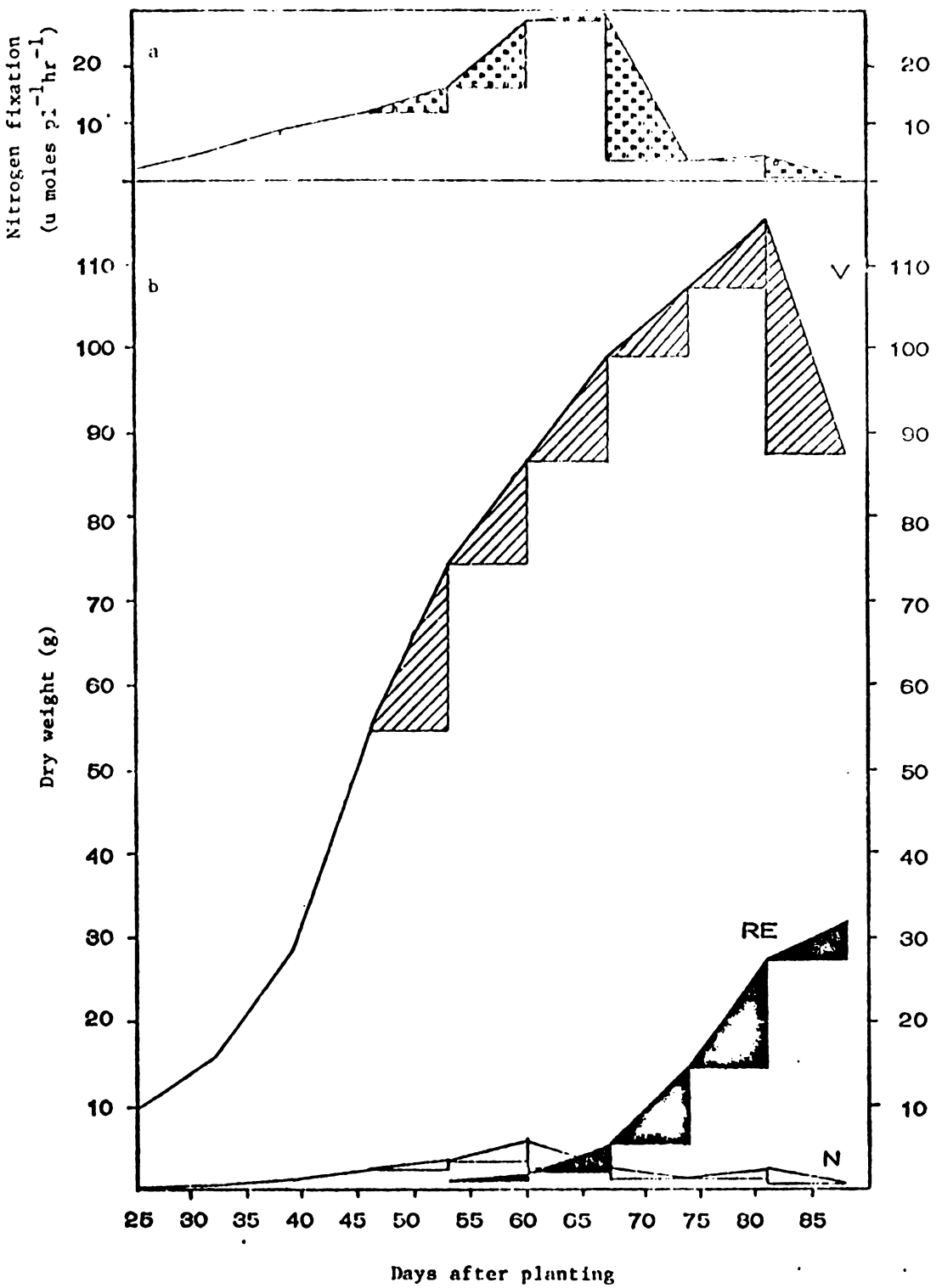

Figure 3. Nitrogen fixation (a) and ontogenetic distribution of dry weight (b) of vegetative (V) and, reproductive (RE) structures and, nodules (N) of the cultivar 72 VUL 26689.

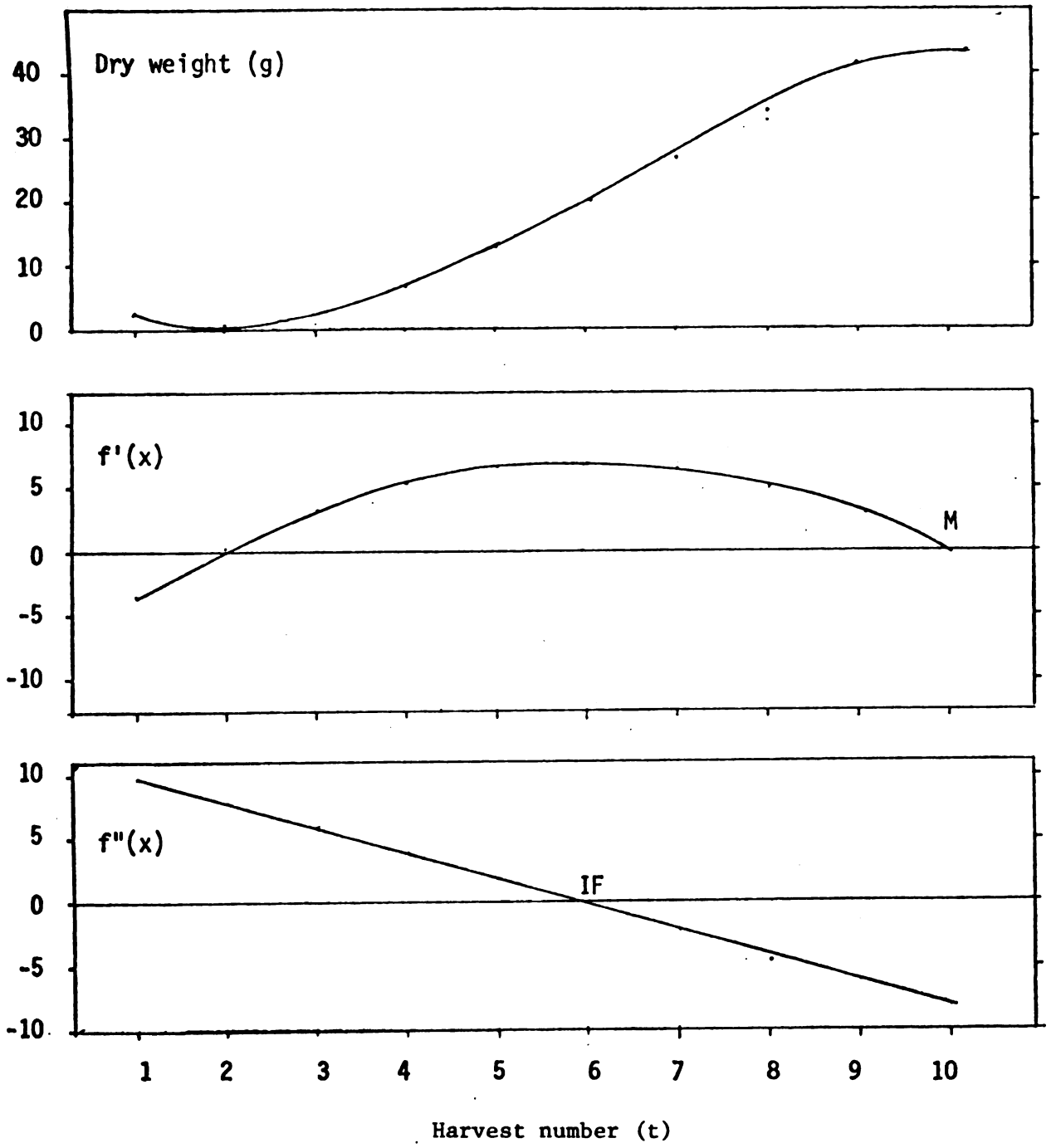

Figure 3a. Ontogenetic distribution of stem dry weight in the variety 72 VUL 26689. Values shown are derived from cubic polynomials. Inflection points (IF) and maxima (M) are shown.

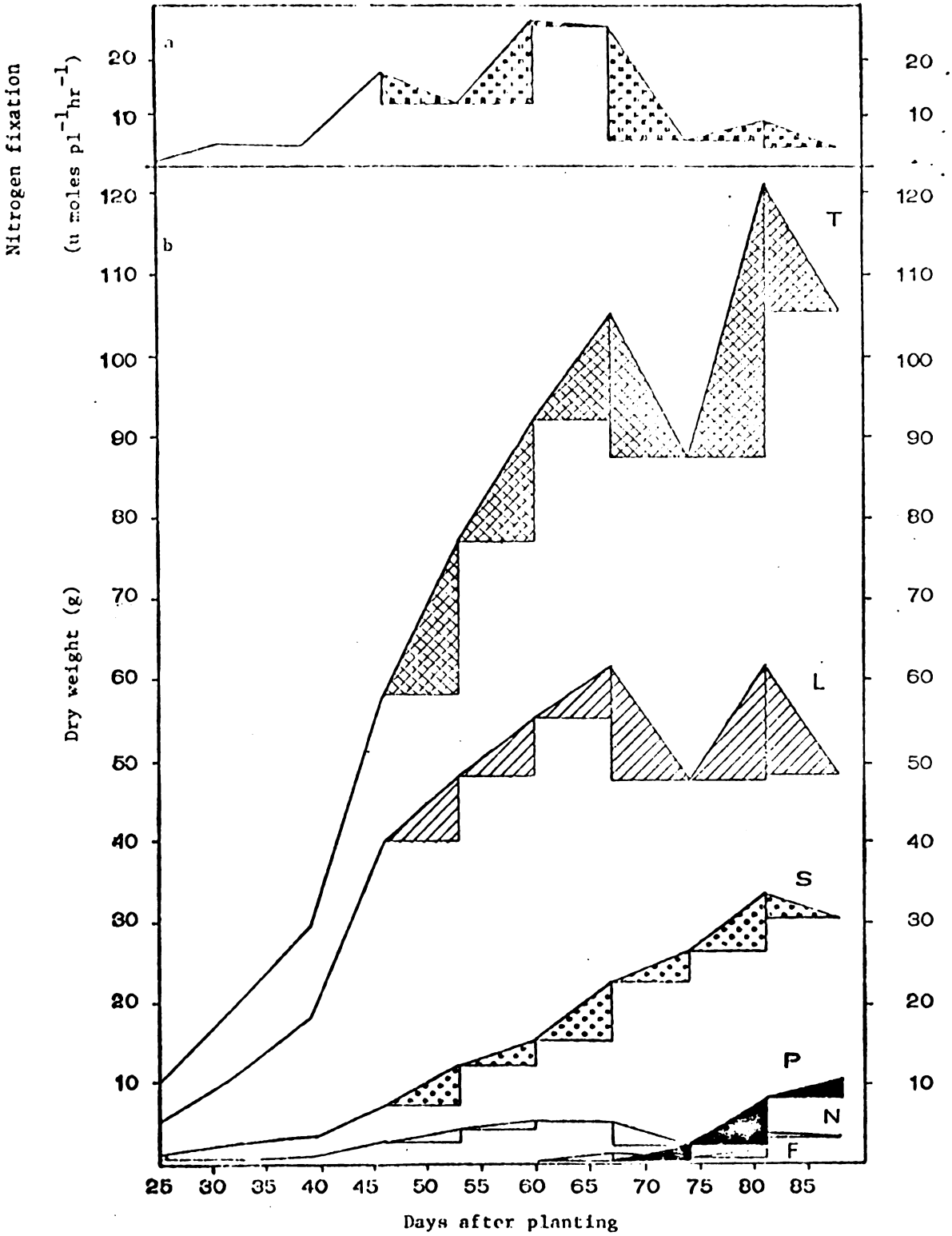

Figure 4. Nitrogen fixation (a) and ontogenetic distribution of dry weight (b): total (T); leaves (L); stems (S); flowers (F); pods (P); and nodules (N) of the cultivar Porrillo Sintetico.

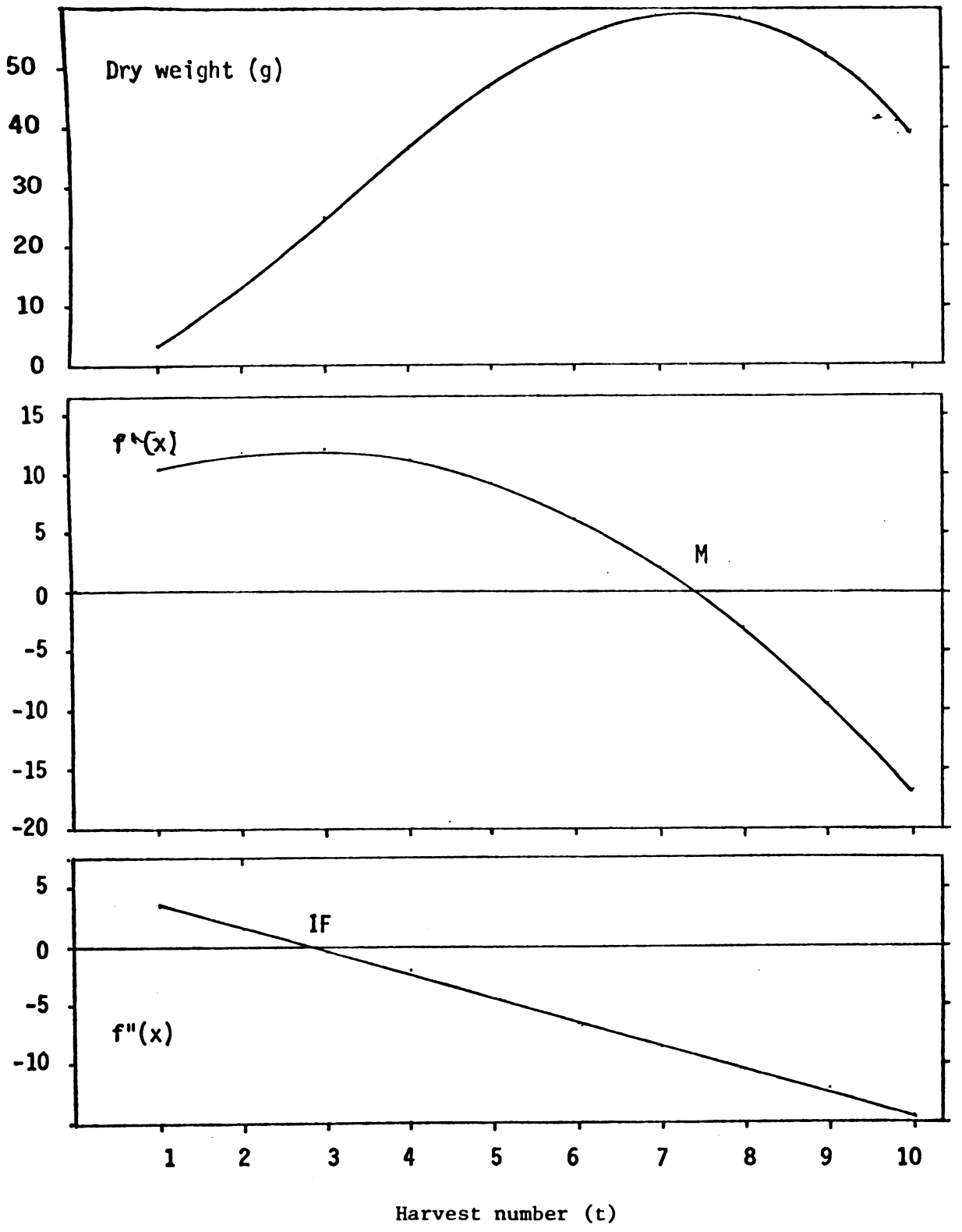

Figure 4a. Ontogenetic distribution of leaf dry weight in the variety 72 VUL 26689. Values shown are derived from cubic polynomials. Inflection points (IF) and maxima (M) are shown.

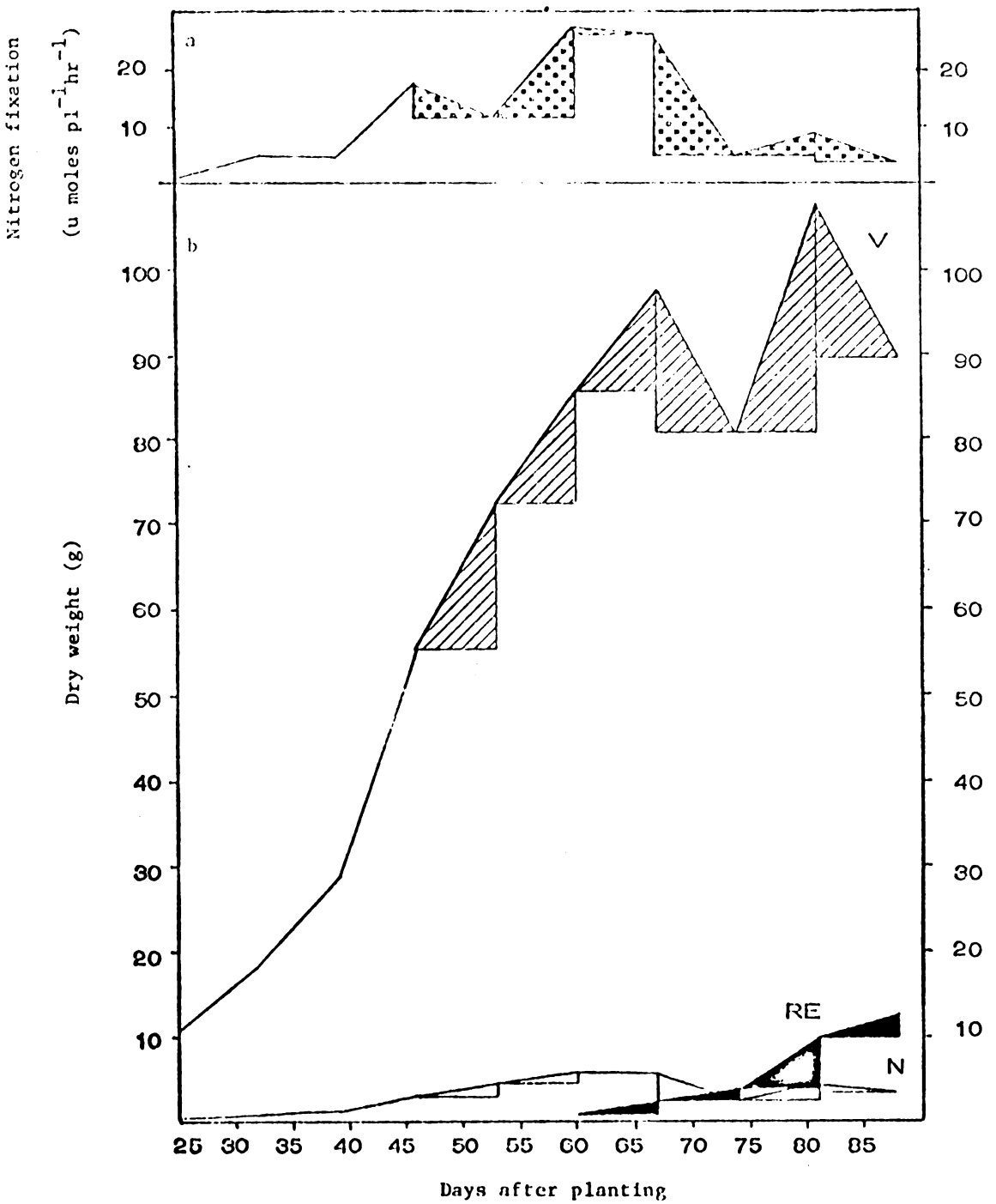

Figure 5. Nitrogen fixation (a) and ontogenetic distribution of dry weight (b) of vegetative (V) and, reproductive (RE) structures and, nodules (N) of the cultivar Porrillo Sintetico.

Stem weight was found to increase continuously until 81 dap (harvest 9), subsequently declining at harvest 10 (88 dap). Corresponding values and Analysis of Variance are given in Appendix Table 3.

Cubic polynomials were fitted to the dry weights of leaves and stems and the results are shown in Table 2. Figures 3a and 4a show the plots of the relative growth rates of these two organs (first derivative of the polynomials) and the plots of acceleration of dry weight (second derivative). It is interesting to observe that leaf dry weight in the cultivar 72 VUL 26689, show a very early inflection point at 37 dap (2.75 harvests) in relative growth rate. This is taken as evidence that allocation of resources favors these structures early on so that the photosynthetic system be established to provide the future demands of other processes, such as nitrogen fixation. Stem dry weight was found to show an inflection point at 60 dap (5.97 harvests) in the same cultivar. As is later shown in this chapter, at 60 dap temporary storage of starch in the stem is initiated at this time. Beyond 60 dap the acceleration of dry weight in stems decreases and clearly reflects the onset of starch storage mentioned above. Also, maximum nitrogen fixation rates occurred at this time. The relative growth rate of leaf dry weight became zero at 70 dap (7.43 harvests), while stems exhibited a zero relative growth rate at 9.99 harvests. Similar results were obtained with the remaining cultivars.

Figures 3 and 5 illustrate the dry weight allocation pattern to vegetative and reproductive structures, nodules, and their correlation in time with N_2 fixation rates. Corresponding values and Analysis of Variance are given in Appendix Tables 5 and 8. Reproductive structures include flowers, pods and the rachis of the racemose inflorescence.

Table 2. Cubic polynomials fitted to stem dry weights (A) and leaf dry weights (B) and harvests (t, independent variable) of four dry bean cultivars (C). First and second derivatives, maximums, and points of inflection are shown.

A. Stem dry weight	R^2
$C_1 = 8.215708 - 8.317555\ t + 2.567223\ t^2 - 0.143593\ t^3$	0.958
$C_2 = 4.172883 - 3.495695\ t + 1.288020\ t^2 - 0.062857\ t^3$	0.991
$C_3 = 3.592725 - 3.208141\ t + 1.298141\ t^2 - 0.072893\ t^3$	0.984
$C_4 = 5.664083 - 5.333215\ t + 1.843301\ t^2 - 0.104465\ t^3$	0.988

First derivatives	$f'(x)=0^1$
$C_1 = -0.430779\ t^2 + 5.13445\ t - 8.317555$	9.9
$C_2 = -0.188570\ t^2 + 2.57604\ t - 3.495695$	12.13
$C_3 = -0.218680\ t^2 + 2.59198\ t - 3.208141$	10.45
$C_4 = -0.313400\ t^2 + 3.68660\ t - 5.333215$	10.07

Second derivatives	$f''(x)=0^1$
$C_1 = -2t + 11.94$	5.97
$C_2 = -2t + 13.66$	6.83
$C_3 = -2t + 11.85$	5.92
$C_4 = -2t + 11.76$	5.88

Table 2. (cont'd.)

B. Leaf dry weight	R^2
$C_1 = -7.556008 + 7.92070\ t + 1.523418\ t^2 - 0.184524\ t^3$	0.936
$C_2 = -5.355783 + 6.94349\ t + 1.097879\ t^2 - 0.118747\ t^3$	0.945
$C_3 = -9.165492 + 11.4805\ t - 0.142661\ t^2 - 0.052869\ t^3$	0.847
$C_4 = -7.475542 + 8.89930\ t + 1.007260\ t^2 - 0.133980\ t^3$	0.931
First derivatives	$f'(x)=0$[1]
$C_1 = 0.553572\ t^2 + 3.04684\ t + 7.92070$	7.31
$C_2 = -0.356242\ t^2 + 2.19576\ t + 6.94349$	8.46
$C_3 = -0.158606\ t^2 - 0.28532\ t + 11.4800$	7.65
$C_4 = -0.401940\ t^2 + 2.01443\ t + 8.89900$	7.84
Second derivatives	$f''(x)=0$[1]
$C_1 = -2t + 5.50$	2.75
$C_2 = -2t + 6.16$	3.08
$C_3 = -2t - 1.80$	0.89
$C_4 = -2t + 5.01$	2.51

1: Values of maximums and inflection points are given as harvests, i.e. 9.9 harvests.

Cultivars: C_1 = 72 Vul 26689, C_2 = ICA Pijao,
C_3 = Nep-2, C_4 = Porrillo Sintetico

Declines in N_2 fixation rates and nodule dry weights coincide with the initial stages of reproductive development. It is important to note that at the time fixation declines, leaf and stem dry weight increases are of a higher magnitude as compared to the increases observed in the reproductive sturcutres. This has lead this author to entertain and evaluate the possibility that it is not "competition" by reproductive organs which explains the observed drop in fixation for the dry bean cultivars under consideration.

The allocation of soluble sugars and starch

Carbohydrate levels (ethanol-soluble sugars and starch) were determined in all organs previously mentioned, with the exception of rachis and flowers. Chemical determinations were made from 39 dap (harvest 3) for nodules and from 53 dap (harvest 5) for all other organs or structures, until 88 dap (harvest 10).

Figures 6, 8, 10, and 12, illustrate the values obtained in terms of total content of carbohydrate per plant. Corresponding values and Analyses of Variance are given in Appendix Tables 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34. Figures 7, 9, 11, 13, illustrate carbohydrate levels expressed as concentration, i.e., mg of carbohyderate/g dry weight. Corresponding values and Analyses of Variance are given in Appendix Tables 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35. Both kinds of information are included since they represent complementary aspects that relate directly to the hypothesis under consideration.

Figures 6 and 7 and, 8 and 9, show the trends of the carbohydrate levels found in nodules, stems, and leaves for 72 VUL 26689 and Porrillo Sintetico, respectively. Total content of starch and sugars in nodules is exhibited by Porrillo Sintetico, NEP-2, and ICA Pijao,

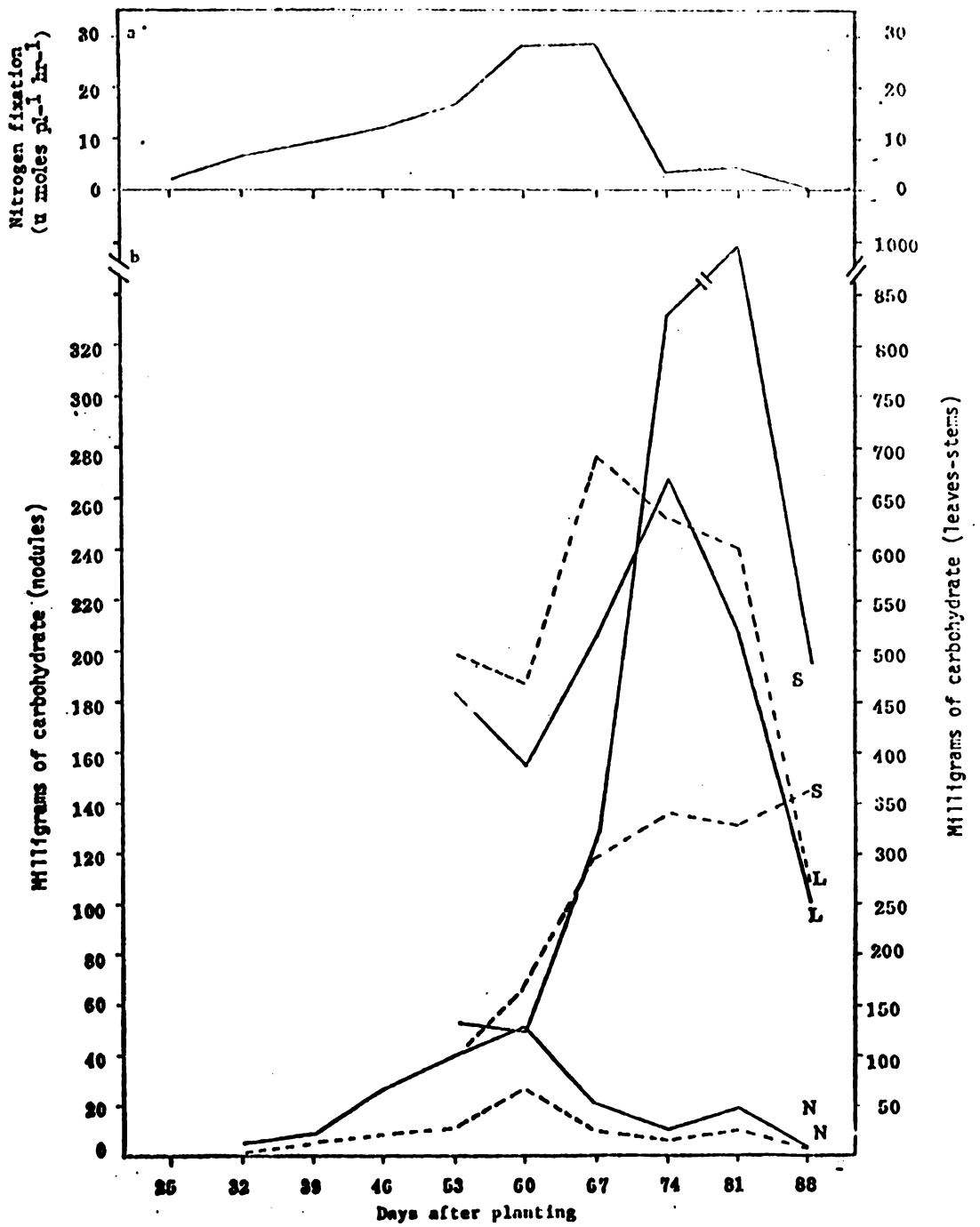

Figure 6. Nitrogen fixation (a) and content of ethanol-soluble carbohydrates (broken line) and starch (solid line) (b) in leaves (L), stems (S), and nodules (N) of the cultivar 72 VUL 26689.

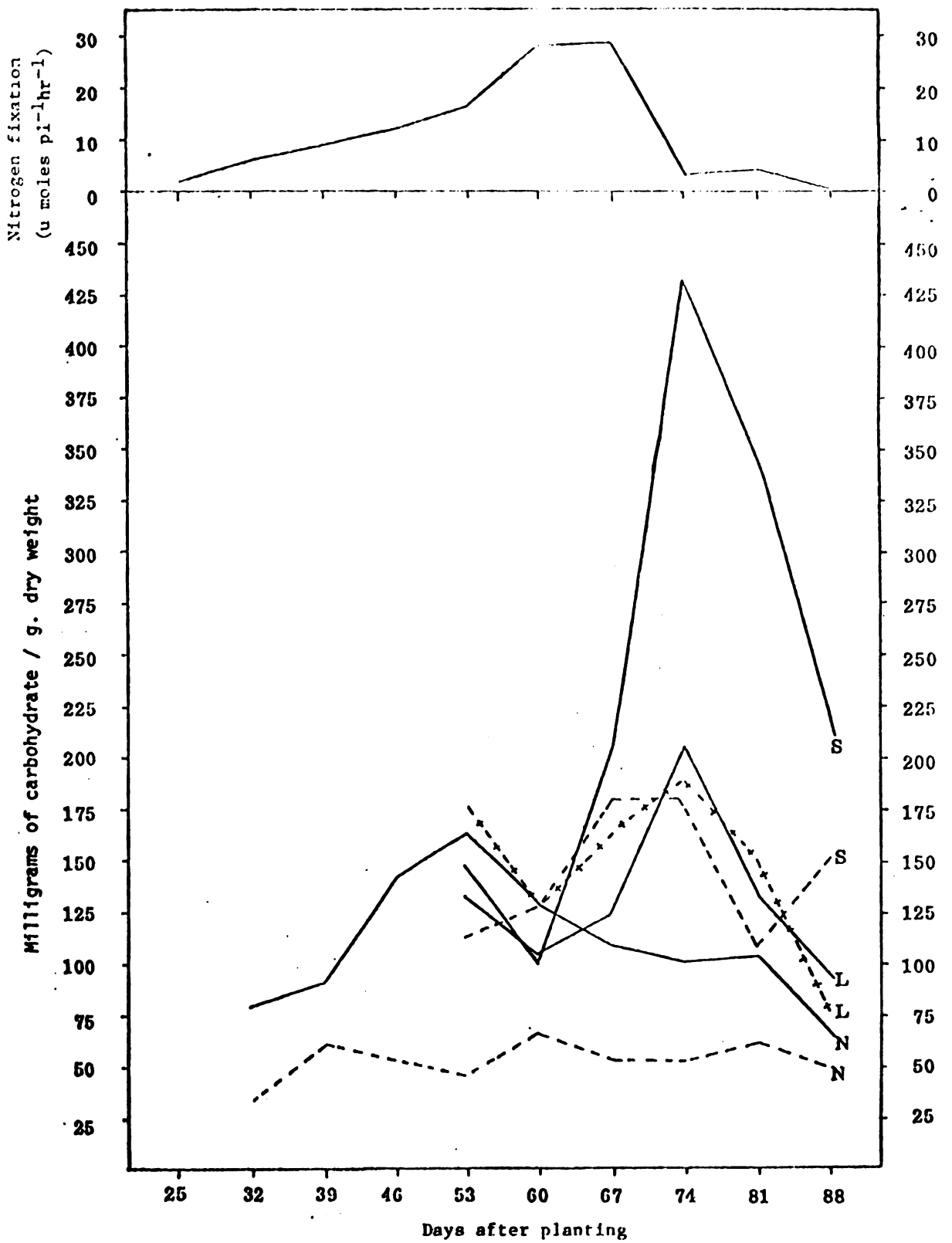

Figure 7. Nitrogen fixation (a) and concentration of ethanol-soluble carbohydrates (broken line) and starch (solid line) (b) in leaves (L), stems (S), and nodules (N) of the cultivar 72 VUL 26689.

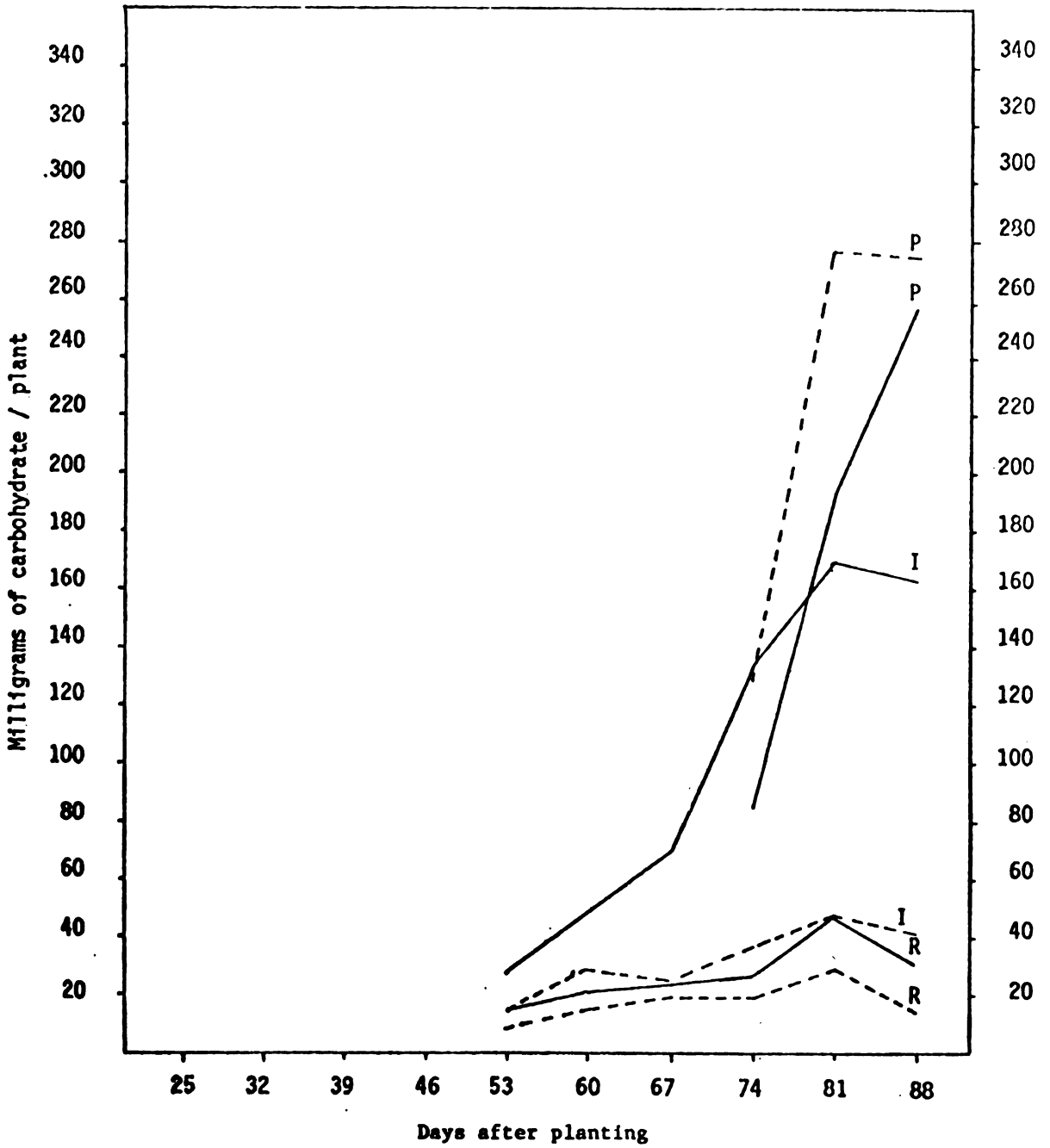

Figure 8. Content of ethanol-soluble carbohydrates (broken lines) and starch (solid line) in primary roots (I), secondary roots (R), and pods (P) of the cultivar 72 VUL 26689.

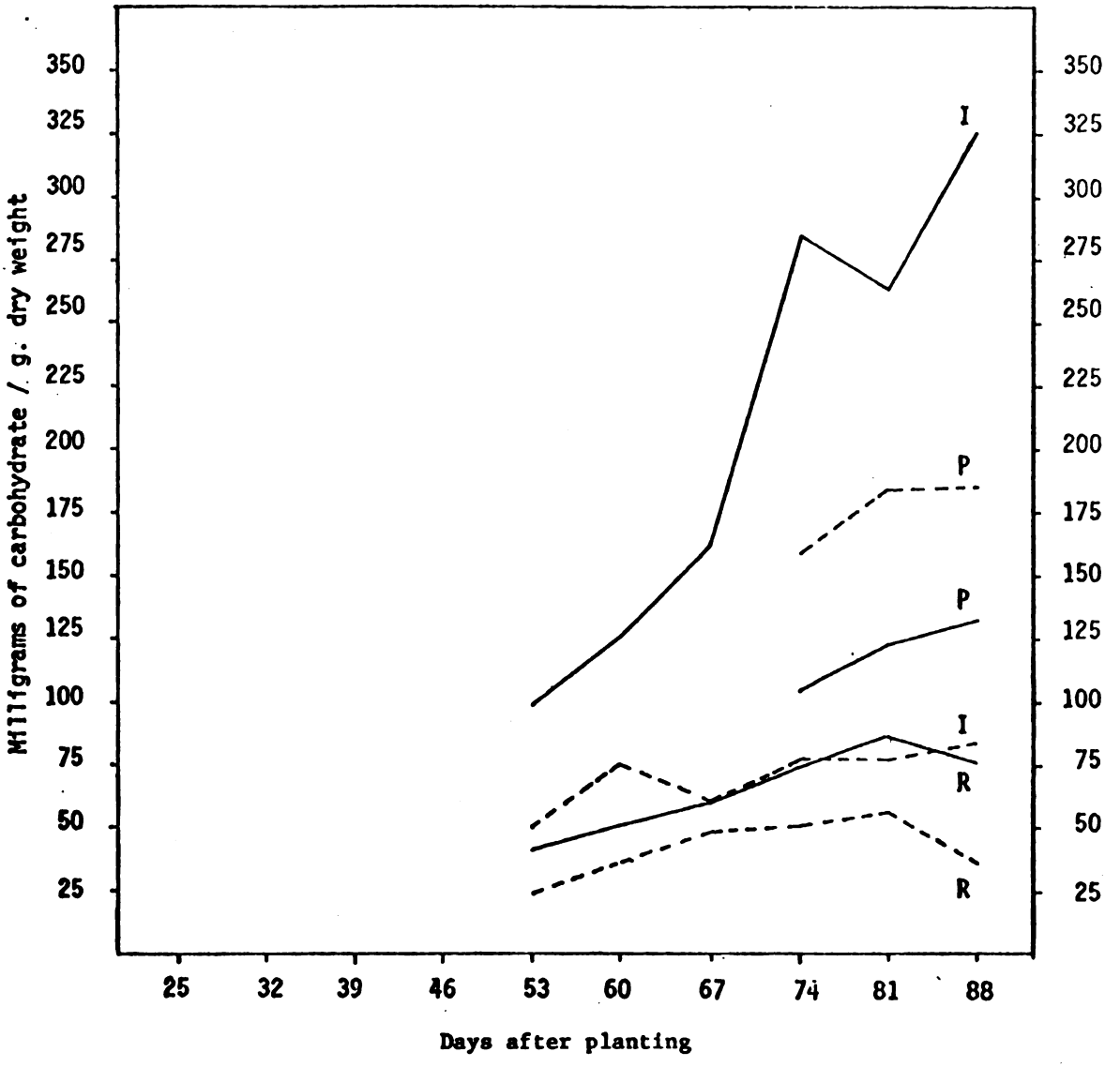

Figure 9. Concentration of ethanol-soluble carbohydrates (broken line) and starch (solid line) in primary roots (I), secondary roots (R), and pods (P) of the cultivar 72 VUL 26689.

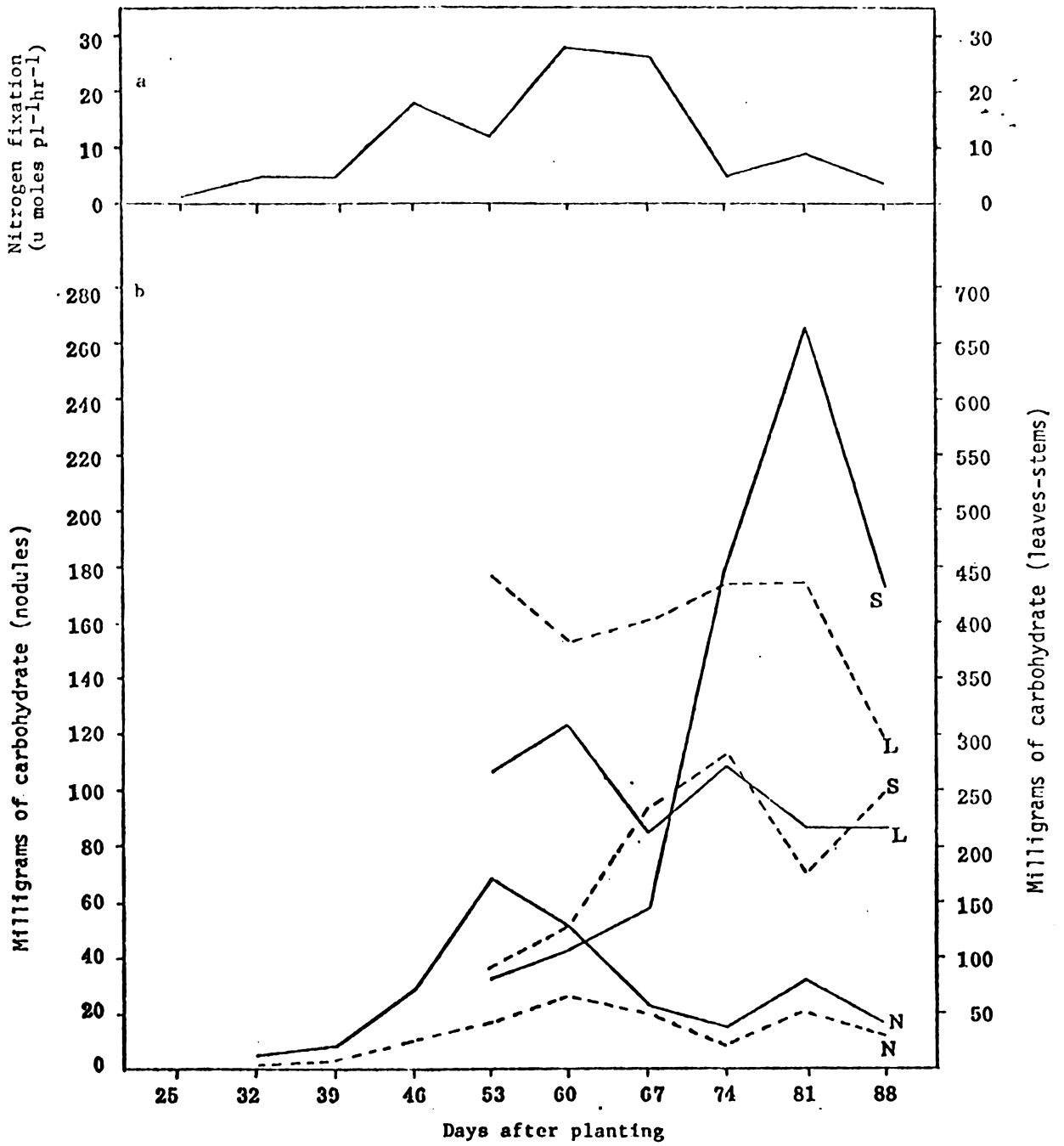

Figure 10. Nitrogen fixation (a) and content of ethanol-soluble carbohydrates (broken line) and starch solid line) (b) in leaves (L), stems (S), and nodules (N) of the cultivar Porrillo Sintetico.

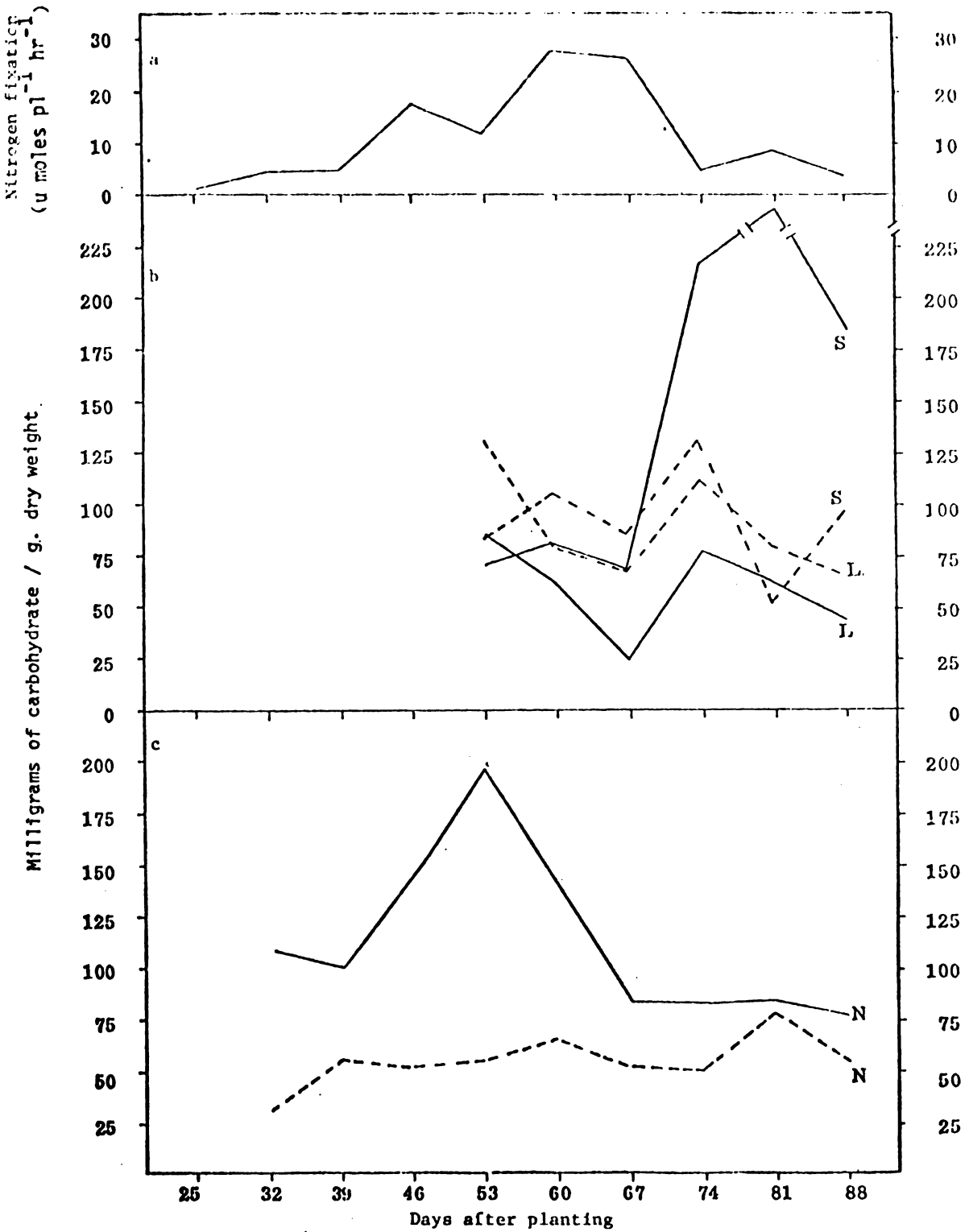

Figure 11. Nitrogen fixation (a) and concentration of ethanol-soluble carbohydrates (broken line) and starch (solid line) (b, c) in leaves (L), stems (S), and nodules (N) of the cultivar Porrillo Sintetico.

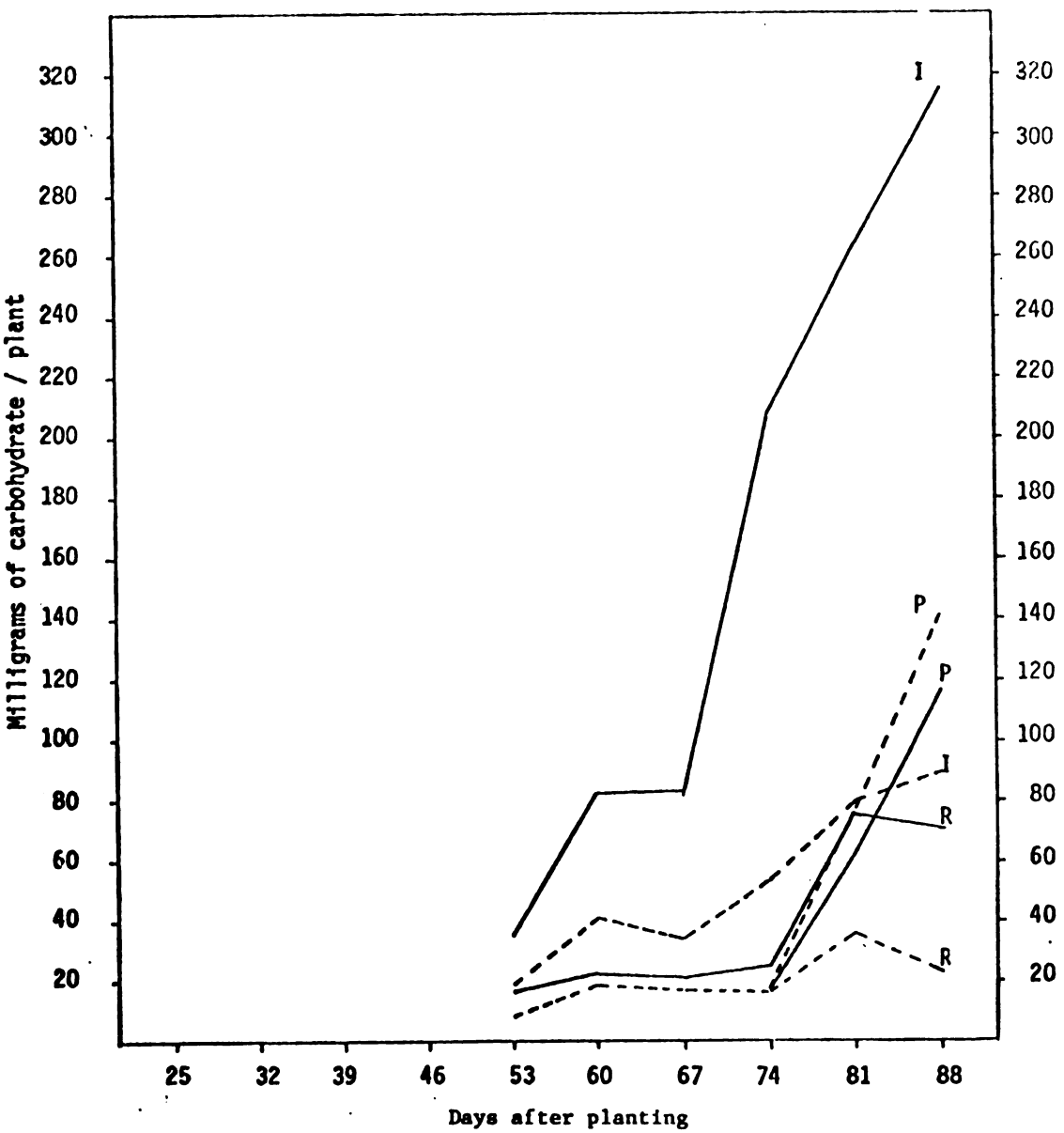

Figure 12. Content of ethanol-soluble carbohydrates (broken lines) and starch (solid line) in primary roots (I), secondary roots (R), and pods (P) of the cultivar Porrillo Sintetico.

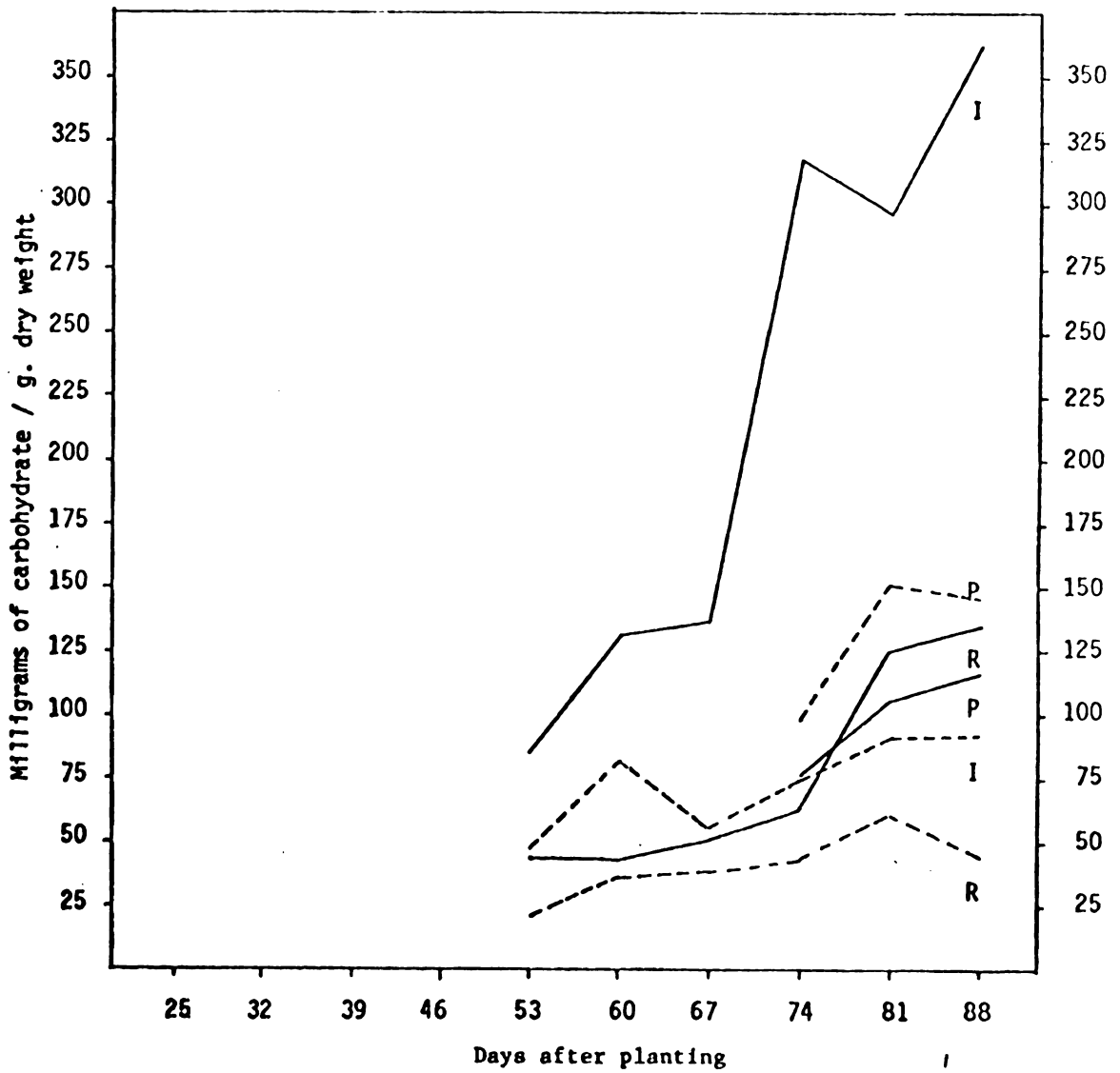

Figure 13. Concentration of ethanol-soluble carbohydrates (broken line) and starch (solid line) in primary roots (I), secondary roots (R), and pods (P) of the cultivar Porrillo Sintetico.

Table 3. Linear regression equations predicting nitrogen fixation rates for a given level of nodule soluble carbohydrate, and a given level of nodule starch.

Cultivar	Linear regression equation (u moles C_2H_4 pl^{-1} hr^{-1})	R^2
72 VUL 26689	1.084 X_1* + 1.962	0.715
	0.477 X_2* + 2.055	0.717
ICA Pijao	1.178 X_1 + 2.314	0.850
	0.566 X_2 - 2.317	0.912
NEP-2	0.822 X_1 - 0.821	0.858
	0.583 X_2 - 2.206	0.913
Porrillo Sintetico	0.923 X_1 - 2.608	0.712
	0.350 X_2 - 2.909	0.619

* X_1 = mg. nodule soluble carbohydrate/plant

X_2 = mg. nodule starch/plant

as shown in Appendix Table 14, is achieved in the cultivar 72 VUL 26689. The sites of starch accumulation in nodules are illustrated in Figures 18, 19, 20, 21, 22, 23. Starch accumulation was found to be restricted to the uninfected cells of bean nodules. Similar observations have been reported by (McCoy, 1929 and Allen and Allen, 1958). Linear regression analysis between fixation activity (umoles C_2H_4 pl^{-1} hr^{-1}) and carbohydrate level in the nodules were performed and the results are shown in Table 3. A very close correlation among these values is seen to exist. It is important to note that total starch and sugar content follow the nodule mass values in synchrony and that the loss in dry weight is evidently followed by a loss in carbohydrate.

The results illustrated in Figures 6 and 10 clearly indicate that at harvest 6 (60 dap), an opposite trend in carbohydrate content of leaves and stems versus nodules is established. Sites of starch storage in stems are illustrated in Figures 24, 25, 26. Starch grains were found to be primarily restricted to parenchyma cells of the axial rays of secondary xylem. Soluble sugars and starch content significantly increase in stems and leaves. Soluble carbohydrate content reaches a maximum level in leaves at 67 dap. (harvest 7), while soluble carbohydrates of stems continue to increase up to 88 dap (harvest 10). Starch in leaves increases up to 74 dap and subsequently rapidly decreases. The trend associated with starch increases following an exponential pattern from 60 dap to 81 dap appears important. This indicates that available sinks (i.e., reproductive sinks) have not reached the stage of development at which carbohydrate and nitrogen demand is maximum. By 88 dap, starch content has decreased in the stems. From this point onwards,

both temporarily stored carbohydrates and photosynthetic assimilates are being directed towards the seeds. This pattern is consistent in qualitative terms for all four varieties.

Figures 7 and 11 illustrate the patterns in the concentration of soluble sugars and starch for 72 VUL 26689 and Porrillo Sintetico. Two trends, in particular, can be discerned in these figures: 1) the concentration of starch in stems is the largest among all structures studied and increases in the way indicated above, and 2) nodules contain large concentrations of carbohydrates, particularly starch. The latter figures also illustrate that nodule starch concentration continuously decreases after reaching a maximum level in harvest 5 (53 dap). This maximum level of starch concentration is seen to occur prior to the time of maximum fixation levels in all varieties. The decrease in the concentration of nodule starch may be an indication that this polysaccharide is used as an indirect energy source for growth and N_2 fixation. The concentration of soluble sugars in nodules has been found to fluctuate only within relatively narrow ranges in all varieties. A consistent rise in concentration in harvest 6 (60 dap), has been observed. It is important to notice, too, that even after the period of maximum N_2 fixation activity, nodule soluble sugar concentration remains relatively constant, but nitrogen fixation subsequently declines. This clearly indicates a nodule population effect is responsible for the high fixation rates that occur during harvests 5 and 6, and that the drop in fixation follows a declining nodule population.

Figures 8 and 9 illustrate the corresponding values for soluble sugar and starch content and concentration for 72 VUL 26689 in the underground portion of stems ("primary roots"), the main root system

("secondary roots"), and pods. Trends in both soluble sugars and starch clearly indicate that these compounds continue to accumulate in the root system up to 81 dap (harvest 9). This trend is consistent with the observed increase in soluble sugars and starch found to be exhibited by nodules at this point and with the small burst of activity in N_2 fixation. These observations would also appear to indicate that carbohydrate is not limiting N_2 fixation. Similar trends for Porrillo Sintetico are illustrated in Figures 12 and 13. Corresponding values and Analyses of Variance for these figures are given in Appendix Tables 16 through 23, and 32 through 35. These trends were also found in NEP-2 and ICA Pijao.

Soluble sugar and starch content of pods is also illustrated in the latter figures. These carbohydrates are seen to increase exponentially from 74 dap onwards. This increase coincides with a buildup of carbohydrates in stems and leaves. Soluble sugars in pods are higher than starch content of pods. This relationship clearly indicates that carbohydrates are primarily being used in pods at this stage for growth and that reproductive structures have not initiated their characteristic storage phase. This also probably indicates a relatively low absolute demand for photosynthetic and temporarily stored reserves.

Distribution of organic nitrogen

The distributions of total organic nitrogen and organic nitrogen concentration in "primary roots", "secondary roots", stems, leaves, and pods, are shown in Figures 14 and 15 for the cultivars 72 VUL 26689 and Porrillo Sintetico. Corresponding values and their Analyses of Variance are given in Appendix Tables 36 through 48. Total nitrogen content for the summation of nitrogen content of roots, stems and

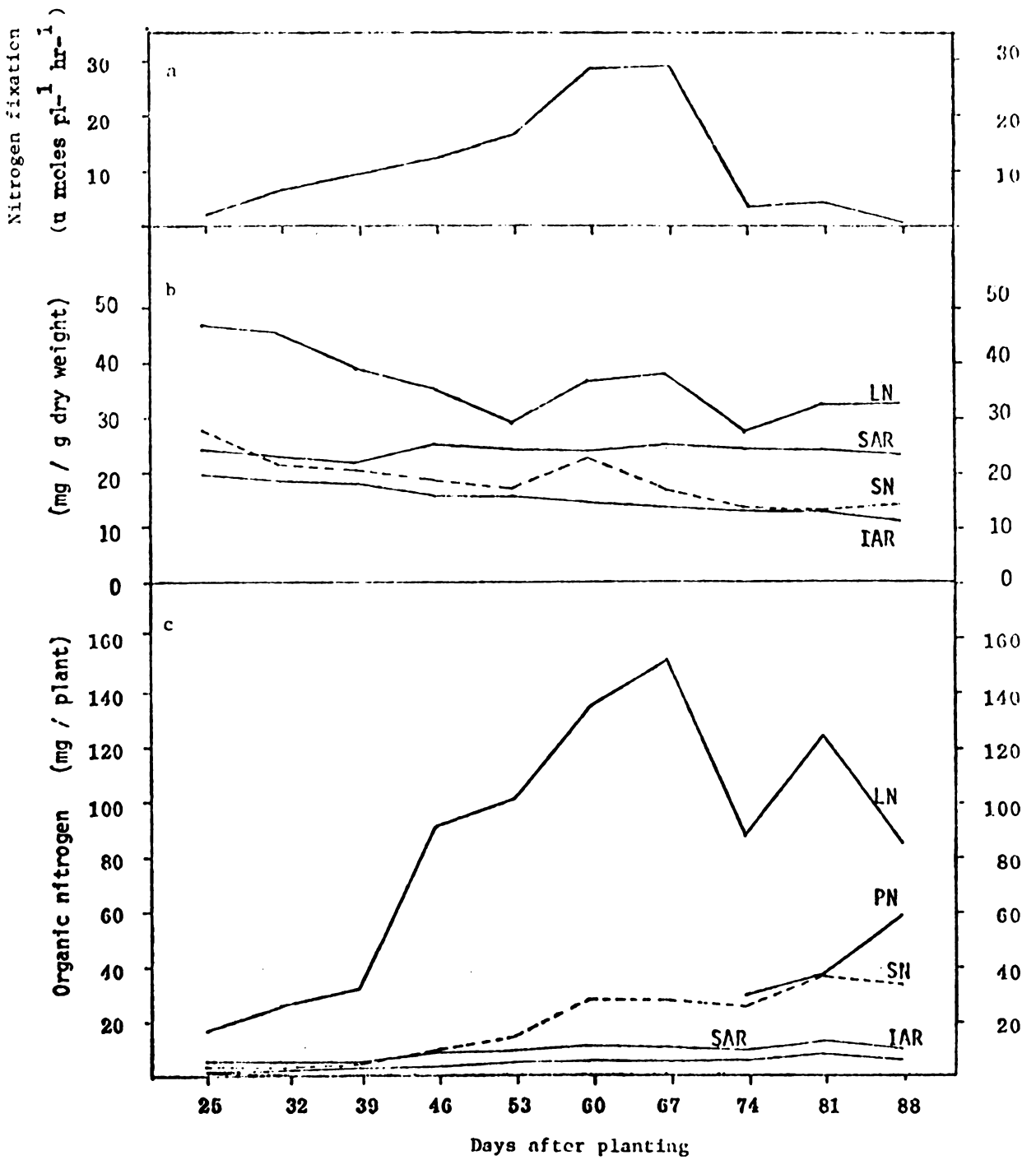

Figure 14. Nitrogen fixation (a), organic nitrogen concentration (b) and content (c) of leaves (LN), stems (SN), primary roots (IAR), secondary roots (SAR), and pods (PN) of the cultivar 72 VUL 26689.

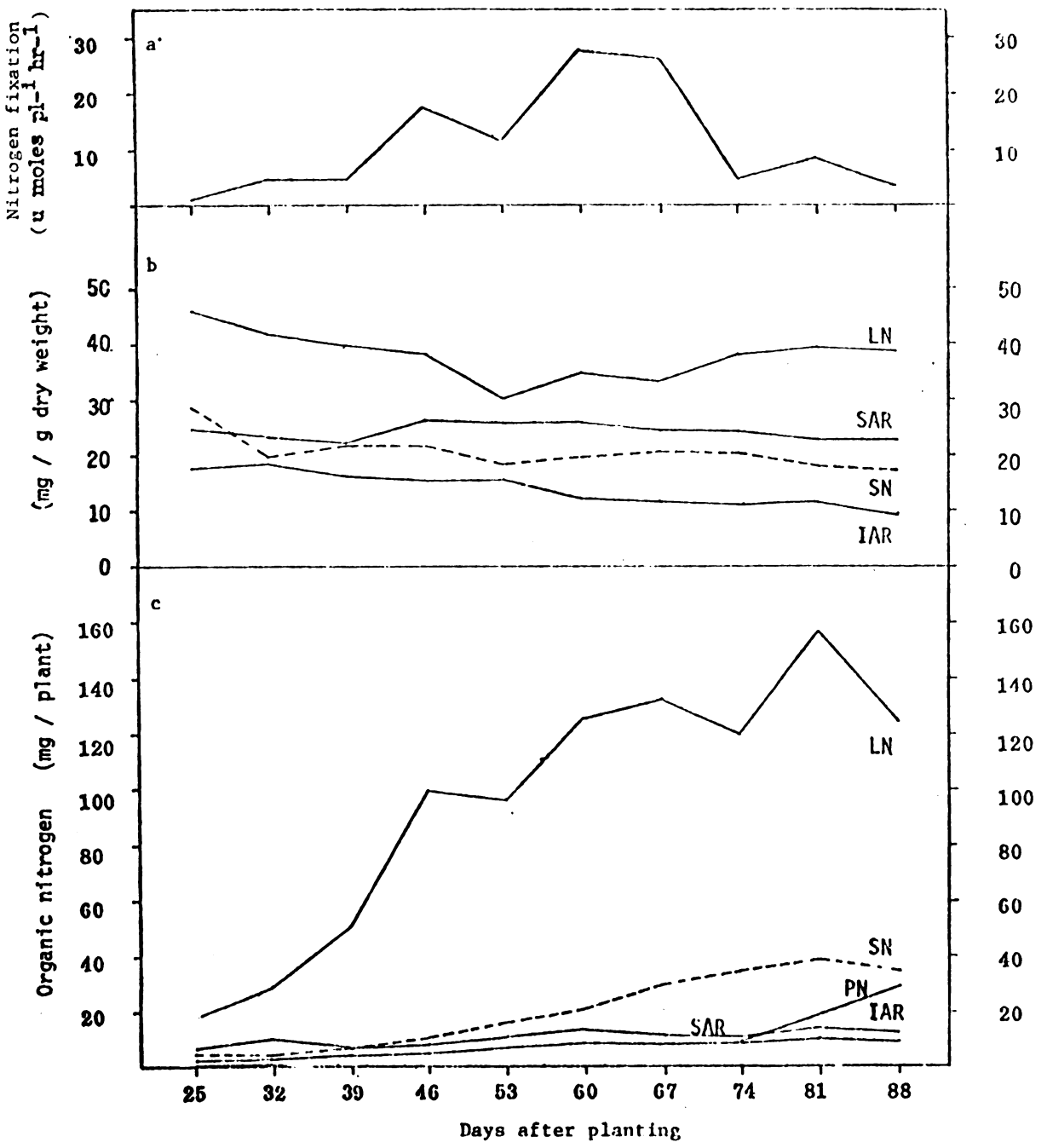

Figure 15. Nitrogen fixation (a), organic nitrogen concentration (b) and content (c) of leaves (LN), stems (SN), primary roots (IAR), secondary roots (SAR), and pods (PN) of the cultivar Porrillo Sintetico.

leaves, is shown in Table 4 and in Appendix Table 44. Total organic nitrogen per plant increases up to 81 dap (at harvest 9), subsequently decreasing. The major portion of the nitrogen present in vegetative tissues is accounted for by leaf nitrogen. The pattern exhibited by the concentration of organic nitrogen in "primary roots", stems and leaves is, in general, a similar one. This pattern is more easily observed in the corresponding tables of the Appendix. It is observed that nitrogen concentration progressivley declines up to about 88 dap. Slight increases during the periods of maximum fixation rates (60 and 67 dap) are exhibited by leaves and stems. Cubic polynomials were fitted to the total nitrogen content of leaves and stems throughout the period studied. Varieties were pooled in this analysis. Curves are obtained for total nitrogen in these structures very similar to the ones obtained when the dry weights were fitted. Fitted curves and the corresponding polynomials are given in Figures 16 and 17, and in Table 5 respectively. An inflection point at 2.68 harvests was found in leaves. A maxima, as indicated by the first derivative going to zero and the sign of the second derivative, was found at 7.29 harvests. Stem total nitrogen exhibited an inflection point at 5.38 harvests and a maxima at 9.34 harvests. The values exhibited by leaves can possibly be taken as evidence that nitrogen is no longer accumulated beyond the 7.29 harvest point and in fact, it may indicate that leaves may be starting to mobilize nitrogen out of them. This view is supported by the seasonal profiles of N_2 fixation.

The level of organic nitrogen in pods is seen to increase from 74 dap to 88 dap, but the level of nitrogen in these structures is low compared to the level of nitrogen usually found in seeds, which is about 38.4 mg N/g. dry weight if one assumes a mean value of protein

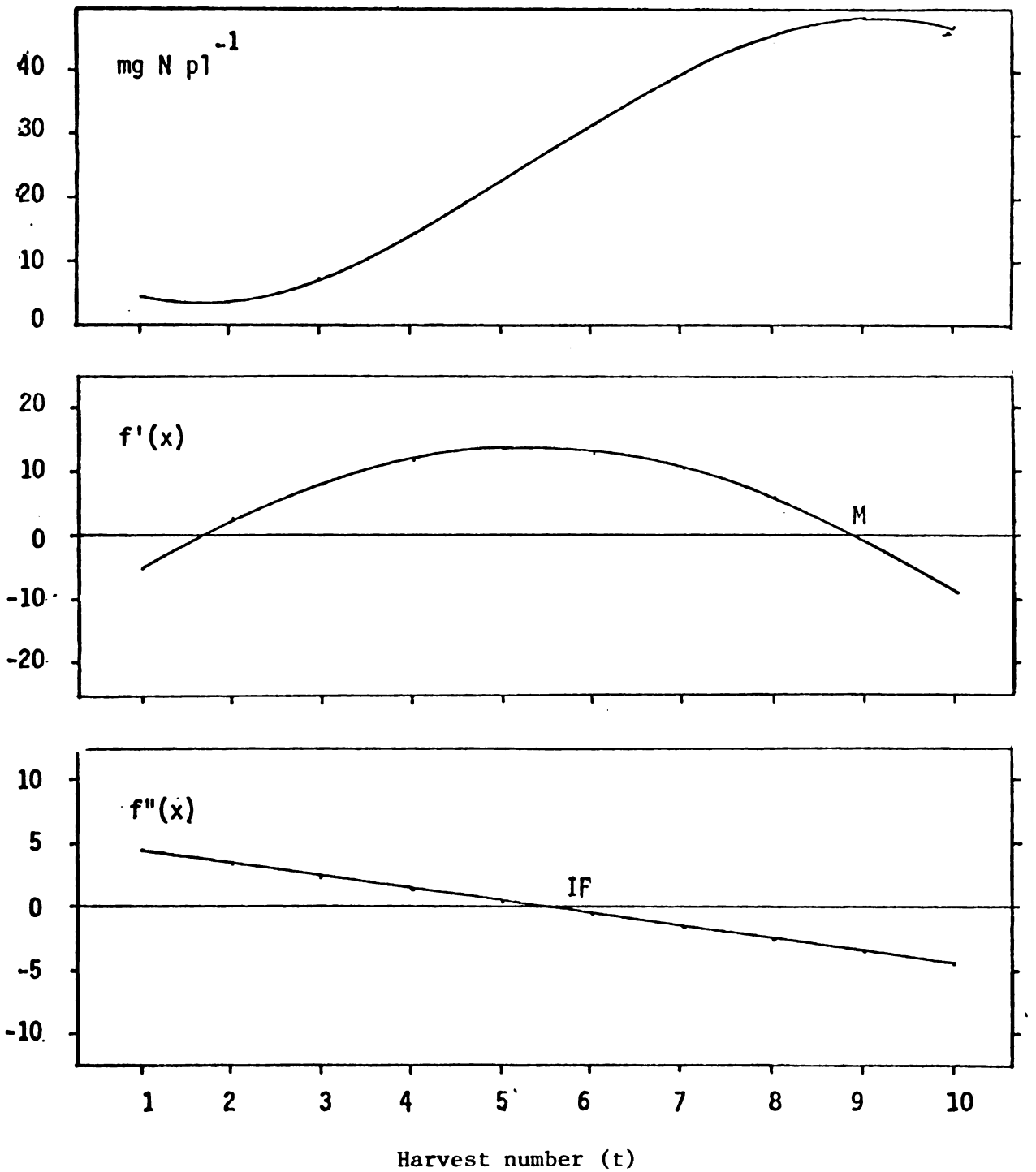

Figure 16. Ontogenetic distribution of stem nitrogen. Values shown are derived from cubic polynomials. Inflection points (IF) and maxima (M) are shown.

200
150
120
80
40
0
30
20
10
0
-10
-20
-25
20
10
0
-10
-20

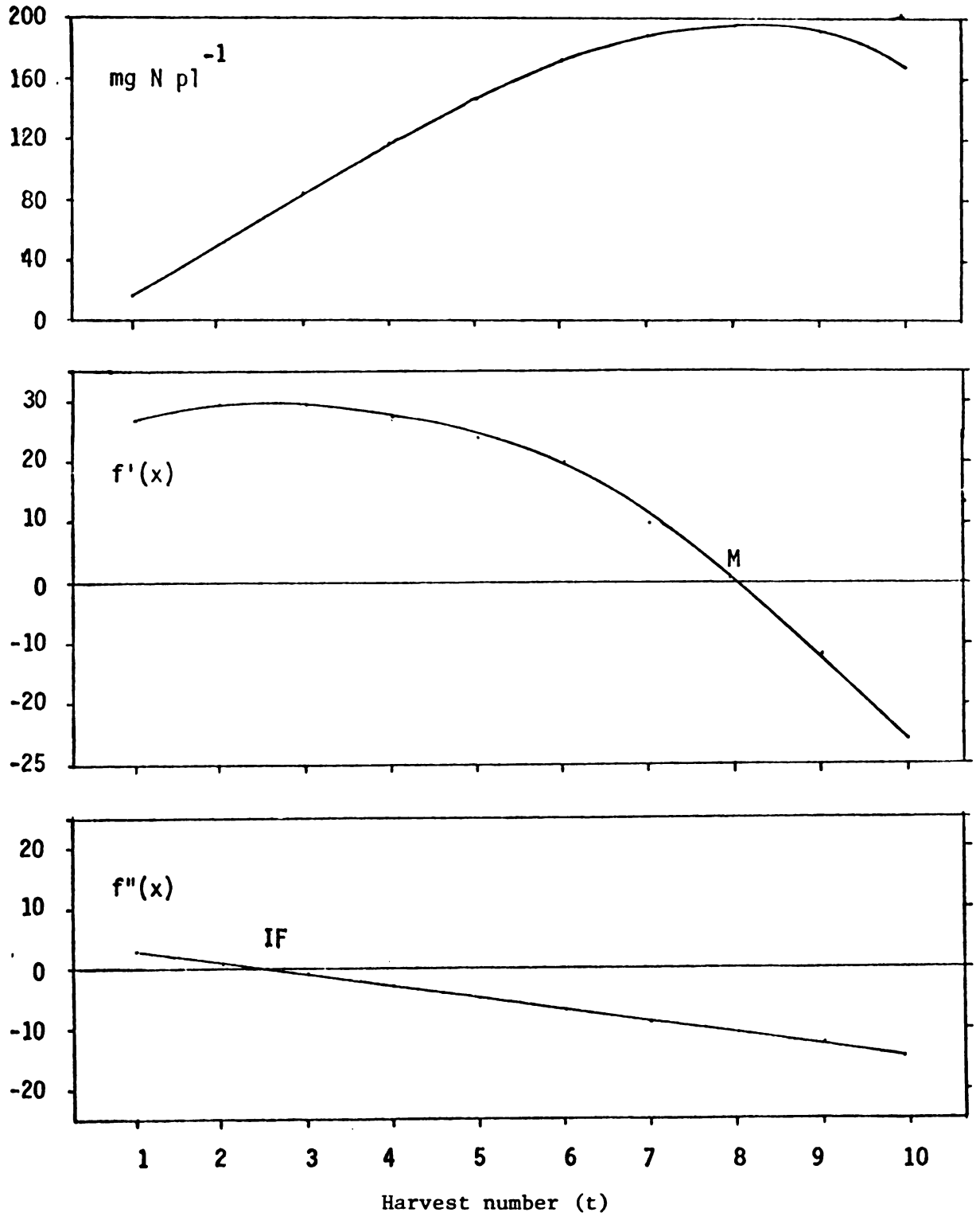

Figure 17. Ontogenetic distribution of leaf nitrogen. Values shown are derived from cubic polynomials. Inflection points (IF) and maxima (M) are shown.

Table 4. Organic nitrogen content of vegetative structures (roots, stems, and leaves) of ten sequential weekly harvests of four dry bean cultivars. Analysis of Variance is presented in Appendix Table 44.

Table 4. Organic nitrogen content of vegetative structures (roots, stems, and leaves) of ten sequential weekly harvests of four dry bean cultivers. Analysis of Variance is presented in Appendix Table 44.

Harvest time (dap)[1]	Cultivar			
	72 Vul 26689 (mg N/plant)	ICA Pijao (mg N/plant)	NEP-2 (mg N/plant)	Porrillo Sintetico (mg N/plant)
25	23.96	24.49	20.68	24.21
32	36.05	32.32	39.20	41.02
39	62.98	68.50	60.06	62.02
46	110.24	116.18	115.62	117.84
53	127.36	101.43	96.27	123.21
60	176.28	161.48	142.51	163.07
67	193.67	190.16	157.74	174.57
74	128.02	178.50	118.45	168.29
89	179.35	215.24	170.52	213.17
88	131.93	190.47	127.58	173.16

1 days after planting

Table 5. Cubic polynomials fitted to organic nitrogen content of stems (A), leaves (B) and, harvests (t independent variable) of four dry bean cultivars. Corresponding 95% confidence upper and lower limits (CL) are given.

A. Stem nitrogen: $R^2 = 0.765$

N content = $10.55269050 - 9.30912632\ t + 3.38087446\ t^2 - 0.20838879\ t^3$

	N content		
Harvest time[1]	Lower 95% CL	(mg/plant	Upper 95% CL
1	0.042	4.416	8.790
2	1.144	3.791	6.438
3	4.675	7.427	10.179
4	11.401	14.073	16.745
5	20.114	22.480	24.846
6	29.039	31.397	33.763
7	36.902	39.574	42.245
8	43.008	45.761	48.513
9	46.059	48.706	51.353
10	42.786	47.160	51.534

Table 5. (cont'd.)

B. Leaf nitrogen: $R^2 = 0.635$

N content = $13.81823221 + 27.36913111\ t + 2.90227790\ t^2 + 0.38194592\ t^3$

	N content		
Harvest time[1]	Lower 95% CL	mg/plant	Upper 95% CL
1	-4.704	16.071	36.847
2	36.902	49.474	62.045
3	71.026	84.097	97.169
4	104.958	117.651	130.342
5	136.603	147.841	159.079
6	161.140	172.378	183.616
7	176.277	188.970	201.662
8	182.253	195.324	208.396
9	176.578	189.150	201.721
10	147.379	168.155	188.930

1: Harvest time (t) given as the harvest number in which samples were collected.

N in seeds of 24%. Increases in plant nitrogen beyond the point of N_2 fixation decline are expected to be small compared to values that are present in the vegetative stage of the plants. This suggests that a major portion of seed nitrogen is derived from mobilization of the nitrogen present in leaves and stems.

Evaluation of the original working hybothesis postulated on the basis of reported research requires that answers be provided to the following questions: 1) does a change occur in the pattern of distribution of carbohydrates to the nodulated system?, i.e., is partitioning of assimilates altered between reproductive and vegetative structures?, and 2) is this change or alteration in the partitioning of carbohydrates correlated with the development of reproductive structures, with a decline in assimilates in the roots and nodules and with a decline in nitrogen fixation?. The approach to answering these questions is a sequential one in time and is as follows.

Soluble carbohydrates and starch in nodules were shown to reach a maximum level at 60 dap in all four cultivars and subsequently declined. Since a similar pattern was exhibited by nodule dry weights the population level of nodules appears to be correlated with high nitrogen fixation rates in the period of 60 to 67 days after planting. Carbohydrate levels (starch and sugars), when expressed as concentration in the nodules, provide a different but complementary kind of information. It would be expected that because of the high energy requirements for N_2 fixation, a higher concentration of soluble carbohydrate would be present during the maximum fixation period. The data collected during the conduct of experiments has failed to support this view as shown in Appendix Table 13. In fact, soluble sugar concentration remains relatively constant. This is taken as an

indication that these figures reflect the level of sugar concentraion required to maintain basal metabolism. This leads to the possibility that soluble carbohydrate translocated to the nodules is very rapidly used in the fixation of nitrogen and, consequently, the data obtained for soluble sugars reflects "basal metabolism status". Starch build-up in nodules has been shown to occur prior to maximum fixation rates, subsequently declining. It is suggested that this decrease in starch concentration is an indication that the products of hydrolysis of starch are being utilized as a supplement to the high energy requirements that have to be met during the period in which maximum fixation rates of nitrogen occur. Most nodules senesce subsequent to the occurrence of the highest fixation rates. This view seems to be supported.

Nodules are borne in larger numbers in the upper portion of the root system of bean plants. Senescence of nodules after the peak of fixation is associated with this major portion of the population of nodules present in the plant. One more aspect deserves consideration. It has been pointed out that lower leaf abscission occurs in dry bean plants between 67 dap and 74 dap, and this coincides with: 1) the drop in nitrogenase activity; 2) the drop in nodule dry weight; 3) a decline in total sugars and starch; and 4) a decline in starch concentration, but not with soluble sugar concentration in nodules. This later aspect, no decline in soluble sugar concentration in nodules, is not surprising since nodules samples were not physically deteriorating.

The evidence reported in the literature, and that presented in this thesis, relating an energy supply to nodules and their activity conflict in the interpretation of the sequence of events that leads to

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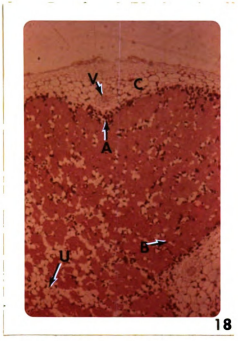
the decline of this activity. The conflict of opinion resides in the reason(s) for the reduction in the supply of carbohydrates to the nodules. Several authors (3, 8, 11, 12, 13, 22) have suggested that the data presented by them indicate that such a decline in activity is a consequence of "competition from reproductive sinks" for photosynthetic assimilates. The data presented in this section indicates that vegetative organs, leaves and stems in particular, become temporary storage sites for carbohydrates. Hence, in all varieties under consideration, carbohydrate levels in stems and leaves show an opposite trend to that found in nodules. It has also been shown that carbohydrate levels in the underground structures of bean plants do not decrease in this period, they in fact show a tendency towards increasing. Since, reproductive structures during the period studied, represent a small sink for carbohydrates and organic nitrogen, it is doubtful that they reduce N_2 fixation primarily by competing for carbohydrates.

Figure 18. Cross section of a Phaseolus vulgaris L. nodule at 53 dap with central bacterial cells (B), peripheral vascular bundle (V), cortex (C) and, uninfected cells (U) containing compound amiloplasts (A). Periodic Acid Schiff - stained section X40.

Figure 19. Cross section of a Phaseolus vulgaris L. nodule at 53 dap illustrating crenula (CR), vascular bundle (V), bacteriod containing cells (B), and uninfected cells (U) containing compound amyloplasts (A). Periodic Acid Schiff - stained section X400.

Figure 20. Cross section of a Phaseolus vulgaris L. nodule at 53 dap with peripheral vascular bundles (V), bacteriod containing cells (B), uninfected cells (U) and, compound amyloplasts. Toluidine Blue-stained section. X100.



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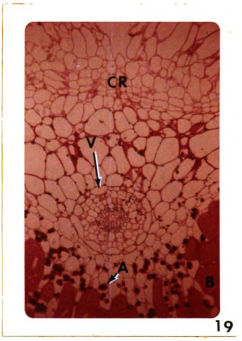

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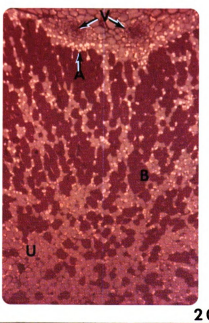

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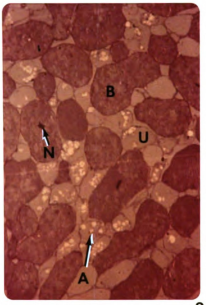
Figure 21. Cross section of a Phaseolus vulgaris L. nodule at 53 dap illustrating bacteriod containing cells (B), nucleous (N), and uninfected (U) cells containing compound amyloplasts (A). Mercuric-Bromphenol Blue - stained section. 560X.

Figure 22. Cross section of a Phaseolus vulgaris L. nodule vascular bundle at 53 dap with endodermis (E), casporian strips (CS), xylem (X), phloem (PH) and, compound amyloplasts (A). 800X.

Figure 23. Bacteriod containing cells (B) and uninfected cells (U) with nucleous (N), nucleolus (ns) and, compound amyloplasts (A). 850X.



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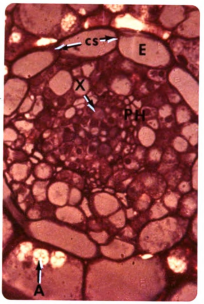

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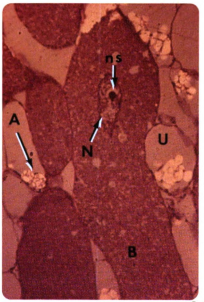

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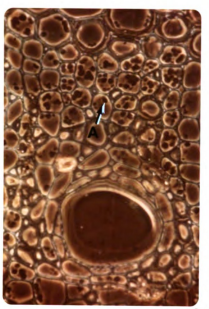
Figure 24. Cross section of stem at third node level illustration amyloplasts (A) in secondary parenchyma cells. Phase-contrast photograph. 400X.

Figure 25. Cross section of stem at the third node level illustrating amyloplasts (A) in parenchyma cells of the pith. Phase-contrast photograph. 400X.

Figure 26. Cross section of stem at the third node level illustrating amyloplasts (A) primarilly located in the porenchyma cells of rays of secondary xylem. Periodic Acid Schiff - stained section. 140X.



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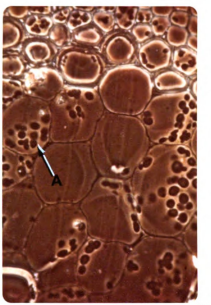

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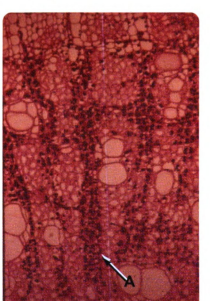

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CHAPTER 3

EFFECTS OF CARBON DIOXIDE ENRICHMENT ON SYMBIOTIC NITROGEN FIXATION IN *PHASEOLUS VULGARIS* L.

Abstract

Dry bean plants exposed to 1200 ppm carbon dioxide at various developmental stages exhibited higher nitrogen fixation rates, higher nodule fresh weights, higher total soluble carbohydrate and slightly higher organic nitrogen contents. Carbon dioxide treatment during the four weeks prior to reproductive growth, was found not to extend the duration, nor prevent the decline of nitrogen fixation. The view that the rate of nitrogen fixation *per se* is limited by photosynthate available to the *entire symbiotic system* is supported.

INTRODUCTION

Studies conducted by several investigators have shown that legumes, such as soybeans, when exposed to carbon dioxide levels higher than those of air fix atmospheric nitrogen at higher rates than plants grown under prevailing carbon dioxide levels in the atmosphere (1, 2, 3, 4, 5, 6). This positive response has been attributed primarily to a reduction of photorespiration (1, 2, 3, 4, 5).

The results reported in this chapter are the outcome of exposing bean plants to 1200 ppm of carbon dioxide during part of their vegetative and reproductive period. Answers to the following questions were sought in this study: a) does carbon dioxide treatment result in a higher level of photosynthetic assimilates in treated plants?· b) are higher levels of soluble carbohydrates in nodules found in CO_2 treated plants?; c) are higher rates of nitrogen fixation attained through CO_2 treatment?; and, d) is the seasonal decline in nitrogen fixation delayed due to carbon dioxide treatment?

MATERIALS AND METHODS

Plants utilized in this study were grown on the experimental grounds of the International Center for Tropical Agriculture (CIAT) in Cali, Colombia in an area 14.5 x 28 m containing a sand-soil mixture (4:1 proportion). Fertilizers were not applied since results of soil analysis indicated adequate nutrient levels for plant growth.

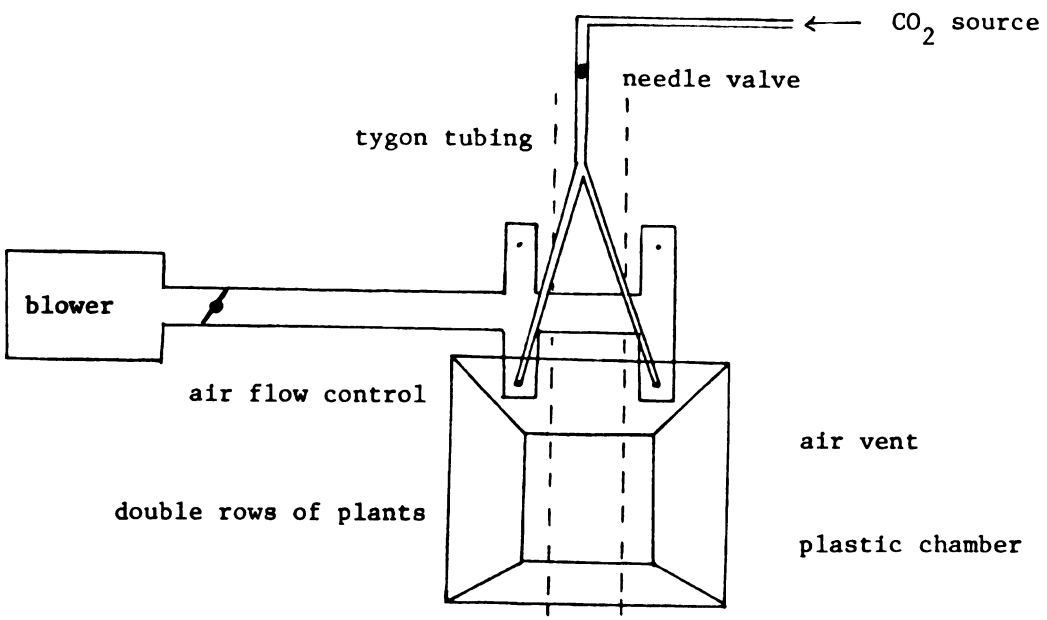
During the month of February, mean temperature at this location is 24 C. Prior to planting, soil temperatures measured at 10 cm depths varied from 25 C at 9 am to as high as 45.5 C by 3 pm. Due to the known

adverse effect of high temperatures on Rhizobium survival and on nodulation a soil mulch of rice hulls (10 cm thick) was used. Mean temperatures at a depth of 10 cm varied from 24.4 C at 9 am to 28.9 C by 3 pm when the mulched was used.

Porrillo Sintetico was the cultivar selected for this study. Seven double rows were planted with seed inoculated with the Rhizobium phaseoli strain CIAT 57 on February 4, 1976. Seeds were wetted with a 10% aqueous solution of sucrose and subsequently the "peat"-(turba)-based inoculum was applied to the wetted seeds. Inoculated seeds were allowed to dry in the sahde prior to hand planting. Planting distances were 35 cm between double the double rows and 1 m between adjacent double rows. Planting density was equivalent to 30 plants/m^2.

Carbon dioxide application

The equipment utilized for delivery and metering of carbon dioxide is illustrated in the diagram shown below:



The chambers used consisted of aluminum frames lined with heavy gage transparent plastic. Chambers were open at the top as illustrated. Two chambers, each supported on a wood frame, were supplied with CO_2 through a blower-plastic pipe assembly. Each chamber had a volume of 1 m^3 (1000 l). The supply of air administered by the blowers was calibrated to give a 0.5 change of air volume per minute per chamber. To provide a concentration of 1200 ppm CO_2, 100 ml of carbon dioxide/5 seconds was metered to every pair of chambers used in this experiment. Carbon dioxide was metered with the aid of needle valves and a soap-bubble meter twice a day. Wind velocities at the plastic pipe openings were calibrated with an Alnor meter using tube number 602 for this purpose.

Ten chambers were available for this experiment. Carbon dioxide treatments consisted of metering 1200 ppm CO_2 in the following manner: a) CO_2 was applied for a period of two weeks (18-32 dap), using four chambers for this purpose; b) CO_2 was applied for a 4 week period (18-46 dap) utilizing four more chambers · c) the chambers utilized for the 18-32 dap treatment were transferred on the thirty-second day after planting to the plants that received carbon dioxide from 32 to 46 dap and; d) the remaining two chambers were utilized to expose plants to a 4-week period (18-46 dap) but plants were not harvested until agronomic maturity was reached (95 dap on May 13, 1976). Appropriate controls not having been exposed to CO_2 were included for each treatment comparison. Flowering occurred at 32 days after planting. Plants of the Porrillo Sintetico cultivar were known to flower at 35 dap at this location. The scheduling of the carbon dioxide treatments was made to coincide with the expected decline in nitrogen fixation during the early periods of reproductive growth.

Acetylene - Ethylene reduction assays and sample preparation

Acetylene - ethylene reduction assays were performed in the manner previously described in Chapter 2. Subsequent to completion of the assays the tops and nodulated roots were separated into component parts and dried as indicated previously. After drying, samples were weighed and subsequently ground for organic nitrogen determinations. Since four chambers were used per treatment, the plants of two chambers belonging to a different pair were selected at random for acetylene assays, dry weight and organic nitrogen determinations. The plants in the other two chambers were used for fresh weight determinations and soluble carbohydrate analyses. Each chamber contained 30 plants and the acetylene assays were performed with 5 nodulated roots/assay. Hence, six subsamples/chamber/treatment were available for each type of analysis indicated above.

Fresh weight determinations were made immediately after removing the plants from the chambers and subsequently frozen at -10 C.

Organic nitrogen determinations

These analyses were performed in the same manner indicated in Chapter 2.

Determination of ethanol-soluble carbohydrates

These analyses were performed in the manner indicated in Chapter 2 with the exception of the extraction procedure. Nodules, roots and stems, were extracted with 80% analytical grade alcohol in a proportion of 1:10, i.e., 1 part of frozen tissue to 10 parts of 80% ethanol. Frozen samples were ground in a blender with ethanol and subsequently filtered under vacuum using hardened Whatman No. 1 filter paper. Filtered samples were adjusted to known volumes and stored in a refrig-

erator in teflon-lined screw capped culture tubes. Chromogen development was performed following the procedure described in Chapter 2.

RESULTS AND DISCUSSION

Results concerning the effect of carbon dioxide on nitrogen fixation rates and nodule fresh weights are shown in Table 6. Corresponding analyses of variance are given in Appendix Tables 47 and 48. Statistical evaluation of the data in this section has revealed a problem of a small number of degrees of freedom for testing of hypotheses. It is suggested that this be taken into consideration in the discussion that follows.

Differences in nitrogen fixation rates at 18-32 dap were found not to be statistically significant, in spite of a 100-fold difference between carbon dioxide treated and untreated plants. Nodule fresh weights were found to be higher when plants were exposed to CO_2 during this period. No differences in nitrogen fixation rates or in nodule fresh weights during the 18-46 dap period were found between plants exposed to CO_2 and plants exposed to a normal concentration of carbon dioxide.

The effects of carbon dioxide on fresh weight of roots, stems, leaves and pods are presented in Table 7. Corresponding analyses of variance are given in Appendix Tables 51, 54, 57, and 58. Root and stem fresh weights at 18-32 dap were found to be higher in carbon dioxide treated plants. A similar pattern was observed for leaf fresh weight but this difference is not statistically significant. For the period comprising 18 to 46 dap, no statistically significant differences were obtained for root and leaf fresh weights between CO_2 treated and untreated plants. Pod and stem fresh weights were found to be higher due to an increasing period of exposure to carbon dioxide.

Table 6. Effect of carbon dioxide on nitrogen fixation rates and nodule fresh weight of the dry bean cultivar Porrillo Sintetico.

CO_2 enrichment	N_2 fixation (u moles 5 pl^{-1} hr^{-1})		Nodule fresh weight (g/5 plants)	
	2 weeks	4 weeks	2 weeks	4 weeks
-	30.67	2.40	1.0547	0.3747
+	63.87 ns	5.46	2.0909[1]	0.5680
+*		2.92 ns		0.5255 ns

* these plants received CO_2 enrichment only the last two weeks of the four week period. CO_2 treatments began 18 days after planting.

Table 7. Effect of carbon dioxide on root fresh weight, stem fresh weight, leaf fresh weight, and pod fresh weight of the dry bean cultivar Porrillo Sintetico.

CO_2 enrichment	Fresh weight[1] (g)							
	Roots		Stems		Leaves		Pods	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	6.87	10.12	22.29	74.46	75.22	182.67	----	65.68
+	9.06*	10.53	30.81[2]	84.42	94.62 ns	186.05	----	91.20
+[3]		9.63 ns		83.21**		184.37 ns		69.10**

1 in g/5 plants

ns no statistical significance at 0.10 - probability level

* $\leq$ 0.05 - probability level

** < 0.01 - probability level

2 < 0.09 - probability level

3 these plants received CO_2 enrichment only the last two weeks of the 4 week period. CO_2 treatments began 18 days after planting.

Dry weights from a different set of plants but similarly treated are presented in Table 8. Corresponding analyses of variance are given in Appendix Tables 59, 62, 65 and 68. Exposure of plants to a higher carbon dioxide level than that of air did not have a positive effect on the dry weights of roots, stems and leaves at the time periods studied. Pod dry weight increases due to carbon dioxide treatment confirms the results obtained with of pod fresh weight.

The effect of carbon dioxide on the content and concentration of soluble carbohydrates in nodules, roots and stems are presented in Table 9. Corresponding analyses of variance are presented in Appendix Tables 49, 50, 52, 53, 55, and 56. In the period comprising 18 to 32 dap, roots/soluble carbohydrate contents and nodules were found to be higher in plants exposed to carbon dioxide. In the longer time period (18-46 dap), soluble carbohydrate content in root and stem of CO_2 treated plants was found to be higher than in the plants not exposed to carbon dioxide. However, no statistically significant differences can be associated with this response. Values concerning the concentration of carbohydrates indicate that the concentraion of soluble carbohydrate in nodule differ between CO_2 exposed and unexposed plants in an unexpected way. In this case, at 18-32 dap, soluble concentration of carbohydrates of nodules in CO_2-treated plants is lower than that exhibited by the nodules of plants not exposed to carbon dioxide. In contrast, during the same time period, the concentration of soluble carbohydrates in roots was found to be higher in CO_2-treated plants than in plants not exposed to carbon dioxide. In a manner similar to that found in nodules, the concentration of soluble sugars in stems in this time period was found to be lower in plants exposed to carbon dioxide. At 18 to 46 dap, soluble carbohydrate root

Table 8. Effect of carbon dioxide on root dry weight, stem dry weight, leaf dry weight, and pod dry weight of the dry bean

Table 8. Effect of carbon dioxide on root dry weight, stem dry weight, leaf dry weight, and pod dry weight of the dry bean cultivar Porrillo Sintetico.

CO_2 enrichment	Dry weight[1] (g)							
	Roots		Stems		Leaves		Pods	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	2.17	3.46	3.76	12.79	12.83	22.79	----	6.31
+	2.14 ns	3.90	3.73 ns	16.51	13.43 ns	26.57	----	9.30
+[2]		4.09 ns		16.20 ns		28.38 ns		9.39**

1 in g/5 plants

ns no statistical significance at 0.10 - probability level

2 these plants received CO_2 enrichment only the last two weeks of the 4 week period. CO_2 treatments began 18 days after planting.

Table 9. Effect of carbon dioxide on content and concentration of soluble carbohydrates of nodules, roots, and stems, of the dry bean cultivar Porrillo Sintetico.

CO_2 enrichment	Soluble carbohydrate content (mg/5 plants)					
	Nodules		Roots		Stems	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	36.84	----	82.29	198.17	272.46	1,287.08
+	44.81*	----	116.56*	228.96	282.27ns	1,633.48
+[1]		----		221.31 ns		1,550.70 ns

CO_2 enrichment	Soluble carbohydrate concentration (mg/g fresh weight)					
	Nodules		Roots		Stems	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	37.54	----	12.21	19.32	12.11	17.20
+	22.08*	----	13.00**	22.04	9.21ns	19.63
+[1]		----		22.94 ns		17.20 ns

ns no statistical significance at 0.10 - probability level

* $\leq$ 0.05 - probability level

** $\leq$ 0.01 - probability level

1 these plants received CO_2 enrichment only the last two weeks of the four week period. CO_2 treatment began 18 days after planting.

and stem levels were found to exhibit a trend of increasing concentration with a longer period of exposure to carbon dioxide. Carbohydrates in nodules were not analyzed during this period due to the smallness of the sample encountered.

Table 10 shows the results obtained on the effect of carbon dioxide on organic nitrogen content and concentration of root, stem, leaf and pod. Corresponding analyses of variance are presented in Appendix Tables: 60, 61, 63, 64, 66, 67, 69, and 70. For the time period comprising 18 to 32 dap, nitrogen content of roots, stems and leaves was found not to differ significantly in a statistical sense between CO_2 treated and untreated plants. A trend toward higher organic nitrogen content as a consequence of exposure to carbon dioxide in these structures can be surmised from the data shown. At 18 to 46 dap no statistically significant differences were detected between carbon dioxide treated and untreated plants in organic nitrogen content of roots, stems and leaves. A possible trend of response to carbon dioxide during 32 to 46 dap can be surmized from the results. In contrast, organic nitrogen content of pods was found to be significantly higher in CO_2 treated plants. A peculiar set of results was found for the treatment period 18 to 46 dap in terms of organic content. Root, stem, and leaf organic nitrogen were found to be higher than the untreated controls but pod nitrogen content was lower in CO_2 treated plants.

The effect of carbon dioxide on organic nitrogen concentration of roots, stems and leaves at 18-32 dap was found not to differ for the two treatments studied. In contrast, plants exposed to carbon dioxide during the 18 to 46 dap time period exhibited a peculiar response. The organic nitrogen concentration of roots, stems, leaves and pods in

Table 10. Effect of carbon dioxide on content and concentration of organic nitrogen of roots, stems, leaves, and pods of the dry bean cultivar Porrillo Sintetico.

CO_2 enrichment	Organic nitrogen content (mg/5 plants)							
	Roots		Stems		Leaves		Pods	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	31.74	43.11	63.66	231.26	517.22	692.11	----	283.88
+	32.32 ns	47.41	66.77 ns	245.37	537.11 ns	753.70	----	373.91
+[1]		51.62 ns		265.29 ns		924.77 ns		393.60**

CO_2 enrichment	Organic nitrogen concentration (mg/g dry weight)							
	Roots		Stems		Leaves		Pods	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
-	14.68	12.45	16.88	18.07	40.23	30.45	----	45.47
+	15.07 ns	12.18	17.79 ns	14.77	40.20 ns	28.28	----	37.84
+[1]		12.55 ns		16.36**		32.56*	----	42.13**

ns no statistical significance at 0.10 - probability level

* $\leq$ 0.05 - probability level

** $\leq$ 0.01 - probability level

1 These plants received CO_2 enrichment only the last two weeks of the four week period. CO_2 treatments began 18 days after planting.

carbon dioxide treated plants was found to be smaller than the corresponding untreated plants. When the values obtained for the concentration of organic nitrogen in control versus carbon dioxide treated plants are compared, the concentration of organic nitrogen in CO_2-treated plants was found to be lower.

In Table 11, the results obtained on the effect of carbon dioxide on various parameters at maturity are presented. No differences were found between plants not exposed to carbon dioxide and plants exposed to CO_2 for a 4-week period. This result is not surprising since carbon dioxide treatment was terminated at 46 days after planting and conditions for plant growth were "carbon dioxide limiting".

The trends exhibited by the data presented above indicate a positive response to carbon dioxide in terms of nitrogen fixation in dry beans and that this response is associated with higher net photosynthetic rates as evidence by the fresh and dry weight of plant parts and by total soluble carbohydrate and organic nitrogen content. Comparisons of the total fresh weight, dry weight, mg soluble carbohydrates/5 plants and mg organic nitrogen/5 plants clearly indicate this response to carbon dioxide treatment. For example, for the 18-32 dap, carbon dioxide treatment resulted in the following values: 136.58 g fresh weight, 19.3 g dry weight, 443.64 mg of soluble carbohydrate and 636.20 mg of organic nitrogen. In contrast, untreated plants for the same time period totalled: 105.47 g of fresh weight, 18.76 g dry weight, 392.59 mg of soluble carbohydrate and 612.62 mg or organic nitrogen. Similarly, for the 18-46 dap period, untreated plants totalled: 333.30 g fresh weight, 45.35 g dry weight, 1,485.25 mg of soluble carbohydrate and 1,250.36 mg of organic nitrogen. In contrast, plants exposed to carbon dioxide for a period of two weeks

Table 11. Effect of carbon dioxide on components and subcomponents of yield of the dry bean variety Porrillo Sintetico at maturity.

Trait	Treatment[1]	
	$-CO_2$ (95 d.a.p.)	$+CO_2$ (18-46 d.a.p.)[2]
Dry weight of pods	10.88	9.46
Seed yield (W)	8.11	7.30
No. pods (X)	6.75	5.88
No. seeds/pod (Y)	6.19	5.85
Weight of 100 seeds (Z)	19.63	20.33
Stem and root dry weight	3.31	3.95
Node number	11.07	11.05

1 no statistical significance in any of the trait comparisons was obtained.

2 treatment consisted of CO_2 exposure of plants from 18 to 46 d.a.p. and harvested at 95 d.a.p.

(32-46 dap) totalled: 346.48 g fresh weight, 58.06 g dry weight, 1,772.01 mg soluble carbohydrate and 1,635.28 mg of organic nitrogen. For the 4-week carbon dioxide treatment (18.-46 dap) plants totalled: 372.72 g fresh weight, 56.88 g dry weight, 1,862.44 mg soluble carbohydrate and 1,420.39 mg of organic nitrogen.

Previously, it had been shown that the concentration of soluble carbohydrate in nodules of plants exposed to carbon dioxide at 18-32 dap was lower than that of untreated plants. An opposite trend has been found for soluble carbohydrate content in nodules for the same treatment comparison. Considering this information with the values obtained for fresh weight of nodules one can suggest that such a response reflects the possibility that carbohydrate is being used at this point for the build-up of nodule mass and that possibly, carbon dioxide treated plants utilize their energy supply at a faster rate to drive the fixation process.

The questions posed at the beginning of this section can now be answered. It is suggested that the data presented in this chapter provides a sound basis to propose that carbon dioxide treatment does result in higher levels of photosynthetic products in bean plants. Higher total amounts of soluble carbohydrate in nodules have been found in carbon dioxide treated plants. The concentration of soluble carbohydrates in nodules is lower in carbon dioxide treated than in untreated plants. Higher rates of nitrogen fixation were found in plants exposed to a concentration of carbon dioxide higher than that found in the atmosphere. In contrast to the findings of Hardy and Havelka (1973, 1975) with soybeans, the duration of nitrogen fixation was not extended through carbon dioxide treatment nor was the decline in the rate of nitrogen fixation delayed.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

The ontogenetic development of four dry bean cultivars has been studied with reference to the relationships that may exist between symbiotic nitrogen fixation and the energy supply (in the form of carbohydrates) to the nodules. Particular emphasis has been placed in these studies on accumulating kinds of information that allow deductions to be made concerning the sequence of events that lead to the observed decline in the fixation of molecular nitrogen in bean plants.

The data that previously have been presented are consistent with the hypothesis that carbohydrate supply to the nodules is a limiting factor to the fixation process. It was also shown that an increase of total photosynthate available to the symbiotic system (through CO_2 enrichment) results in higher rates of nitrogen fixation.

The possibility that nodules and reproductive sinks "compete" for energy has been entertained as a possible explanation for the decline in nitrogen fixation rate in the bean cultivars studied. Spiegelman (1945), has clearly defined the concept of physiological competition: "Every group of biological units (species, individual, cells) are in physiological competition if,

a) they require and draw upon a common food source (substrate) and/or,

b) excrete harmful metabolites into a common environment".

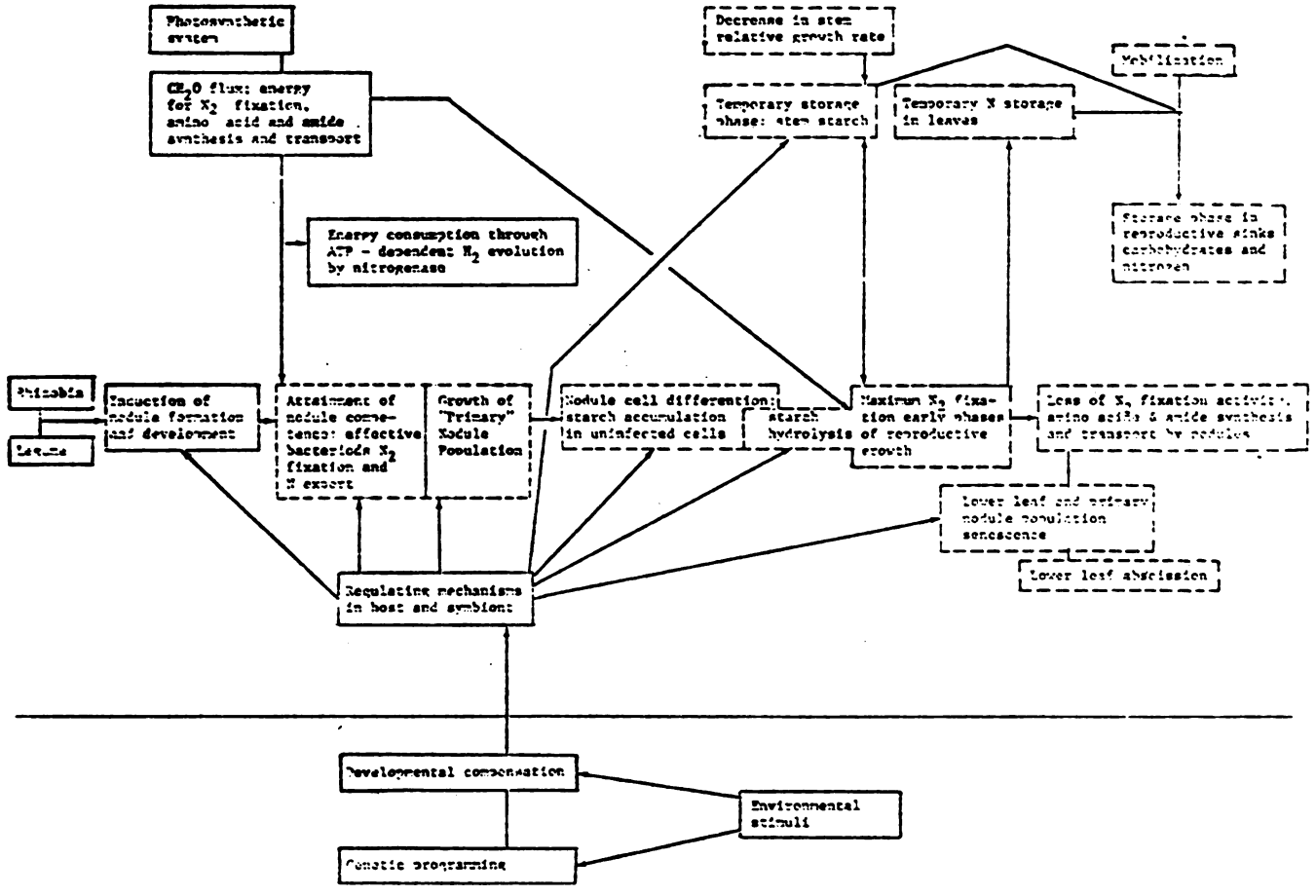
The premise given in point "a" has served as a basis for elucidating whether competition can be utilized as an explanatory concept of the interacting relationships in a symbiotic system in terms of energy requirements in dry bean plants.

The basic assumption that a common substrate supply is available to nodules and reproductive structures is suggested not to be upheld by the responses observed in the cultivars used in this study. The data presented in previous chapters have been interpreted as indicating a nutritional dependency upon photosynthate produced in lower leaves by the main nodule population. Events that have been found to be correlated in time with the decline in N_2 activity are illustrated in Figure 27. It is suggested that whether a selected pathway is entered rests upon whether or not activating stimuli (genetically and developmentally programmed stimuli) are received: if they are, as may be the case in the decline of N_2 fixing activity, senescence of the primary nodule population and lower leaves ensues. It is this author's opionion that a developmentally compensated genetic program predisposes the nodules to the observed decline in N_2 fixation. Thus, loss of nodule competency in N_2 fixation may not be triggered by a reduction in carbohydrate movement to such structures but by an activating signal(s) affecting both nodule and lower leaf senescence, probably hormonal in nature. This interpretation is also compatible with the results obtained concerning the small burst of nodule activity exhibited subsequent to the decline in N_2 fixation, and the increase in carbohydrates in the root system after this decline. The carbohydrate source that accounts for this latter observation is suggested to be different than that due to the lower leaves of the plant, i. e., a distinct "physiological space and time" is shared by the main nodule

population and the lower leaves. These spatial and temporal events are consequences of the unfolding of a genetic program that leads to a dynamic regulation of growth in symbiotic systems. The nature of this regulation remains unknown. Data presented have been interpreted as supporting the ontogenetic sequence of events outlined by broken lines in Figure 27.

Evidence has also been presented concerning the relevance of the symbiotic fixation of molecular nitrogen to legumes. This phenomenon can certainly be considered the insurance of survival of a legume. It has been shown, for example, that nitrogen in the bean plant is stored temporarily in the leaves and it is suggested that mobilization of this nitrogen to the seeds is primarily derived from the ageing processes of bean leaves. A similar phenomenon of mobilization of carbohydrates (temporarily stored in the stems and leaves) can be surmized from the data presented in this thesis.

Figure 27. CORRELATED SEQUENCE OF EVENTS IN NITROGEN FIXATION



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APPENDIX

Least significant differences, henceforth referred to as LSD, refer to the following treatment comparisons:

LSD_1: difference between two harvest means.

LSD_2: difference between two variety means.

LSD_3: difference between two variety means at the same harvest.

LSD_4: difference between two harvest means at the same variety.

*: 0.01 - probability level
**: 0.001 - probability level

Appendix Table 1. Dry weights (in grams/15 plants) of primary roots of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	0.85	0.79	0.84	0.71	0.80
2	1.50	1.39	1.25	1.53	1.42
3	1.91	2.60	2.06	2.48	2.26
4	2.97	4.33	3.64	3.93	3.72
5	4.21	5.07	4.48	6.13	4.97
6	5.74	7.51	6.73	9.32	7.33
7	6.24	9.94	8.83	9.04	8.52
8	6.99	12.14	8.94	10.66	9.68
9	9.40	12.48	10.25	13.07	11.30
10	7.45	12.73	8.93	12.98	10.52
Cultivar Means	4.73	6.90	5.59	6.98	

$LSD_1 = 1.65$; $LSD_2 = 0.52$; $LSD_3 = 1.64$; $LSD_4 = 2.17$

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	16.40
Harvest (H)	9	246.07**
Error (a)	27	5.20
Cultivar(V)	3	47.36**
H X V	27	4.35**
Error (b)	90	1.36

Appendix Table 2. Dry weights of "secondary roots" (in grams/15 plants) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	2.66	3.09	1.90	2.96	2.65
2	4.71	3.62	4.97	6.59	4.97
3	3.88	3.69	3.40	3.83	3.70
4	4.91	4.19	3.32	3.81	4.06
5	5.32	5.12	5.54	5.69	5.42
6	6.51	6.08	4.13	7.60	6.08
7	5.99	6.62	4.81	6.54	5.99
8	5.87	5.65	5.15	5.77	5.61
9	8.04	5.46	5.97	8.62	7.02
10	6.30	7.39	5.96	7.67	6.83
Cultivar Means	5.42	5.09	4.51	5.91	

$LSD_1 = 1.90$; $LSD_2 = 0.61$; $LSD_3 = 1.93$; $LSD_4 = 2.52$

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	8.65
Harvest (H)	9	31.41**
Error (a)	27	6.85
Cultivar (V)	3	13.71**
H X V	27	2.17
Error (b)	90	1.88

Appendix Table 3. Cubic polynomials fitted to stem dry weight (dependent variable) of four dry bean cultivars, and corresponding Analysis of Variance.

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S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.958
Regression	3	658.35**	
Error	6	14.44	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	8.21570833	1.114	0.308
H	- 8.31755522	- 1.504	0.183
H^2	2.56722305	2.251	0.065
H^3	- 0.143.59295	- 2.100	0.080

ICA Pijao

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.991
Regression	3	465.64	
Error	6	2.052**	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	4.17288333	1.501	0.184
H	- 3.49569464	- 1.678	0.144
H^2	1.28802054	2.997	0.024
H^3	- 0.06285679	- 2.439	0.050

Appendix Table 3. (continuation).

NEP-2

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.984
Regression	3	318.35**	
Error	6	2.56	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	3.59272500	1.156	0.291
H	- 3.20814117	- 1.378	0.217
H^2	1.29599184	2.698	0.036
H^3	- 0.07289350	- 2.531	0.045

Porrillo Sintetico

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.988
Regression	3	439.02	
Error	6	2.68	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	5.66408333	1.781	0.125
H	- 5.33321465	- 2.238	0.067
H^2	1.84330114	3.750	0.009
H^3	- 0.10446528	- 3.544	0.012

Appendix Table 4. Dry weights of leaves (in grams 15/plants) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Anaylsis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	5.17	5.26	4.82	5.46	5.18
2	8.48	7.33	8.78	10.14	8.68
3	20.40	19.37	17.86	18.32	18.98
4	39.78	40.10	41.31	40.35	40.38
5	52.19	38.48	31.97	48.22	42.72
6	55.20	52.94	47.56	55.21	52.73
7	61.44	57.06	48.06	61.34	56.98
8	48.05	51.45	35.95	47.81	45.82
9	58.55	66.12	51.50	61.96	59.33
10	39.16	53.69	37.10	48.38	44.58
Cultivar Means	38.84	39.18	32.49	39.72	

LSD_1 = 8.68; LSD_2 = 4.52; LSD_3 = 14.31; LSD_4 = 15.08

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	384.31
Harvest (H)	9	6,168.69**
Error (a)	27	143.08
Cultivar (V)	3	461.56*
H X V	27	75.50
Error (b)	90	103.67

Appendix Table 4a. Cubic polynomials fitted to leaf dry weight (dependent variable) of four cultivars, and corresponding Analysis of Variance.

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S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.936
Regression	3	1,194.40**	
Error	6	40.79	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	- 7.55600833	- 0.609	0.564
H	7.92070013	0.852	0.427
H^2	1.52341856	0.795	0.457
H^3	- 0.18452399	- 1.606	0.159

ICA Pijao

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.945
Regression	3	1,308.50**	
Error	6	38.25	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	- 5.35578333	- 0.446	0.671
H	6.94349747	0.772	0.470
H^2	1.09787879	0.592	0-576
H^3	- 0.11874747	- 1.067	0.327

Appendix Table 4a. (Continuation).

NEP-2

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.848
Regression	3	701.89**	
Error	6	63.09	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	- 9.16549167	- 0.59458	0.574
H	11.48045770	0.99360	0.359
H^2	- 0.14266098	- 0.05986	0.954
H^3	- 0.05286869	- 0.36995	0.724

Porrillo Sintetico

S.O.V.[1]	d.f.	M.S.	R^2
Total	9		0.931
Regression	3	1,216.80	
Error	6	45.26	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	- 7.47554167	- 0.572	0.589
H	8.89930250	0.909	0.398
H^2	1.00721606	0.499	0.636
H^3	- 0.13398038	- 1.107	0.311

Appendix Table 5. Dry weights of vegetative structures (in grams/15 plants) of ten sequential harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	10.52	11.14	9.04	10.75	10.36
2	17.21	14.60	17.51	21.34	17.66
3	30.55	30.40	27.63	29.24	29.46
4	57.27	58.85	57.60	58.22	57.99
5	78.21	61.33	53.43	76.86	67.46
6	92.56	86.90	79.19	93.82	88.12
7	101.94	99.10	83.73	105.14	97.48
8	91.39	100.97	73.79	93.43	89.89
9	122.91	117.68	99.92	121.49	115.50
10	88.62	110.89	80.69	103.19	95.85
Cultivar Means	69.12	69.19	58.25	71.35	

LSD_1 = 15.84; LSD_2 = 7.02; LSD_3 = 22.19; LSD_4 = 24.81

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	1,458.26
Harvest (H)	9	21,717.77**
Error (a)	27	476.53
Cultivar (V)	3	1,395.98**
H X V	27	166.73
Error (b)	90	249.36

Appendix Table 6. Dry weights of flowers and rachis (in grams/15 plants) of ten sequential harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	.994	.505	.517	.489	.626
6	2.070	1.042	1.414	.729	1.314
7	2.764	1.268	1.832	1.200	1.766
8	2.531	1.353	2.206	.994	1.771
9	4.792	3.167	3.765	1.853	3.395
10	3.371	3.326	3.474	1.738	2.977
Cultivar Means	2.734	1.777	2.202	1.167	

LSD_1 = .910; LSD_2 = .400; LSD_3 = .979; LSD_4 = 1.216

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	3.509
Harvest (H)	5	17.152**
Error (a)	15	1.458
Cultivar (V)	3	10.797**
H X V	15	.740
Error (b)	54	.477

Appendix Table 7. Dry weight of pods (in grams/15 plants) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
7	2.823	.653	.622	1.276	1.343
8	11.891	4.552	6.488	2.801	6.433
9	22.885	13.958	18.075	8.124	15.761
10	28.441	27.909	31.305	10.596	24.563
Cultivar Means	16.51	11.768	14.123	5.699	

$LSD_1 = 5.465$; $LSD_2 = 4.612$; $LSD_3 = 9.224$; $LSD_4 = 9.567$

S.O.V.[1]	d.f.	M.S.
Total	63	
Block	3	112.850
Harvest (H)	3	1,688.076**
Error (a)	9	46.705
Cultivar (V)	3	344.520**
H X V	9	78.116
Error (b)	36	41.577

Appendix Table 8. Dry weights of reproductive structures (in grams/15 plants) of ten sequential harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	.994	.505	.517	.489	.627
6	2.070	1.042	1.414	.729	1.314
7	5.587	1.921	2.455	2.476	3.110
8	14.421	5.905	8.695	3.795	8.204
9	27.677	17.126	21.841	9.977	19.155
10	31.812	31.235	34.779	12.334	27.540
Cultivar Means	13.760	9.622	11.617	4.967	

LSD_1 = 5.160; LSD_2 = 3.300; LSD_3 = 8.083; LSD_4 = 8.637

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	110.85
Harvest (H)	5	1,937.53**
Error (a)	15	49.90
Cultivar (V)	3	337.85**
H X V	15	80.94
Error (b)	54	32.50

Appendix Table 9. Total dry weights of plants (in grams/15 plants) of ten sequential harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	10.52	11.14	9.04	10.75	10.36
2	17.21	14.60	17.51	21.34	17.66
3	30.55	30.40	27.63	29.24	29.46
4	57.27	58.85	57.60	58.22	57.99
5	79.21	61.84	53.95	77.35	68.09
6	94.63	87.94	88.60	94.55	89.43
7	107.52	101.02	86.18	107.62	100.59
8	105.81	106.87	82.48	97.22	98.10
9	150.58	134.80	121.76	131.47	134.66
10	120.44	142.12	155.47	115.52	123.39
Cultivar Means	77.37	74.96	65.22	74.33	

LSD_1 = 18.61; LSD_2 = 7.75; LSD_3 = 24.51; LSD_4 = 28.13

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	2,086.48
Harvest (H)	9	30,456.57**
Error (a)	27	658.41
Cultivar (V)	3	1,136.39*
H X V	27	201.90
Error (b)	90	304.31

Appendix Table 10. Nitrogen fixation rates (u moles C_2H_4 $5pl^{-1}$ hr^{-1}) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	10.05	9.21	2.89	5.16	6.83
2	31.86	7.97	10.18	23.94	18.49
3	46.51	40.34	13.02	21.63	30.37
4	60.80	46.55	43.96	86.08	59.35
5	81.39	61.05	32.81	57.48	58.18
6	142.08	153.71	108.64	135.32	134.96
7	146.42	96.79	48.14	130.74	105.52
8	19.24	24.72	11.35	23.58	19.72
9	21.50	36.82	41.88	43.60	35.94
10	1.47	13.02	13.98	17.90	11.59
Cultivar Means	56.13	49.02	32.69	54.54	

LSD_1 = 31.44; LSD_2 = 13.30; LSD_3 = 42.08; LSD_4 = 48.50

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	950.10
Harvest (H)	9	28,886.16**
Error (a)	27	1,878.68
Cultivar (V)	3	4,591.64**
H X V	27	1,090.24
Error (b)	90	896.85

** = $\leq$ .01 - probability level

* = $\leq$ .05 - probability level

Appendix Table 11. Nodule dry weights (in grams/15 plants) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	.4560	.4417	.2014	.2001	.3248
2	.7276	.2870	.2614	.6328	.4772
3	1.4379	.7859	.6567	1.0468	.9818
4	2.5068	2.3003	1.7847	2.9301	2.3805
5	3.7156	2.8615	2.4737	4.3045	3.3388
6	5.8262	5.0052	3.8700	5.8297	5.1328
7	2.8484	5.4224	2.8046	5.3364	4.1029
8	1.6524	2.6344	1.7574	2.6445	2.1722
9	2.6257	3.0136	3.4171	4.0176	3.2685
10	.8156	2.1934	1.3404	3.4278	1.9443
Cultivar Means	2.2612	2.4945	1.8567	3.0370	

LSD_1 = 1.1812; LSD_2 = .4274; LSD_3 = 1.3514; LSD_4 = 1.6562

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	1.82
Harvest (H)	9	39.60**
Error (a)	27	2.65
Cultivar (V)	3	9.71*
H X V	27	1.63
Error (b)	90	.92

Appendix Table 12. Total content of ethanol soluble carbohydrates in nodules (in mg/15 plants) of eight sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
3	83.855	45.787	39.447	53.850	55.735
4	124.010	123.09	117.097	157.750	130.487
5	170.477	151.95	147.375	238.835	177.161
6	398.817	361.325	327.422	391.492	369.764
7	150.270	281.625	182.250	298.137	228.071
8	89.300	145.805	94.587	133.827	115.880
9	162.612	199.710	276.907	316.862	239.023
10	40.647	132.575	79.83	177.672	107.681
Cultivar Means	152.499	180.234	158.115	221.053	

LSD_1 = 97.366; LSD_2 = 36.549; LSD_3 = 103.375; LSD_4 = 131.506

S.O.V.[1]	d.f.	M.S.
Total	127	
Block	3	13,127.55
Harvest (H)	7	157.748.60**
Error (a)	21	17,529.74
Cultivar (V)	3	30,979.48**
H X V	21	5,993.84
Error (b)	72	5,380.81

Appendix Table 13. Concentration of ethanol soluble carbohydrates in nodules (in mg/g nodule dry wt.) of eight sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
3	62.85	60.97	58.18	55.93	59.48
4	54.03	50.95	60.78	52.47	54.56
5	45.92	50.92	60.16	54.67	52.92
6	65.83	73.50	60.07	62.23	66.16
7	52.51	51.68	61.37	51.91	54.37
8	52.81	54.90	53.95	51.28	53.24
9	61.65	69.82	80.22	78.07	72.44
10	49.00	60.72	62.00	55.85	56.89
Cultivar Means	55.58	59.18	62.09	58.18	

S.O.V.[1]	d.f.	M.S.
Total	127	
Block	3	345.12
Harvest (H)	7	794.40
Error (a)	21	355.72
Cultivar (V)	3	232.08
H X V	21	95.90
Error (b)	72	121.47

Appendix Table 14. Total starch content in nodules (in mg/15 plants) of eight sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
3	131.580	80.880	67.747	141.795	105.501
4	406.220	369.402	247.982	430.502	363.527
5	609.610	435.530	342.777	835.820	555.934
6	780.745	773.420	520.505	775.200	712.467
7	315.607	585.837	321.362	427.792	412.650
8	164.370	284.305	147.667	217.030	203.343
9	285.990	259.565	317.177	363.527	306.565
10	54.040	207.427	103.265	239.615	151.088
Cultivar Means	343.520	374.546	258.561	428.910	

LSD_1 = 161.892; LSD_2 = 87.016; LSD_3 = 246.120; LSD_4 = 266.394

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	98,968.66
Harvest (H)	7	687.144.09**
Error (a)	21	48,463.50
Cultivar (V)	3	162,398.09**
H X V	21	34,338.43
Error (b)	72	30,500.59

Appendix Table 15. Concentration of starch in nodules (mg/g dry weight) of eight sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
3	90.94	80.58	83.12	99.58	88.56
4	144.87	163.94	138.89	144.87	148.14
5	163.97	163.65	152.17	198.12	169.48
6	129.82	150.35	133.45	143.27	139.22
7	109.97	107.60	113.12	82.38	103.27
8	100.95	104.55	82.30	83.52	92.83
9	105.87	88.37	85.27	84.05	90.89
10	65.77	88.15	80.25	78.10	78.07
Cultivar Means	114.02	118.40	108.57	114.24	

LSD_1 = 26.358; LSD_4 = 46.521

S.O.V.[1]	d.f.	M.S.
Total	127	
Block	3	6,451.91
Harvest (H)	7	18,092.97**
Error (a)	21	1,284.65
Cultivar (V)	3	519.64
H X V	21	609.16
Error (b)	72	998.83

Appendix Table 16. Total ethanol soluble carbohydrate content of primary roots (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	21.645	25.542	22.81	28.510	24.627
6	43.252	54.605	46.045	61.437	51.335
7	37.415	55.087	61.527	51.182	51.303
8	55.797	87.362	70.040	80.530	73.432
9	71.730	106.435	91.067	119.337	97.142
10	61.827	108.137	79.562	127.965	94.373
Cultivar Means	48.611	72.862	61.842	78.160	

LSD_1 = 19.304; LSD_2 = 8.372; LSD_3 = 20.506; LSD_4 = 26.023

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	2,458.31
Harvest (H)	5	12,705.72**
Error (a)	15	656.46
Cultivar (V)	3	4,104.17**
H X V	15	504.75
Error (b)	54	209.21

Appendix Table 17. Concentration of ethanol-soluble carbohydrates of primary roots (mg/g dry weight) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	5.056	4.892	5.027	4.794	4.942
6	7.509	7.272	6.746	6.618	7.036
7	6.017	5.601	6.906	5.575	6.032
8	7.851	7.266	7.527	7.496	7.535
9	7.751	8.504	8.830	9.145	8.558
10	8.511	8.478	8.943	9.835	8.942
Cultivar Means	7.117	7.007	7.330	7.244	

LSD_1 = 1.291; LSD_4 = 1.831

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	.557
Harvest (H)	5	36.721**
Error (a)	15	2.937
Cultivar (V)	3	.483
H X V	15	1.012
Error (b)	54	1.156

Appendix Table 18. Total starch content of primary roots (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	41.890	52.857	46.870	53.057	48.669
6	73.377	69.125	69.010	124.445	83.989
7	104.500	152.260	138.910	124.585	130.064
8	200.665	344.327	269.062	314.822	282.219
9	254.325	297.097	264.550	393.540	302.378
10	244.870	348.625	265.097	472.260	332.713
Cultivar Means	153.271	210.715	175.583	247.118	

LSD_1 = 66.845; LSD_2 = 33.202; LSD_3 = 81.327; LSD_4 = 96.356

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	20,512.52
Harvest (H)	5	243,322.63**
Error (a)	15	7,871.55
Cultivar (V)	3	40,563.26**
H X V	15	7,720.12
Error (b)	54	3,290.57

Appendix Table 19. Concentration of starch of primary roots (mg/g dry weight) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	9.824	10.993	10.251	8.537	9.901
6	12.722	9.572	9.941	13.214	11.362
7	16.338	15.401	15.416	13.652	15.202
8	28.525	28.228	29.926	29.652	29.083
9	26.341	23.541	25.269	29.738	26.222
10	32.628	27.224	30.481	36.391	31.681
Cultivar Means	21.063	19.160	20.214	21.864	

LSD_1 = 2.055; LSD_4 = 5.733

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	32.328
Harvest (H)	5	1,456.921**
Error (a)	15	14.884
Cultivar (V)	3	32.261
H X V	15	16.054
Error (b)	54	16.431

Appendix Table 20. Total ethanol soluble carbohydrate content of "secondary roots" (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	110.005	141.417	173.127	125.285	137.459
6	237.080	243.640	202.695	287.117	242.633
7	300.802	328.015	240.295	249.047	279.540
8	292.722	301.932	254.297	244.877	273.457
9	434.830	341.020	372.930	539.662	422.111
10	225.957	305.257	171.305	347.527	262.512
Cultivar Means	266.900	276.880	235.775	298.920	

$LSD_1 = 65.639$; $LSD_2 = 45.819$; $LSD_3 = 112.233$; $LSD_4 = 116.593$

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	28,672.07
Harvest (H)	5	133,158.20**
Error (a)	15	7,590.01
Cultivar (V)	3	16,512.51
H X V	15	11,395.57
Error (b)	54	6,266.79

Appendix Table 21. Concentration of ethanol-soluble carbohydrates of "secondary roots" (mg/g dry weight) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	21.18	26.91	32.06	21.38	25.38
6	37.15	40.62	47.97	39.91	40.66
7	49.45	49.64	50.28	38.49	46.96
8	50.27	55.93	50.11	44.16	50.12
9	56.67	64.30	61.58	61.97	61.13
10	36.62	40.82	28.56	45.10	37.77
Cultivar Means	41.89	46.37	45.09	41.34	

LSD_1 = 8.63; LSD_4 = 12,837

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	518.39*
Harvest (H)	5	2,353.50**
Error (a)	15	131.32
Cultivar (V)	3	143.29
H X V	15	106.21
Error (b)	54	61.52

Appendix Table 22. Total content of starch of "secondary roots" (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	224.297	258.965	218.370	271.057	243.172
6	319.977	316.952	194.457	332.605	290.998
7	363.605	366.880	227.03	319.982	319.374
8	402.840	384.037	401.54	377.215	391.408
9	717.282	518.135	481.265	1,137.427	713.527
10	492.125	588.175	396.817	1,054.515	632.908
Cultivar Means	420.021	405.524	319.914	582.134	

LSD_1 = 121.781; LSD_2 = 120.259; LSD_3 = 294.573; LSD_4 = 281.611

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	64,642.20
Harvest (H)	5	606,372.10**
Error (a)	15	26,126.57
Cultivar (V)	3	287.583.11**
H X V	15	90,465.96
Error (b)	54	43,170.48

Appendix Table 23. Concentration of starch of "secondary roots" (mg/g dry weight) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	41.47	50.45	42.32	48.20	45.61
6	50.95	52.76	46.09	43.07	48.22
7	60.64	55.06	47.96	51.08	53.69
8	70.14	72.08	77.56	64.60	71.09
9	63.24	94.01	81.39	43.10	70.44
10	76.19	78.73	66.96	82.04	75.98
Cultivar Means	60.44	67.18	60.38	55.35	

LSD_1 = 17.66; LSD_4 = 32.39

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	419.92
Harvest (H)	5	2,780.24**
Error (a)	15	549.73
Cultivar (V)	3	566.19
H X V	15	391.74
Error (b)	54	477.98

Appendix Table 24. Total content of ethanol soluble carbohydrates of stems (in mg/15 plants) of six sequential weekly harvests, of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
5	1,479.62	1,164.62	945.70	1,383.00	1,243.24
6	2,503.10	1.832.40	2,757.72	2,082.90	2,294.03
7	4,427.97	2,081.80	3,289.87	2,852.37	3,163.01
8	5,095.32	3,500.60	3,591.27	4,204.77	4,097.99
9	4,911.45	2,778.70	3,218.27	2,641.25	3,387.42
10	5,432.42	4,163.30	4,159.15	3,722.75	4,369.41
Cultivar Means	3,974.98	2,586.90	2,993.67	2,814.51	

LSD_1 = 1,240.06: LSD_2 = 655.52; LSD_3 = 1,605.93; LSD_4 = 1,848.86

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	11,740,198.8
Harvest (H)	5	21,730,494.7**
Error (a)	15	2,708,964.9
Cultivar (V)	3	8,971,611.0**
H X V	15	899,391.3
Error (b)	54	1,283.093.1

Appendix Table 25. Concentration of ethanol soluble carbohydrates of stems (mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar				Harvest Means
	1	2	3	4	
5	111.18	108.87	104.74	107.51	108.83
6	127.28	127.43	165.39	130.41	137.63
7	180.10	105.86	165.60	110.40	140.49
8	175.19	121.98	153.49	156.22	151.72
9	109.37	87.30	114.71	77.10	97.12
10	158.54	117.56	152.79	122.91	137.95
$\bar{V}$	143.61	111.50	143.29	117.43	

$LSD_1 = 42.01$; $LSD_2 = 20.18$; $LSD_3 = 49.42$; $LSD_4 = 59.51$

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	2,008.85
Harvest (H)	5	7,123.23
Error (a)	15	3,109.54
Cultivar (V)	3	6,861.36**
H X V	15	1,040.26
Error (b)	54	1,215.24

Appendix Table 26. Total content of starch of stems (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	1,979.66	662.03	834.22	1,212.23	1,172.04
6	1,837.83	1,293.63	1,449.63	1,639.21	1,555.08
7	4,839.71	2,232.29	2,237.88	2.185.46	2,873.84
8	12,394.75	5,605.19	4,232.45	6,863.23	7,273.91
9	15,289.49	6,947.05	7,413.88	9,937.78	9,897.05
10	7,280.97	5,815.17	4,924.71	6,414.67	6,108.88
Cultivar Means	7,270.40	3,759.23	3,515.46	4,708.77	

LSD_1 = 3,053.25; LSD_2 = 1,020.01; LSD_3 = 2,498.50; LSD_4 = 3,713.03

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	14,817,867.0
Harvest (H)	5	195,884,735.0**
Error (a)	15	16,422,812.0
Cultivar (V)	3	70,749,829.0**
H X V	15	10,298.160.0**
Error (b)	54	3,105,707.0

Appendix Table 27. Concentration of starch in stems (mg/g dry weight of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	150.06	67.67	99.90	94.75	103.09
6	98.89	99.60	92.10	105.77	99.09
7	200.80	105.91	116.63	90.05	124.35
8	430.98	187.61	199.11	239.66	264.34
9	344.99	225.75	258.84	278.90	275.62
10	213.59	174.61	177.73	207.26	191.94
Cultivar Means	241.03	143.52	156.39	169.40	

LSD_1 = 64.10; LSD_2 = 33.72; LSD_3 = 82.61; LSD_4 = 95.31

S.O.V.[1]	d.f.	M.S.
Total	94[2]	
Block	3	1,758.51
Harvest (H)	5	100,681.00**
Error (a)	15	7,238.23
Cultivar (V)	3	44,260.14**
H X V	15	6,729.78*
Error (b)	53[2]	3,402.67

2 = correction 1 d.f. due to missing observation.

Appendix Table 28. Total content of ethanol soluble carbohydrates of leaves (in mg/15 plants of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	7,439.22	5,194.77	4,814.52	7,351.45	6,199.99
6	7,037.27	5,130.70	4,953,45	5,703.87	5,706.32
7	10,383.52	5,062,35	8,859.00	6,014.30	7,579.79
8	9,453,87	6,455.85	4,771.75	6,530.05	6,802.88
9	9,006.30	6,880.60	4,855.62	6,526.97	6,817.37
10	3,234.57	4,027.05	2,663.20	4,432.30	3,589.28
Cultivar Means	7,759.13	5,458.55	5,152.92	6.093.16	

LSD_1 = 2,585.97; LSD_2 = 1,429.22; LSD_3 = 3,500.85; LSD_4 = 3,955.25

S.O.V[1]	d.f.	M.S.
Total	95	
Block	3	4,779.228.6
Harvest (H)	5	30,929,987.4
Error (a)	15	11,780,640.6
Cultivar (V)	3	32,481,149.3**
H X V	15	6,492.779.2
Error (b)	54	6,097,453.8

Appendix Table 29. Concentration of ethanol soluble carbohydrates of leaves (mg /g dry weight) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	138.62	133.70	150.81	157.30	145.11
6	128.16	107.07	105.87	105.12	111.56
7	166.06	87.32	179.18	91.91	131.12
8	190.50	117.16	132.43	136.74	144.21
9	151.58	89.13	93.97	103.40	109.52
10	76.11	75.30	75.79	91.74	79.74
Cultivar Means	141.84	101.61	123.01	114.37	

LSD_1 = 35.41; LSD_2 = 19.45; LSD_3 47.66; LSD_4 = 53.97.

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	1,953.26
Harvest (H)	5	10,054.27**
Error (a)	15	2,209.37
Cultivar (V)	3	6,845.11**
H X V	15	2,213.68
Error (b)	54	1,129.88

Appendix Table 30. Total content of starch of leaves (in mg/15 plants) of six sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
5	6,884.65	4,215.52	4,181.88	3,972.62	4,813.67
6	5,763.80	2,781.96	4,289.94	4,788.73	4,406.110
7	7,751.48	3,093.01	4,071.49	3,152.93	4,517.23
8	9,995.88	4,385.84	3,211.73	4,076.40	5,417.46
9	7,692.27	3,244.22	2,705.00	5,504.57	4,786.52
10	3,718.87	2,524.60	2,327.40	3,362.62	2,958.37
Cultivar Means	6,967.83	3.374.19	3,464.58	4,126.31	

LSD_1 = 1,903.85; LSD_2 = 986.14; LSD_3 = 2,415.53; LSD_4 = 2,806.24

S.O.V.[1]	d.f.	M.S.
Total	95	
Block	3	10,271,616.5
Harvest (H)	5	10,900,021.9
Error (a)	15	6,385.382.4
Cultivar (V)	3	68,545,884.0**
H X V	15	5,106,983.8
Error (b)	54	2,902,873.4

Appendix Table 32. Total content of ethanol-soluble carbohydrates of pods (in mg/15 plants) of three sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
8	1,925.82	561.67	935.57	284.35	926.86
9	4,151.62	3,108.00	4,322.00	1,158.80	3,185.11
10	4,114.25	4,895.55	8,180.37	2,114.65	4,826.21
Cultivar Means	3,397.23	2,855.07	4,479.32	1,185.93	

LSD_1 = 1,484.45; LSD_2 = 1,580.39; LSD_3 = 2,737.31; LSD_4 = 2,750.62

S.O.V.[1]	d.f.	M.S.
Total	47	
Block	3	5,527,817.0
Harvest (H)	2	61,327,554.0**
Error (a)	6	2,944,101.0
Cultivar (V)	3	22,625,256.0**
H X V	6	6,685,645.0
Error (b)	27	3,558,946.0

Appendix Table 33. Concentration of ethanol soluble carbohydrates of pods (mg/g dry weight) of three sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
8	159.25	124.36	144.74	98.87	131.81
9	184.28	209.44	243.22	151.68	197.15
10	147.72	178.77	253.88	360.05	231.11
Cultivar Means	163.75	170.86	213.95	203.53	

S.O.V.[1]	d.f.	M.S.
Total	47	
Block	3	14,975.46
Harvest (H)	2	43,685.01
Error (a)	6	13,031.85
Cultivar (V)	3	7,185.60
H X V	6	18,634.77
Error (b)	27	14,074.74

Appendix Table 34. Total starch content of pods (in mg/15 plants) of three sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
8	1,265.49	299.79	676.42	212.34	613.51
9	2,966.12	1,727.90	3,118.85	938.47	2,187.84
10	3,841.19	3,972.71	6,696.46	1,738.80	4,062.29
Cultivar Means	2,690.93	2,000.14	3,497.24	963.21	

LSD_1 = 1,386.16; LSD_2 = 1,451.22; LSD_3 = 2,513.58; LSD_4 = 1,388.33

S.O.V.[1]	d.f.	M.S.
Total	47	
Block	3	7,471,794.0
Harvest (H)	2	47,696,437.0
Error (a)	6	2,567,141.0
Cultivar (V)	3	13,850,282.0**
H X V	6	3.959.992.0
Error (b)	27	3,000,965

Appendix Table 35. Concentration of starch in pods (mg/g dry weight) of three sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
8	104.32	65.35	98.82	77.14	86.41
9	126.54	112.31	170.33	106.19	128.84
10	131.41	141.65	197.42	342.46	203.23
Cultivar Means	120.76	106.44	155.52	175.26	

S.O.V.[1]	d.f.	M.S.
Total	47	
Block	3	20,820.11
Harvest (H)	2	55,956.17
Error (a)	6	15,505.24
Cultivar (V)	3	11,921.16
H X V	6	15,297.07
Error (b)	27	17,076.19

Appendix Table 36. Total content of organic nitrogen in "primary roots" (in mg/plant) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	1.1599	1.0404	.9901	.7804	.9928
2	1.7985	1.8567	1.5201	1.6810	1.7140
3	2.3656	3.1591	2.4397	2.4999	2.6161
4	3.0813	3.9533	3.5994	3.7296	3.5909
5	4.3808	4.5102	4.2452	5.8987	4.7587
6	5.4326	5.6260	5.2531	7.0412	5.8382
7	5.7058	8.0009	6.1258	6.6575	6.6225
8	5.8159	8.5436	5.7993	7.1748	6.8347
9	7.7050	8.4167	5.6019	9.1353	7.7147
10	5.3606	6.4852	4.5371	7.4042	5.9468
Cultivar Means	4.2806	5.1597	4.0111	5.2002	

LSD_1 = 1.220; LSD_2 = .4430; LSD_3 = 1.401; LSD_4 = 1.748

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	3.844
Harvest (H)	9	86.069**
Error (a)	27	2.829
Cultivar (V)	3	14.753**
H X V	27	2.004
Error (b)	90	.994

Appendix Table 37. Concentration of organic nitrogen in "primary roots" (mg/g dry weight) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	19.50	19.07	17.57	16.30	18.11
2	18.30	20.05	18.47	17.17	18.50
3	18.62	18.27	17.32	15.20	17.36
4	15.67	13.62	14.85	14.32	14.62
5	15.52	13.90	14.00	14.55	14.49
6	14.27	11.15	11.60	11.22	12.06
7	13.42	12.05	10.40	10.95	11.71
8	12.50	10.42	9.82	10.15	10.72
9	12.52	10.17	8.62	10.62	10.49
10	10.60	7.70	7.82	8.62	8.69
Cultivar Means	15.09	13.64	13.05	12.91	

LSD_1 = 2.39; LSD_2 = 0.61; LSD_3 = 1.93; LSD_4 = 2.91

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	4.812
Harvest (H)	9	192.53**
Error (a)	27	10.891
Cultivar (V)	3	39.860**
H X V	27	3.145*
Error (b)	90	1.892

Appendix Table 38. Total organic nitrogen of "secondary roots" (in mg/plant) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	4.249	5.499	3.133	4.611	4.373
2	6.560	5.382	7.154	9.159	7.064
3	5.546	5.730	4.924	5.382	5.395
4	8.347	6.492	5.195	6.460	6.623
5	8.569	8.240	9.921	9.272	9.001
6	10.241	9.715	6.670	12.352	7.745
7	9.974	9.185	7.516	9.990	9.166
8	9.399	8.610	8.126	9.042	8.794
9	12.491	7.855	9.046	12.266	10.414
10	9.501	9.742	8.839	10.817	9.725
Cultivar Means	8.488	7.645	7.052	8.935	

LSD = 2.611; LSD = 1.017; LSD = 3.199; LSD = 3.792

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	19.812
Harvest (H)	9	66.738**
Error (a)	27	12.951
Cultivar (V)	3	28.432**
H X V	27	5.301
Error (b)	90	5.184

Appendix Table 39. Concentration of organic nitrogen in "secondary roots" (mg/g dry weight) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	23.95	26.77	23.72	23.37	24.46
2	22.80	23.32	22.50	22.45	22.77
3	21.60	23.60	22.02	21.05	22.07
4	24.95	24.27	23.45	25.42	24.52
5	23.95	24.27	26.95	24.07	24.81
6	23.12	23.97	24.32	24.37	23.95
7	24.40	20.87	23.37	23.02	22.92
8	23.92	23.10	23.75	23.52	23.57
9	23.55	21.47	22.77	21.77	22.39
10	22.90	19.87	22.12	21.20	21.52
Cultivar Means	23.51	23.15	23.50	23.03	

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	2.512
Harvest (H)	9	20.513
Error (a)	27	15.838
Cultivar (V)	3	2,420
H X V	27	4,996
Error (b)	90	7,220

Appendix Table 40. Total content of organic nitrogen of stems (in mg/plant) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest
Harvest	1	2	3	4	
1	2.574	2.585	2.296	2.598	2.513
2	2.472	3.445	3.615	3.102	3.159
3	3.911	6.084	4.970	5.019	4.999
4	8.851	12.322	10.183	9.805	10.290
5	14.025	11.574	10.526	14.213	12.585
6	27.678	21.844	19.328	19.866	22.179
7	27.233	31.232	19.492	28.098	26.514
8	25.315	38.768	23.226	32.362	29.918
9	35.876	34.183	26.323	36.980	33.340
10	33.203	34.712	23.513	32.690	31.030
Cultivar Means	18.114	19.675	14.347	18.473	

LSD_1 = 5.145; LSD_2 = 2.553; LSD_3 = 8.075; LSD_4 = 8.651

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	123.42
Harvest (H)	9	2,406.85**
Error (a)	27	50.29
Cultivar (V)	3	212.03*
H X V	27	40.96
Error (b)	90	33.03

Appendix Table 40a. Cubic polynomials fitted to stem nitrogen content (dependent variable), and corresponding Analysis of Variance.

S.O.V.[1]	d.f.	M.S.	R^2
Total	159		0.765
Regression	3	16,125.92**	
Error	156	95.94	

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	10.55269059	2.229	0.027
H	- 9.3091.2632	- 2.623	0.010
H^2	3.38087446	4.618	0.0001
H^3	- 0.20838879	- 4.747	0.001

Appendix Table 41. Concentration of organic nitrogen in stems (mg/g dry weight) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	27.12	23.90	25.37	27.50	25.97
2	21.27	26.15	23.52	19.20	22.54
3	20.27	23.40	20.55	20.87	21.27
4	18.20	23.10	20.40	20.65	20.59
5	16.77	18.90	17.45	17.20	17.58
6	22.30	20.32	17.07	18.80	19.62
7	16.27	23.57	15.80	19.80	18.86
8	13.20	19.97	15.97	19.05	17.05
9	12.37	17.02	14.17	17.27	15.21
10	13.67	15.12	13.32	15.92	14.51
Cultivar Means	18.15	21.15	18.36	19.63	

LSD_1 = 3.728; LSD_2 = 1.410; LSD_3 = 4.460; LSD_4 = 5.356

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	47.049
Harvest (H)	9	192.946**
Error (a)	27	26.408
Cultivar (V)	3	76.281**
H X V	27	15.161
Error (b)	90	10.076

Appendix Table 42. Total content of organic nitrogen of leaves (in mg/plant) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

Harvest	Cultivar 1	2	3	4	Harvest Means
1	15.974	15.365	14.257	16.218	15.454
2	25.216	21.638	26.912	27.078	25.212
3	51.161	53.528	47.727	49.117	50.383
4	89.958	93.412	96.643	97.841	94.463
5	100.381	77.110	71.579	93.831	85.725
6	132.929	124.292	111.554	123.812	123.147
7	150.758	141.746	124.604	129.824	136.733
8	87.487	122.576	81.298	119.717	102.769
9	123.279	164.787	129.546	154.793	143.101
10	83.871	139.532	90.694	122.247	109.086
Cultivar Means	86.101	95.399	79.481	93.448	

LSD_1 = 24.44; LSD_2 = 11.90; LSD_3 = 37.64; LSD_4 = 40.60

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	582.23
Harvest (H)	9	31,951.58**
Error (a)	27	1,134.89
Cultivar (V)	3	2,121.48*
H X V	27	640.34
Error (b)	90	717.52

Appendix Table 42a. Cubic polynomials fitted to leaf nitrogen (dependent variable), and corresponding Analysis of Variance.

S.O.V.[1]	d.f.	M.S.	R^2
Total	159	194,413.50	0.635
Regression	3	2,148.55	
Error	156		

S.O.V.[1]	B Values	T for Ho: B ≠ 0	Prob T
Intercept	- 13.81823221	- 0.614	0.540
H	27.36913111	1.624	0.106
H^2	2.90227709	0.835	0.405
H^3	- 0.38194592	- 1.832	0.069

Appendix Table 43. Concentration of organic nitrogen in leaves (mg/g dry weight) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	46.100	43.825	44.500	44.425	44.712
2	44.925	45.200	45.925	40.775	44.206
3	38.250	41.550	40.925	39.250	39.994
4	34.750	34.625	35.425	36.700	35.375
5	28.600	31.200	33.025	29.650	30.619
6	36.175	35.025	34.925	33.475	34.900
7	37.225	36.875	38.875	32.400	36.343
8	27.775	35.250	33.775	37.625	33.606
9	31.650	37.200	38.450	38.425	36.431
10	32.075	39.150	36.350	37.900	36.369
Cultivar Means	35.752	37.990	38.217	37.062	

$LSD_1 = 4.510$; $LSD_2 = 1.789$; $LSD_3 = 5.658$; $LSD_4 = 6.632$

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	150.975
Harvest (H)	9	320.290**
Error (a)	27	386.387
Cultivar (V)	3	50.149*
H X V	27	21.006
Error (b)	90	16.216

Appendix Table 44. Total content of organic nitrogen of vegetative structures (in mg /plant) of ten sequential weekly harvests of four dry bean cultivars, and corresponding Analysis of Variance.

	Cultivar				Harvest Means
Harvest	1	2	3	4	
1	23.96	24.49	20.68	24.21	23.33
2	36.05	32.32	39.20	41.02	37.15
3	62.98	68.50	60.06	62.02	63.39
4	110.24	116.18	115.62	117.84	114.97
5	127.36	101.43	96.27	123.31	112.07
6	176.28	161.48	142.51	163.07	160.91
7	193.67	190.16	157.74	174.57	179.04
8	128.02	178.50	118.45	168.29	148.32
9	179.35	215.24	170.52	213.17	194.67
10	131.93	190.47	127.58	173.16	155.79
Cultivar Means	116.98	127.88	104.89	126.05	

LSD_1 = 29.20; LSD_2 = 14.43: LSD_3 = 45.65; LSD_4 = 48.97

S.O.V.[1]	d.f.	M.S.
Total	159	
Block	3	1,157.81
Harvest (H)	9	57,409.65**
Error (a)	27	1,619.72
Cultivar (V)	3	4,422.68*
H X V	27	885.40
Error (b)	90	1,055.56

Appendix Table 45. Total content of organic nitrogen (A) of flowers and rachis (in mg/plant) and concentration (B) (mg/g dry weight) of three sequential harvests of four dry bean cultivars.

A. Organic nitrogen content

Harvest	Cultivar 1	2	3	4	Harvest Means
8	5.758 (4)	4.138 (2)	5.648 (2)	2.001 (1)	4.386
9	9.826 (3)	6.378 (4)	7.246 (4)	4.849 (3)	7.075
10	8.033 (3)	7.321 (4)	6.987 (4)	4.138 (4)	6.620
Cultivar Means	7.872	5.946	6.627	3.663	

B. Concentration of organic nitrogen

Harvest	Cultivar 1	2	3	4	Harvest Means
8	33.70	42.60	33.40	34.50	36.05
9	30.94	31.17	27.75	36.40	31.565
10	34.47	33.10	30.60	36.57	33.685
Cultivar Means	33.037	35.623	30.583	35.823	

[1]Values shown in parenthesis are the number of observations in which means are based.

Appendix Table 46. Total content of organic nitrogen (A) of pods (in mg/plant) and concentration (B) (mg/g dry weight) of three sequential harvests of four dry bean cultivars.

A. Organic nitrogen content

	Cultivar				Harvest Means
Harvest	1	2	3	4	
8	29.33	13.26	14.59	7.07	16.062
9	35.80	25.33	29.09	17.56	26.995
10	57.55	37.64	49.58	26.94	42.927
Cultivar Means	40.893	25.410	31.087	17.190	

B. Concentration of organic nitrogen

	Cultivar				Harvest Means
Harvest	1	2	3	4	
8	24.65	35.10	31.43	32.90	31.02
9	23.77	29.75	26.10	26.10	26.43
10	24.65	21.40	22.32	27.30	23.917
Cultivar Means	24.357	38.750	26.617	28.767	

Appendix Table 47. Analysis of variance of the effect of carbon dioxide on nitrogen fixation rates (u moles C_2H_4 $5pl^{-1}$ hr^{-1}) of the dry bean cultivar Porrillo Sintético of two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	6,619.82
Chamber within treatment	2	1,011.59*
Subsample within chamber	20	92.62

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	32.14
Chamber within treatment	3	38.88*
Subsample within chamber	30	13.60

Appendix Table 48. Analysis of variance of the effect of carbon dioxide on nodule fresh weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico of two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	6.44
Chamber within treatment	2	0.66*
Subsample within chamber	20	.19

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	0.12
Chamber within treatment	3	0.33**
Subsample within chamber	30	0.04

Appendix Table 49. Analysis of variance of the effect of carbon dioxide on nodule soluble carbohydrate concentration (mg./g. fresh weight) of the dry bean cultivar Porrillo Sintetico at 18 to 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	1,434.08**
Chamber within treatment	2	27.08
Subsample within chamber	20	78.54

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	1,434.08**
Pooled Error	22	81.25

Appendix Table 50. Analysis of variance of the effect of carbon dioxide on nodule soluble carbohydrate (in gm./5 plants) of the dry bean cultivar Porrillo Sintetico at 18 to 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	381.35
Chamber within treatment	2	143.88
Subsample within chamber	20	116.15

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	381.35*
Pooled Error	22	118.67

Appendix Table 51. Analysis of variances of the effect of carbon dioxide on root fresh weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico of two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	28.60*
Chamber within treatment	2	1.18
Subsample within chamber	20	0.95

B. 18 - 36 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	2.43
Chamber within treatment	3	1.07
Subsample within chamber	30	0.26

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	2.43
Pooled Error	33	3.83

Appendix Table 52. Analysis of variance of the effect of carbon dioxide on root soluble carbohydrate concentration (mg./g. fresh weight) of the dry bean cultivar Porrillo Sintetico of two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	3.74**
Chamber within treatment	2	0.02
Subsample within chamber	20	5.34

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	42.66
Chamber within treatment	3	59.54
Subsample within chamber	30	28.78

Appendix Table 53. Analysis of variance of the effect of carbon dioxide on root soluble carbohydrate (in mg./5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	6,640.80*
Chamber within treatment	2	276.62
Subsample within chamber	20	

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	3,082.99
Chamber within treatment	3	10,390.41
Subsample within chamber	30	4,508.59

Appendix Table 54. Analysis of variance of the effect of carbon dioxide on stem fresh weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	435.20
Chamber within treatment	2	42.66*
Subsample within chamber	20	9.53

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	354.75
Chamber within treatment	3	113.08
Subsample within chamber	30	185.25

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	354.74**
Pooled Error	33	0.63

Appendix Table 55. Analysis of variance of the effect of carbon dioxide on stem soluble carbohydrate concentration (mg./g. fresh weight) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	50.27
Chamber within treatment	2	16.18**
Subsample within chamber	20	2.13

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	18.27
Chamber within treatment	3	11.21
Subsample within chamber	30	18.00

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	18.27
Pooled error	33	17.39

Appendix Table 56. Analysis of variance of the effect of carbon dioxide on stem soluble carbohydrate (in mg./5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	578.07
Chamber within treatment	2	21,337.50**
Subsample within chamber	20	2,283.85

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	392,685.79
Chamber within treatment	3	116,846.46
Subsample within chamber	30	138,359.40

Appendix Table 57. Analysis of variance of the effect of carbon dioxide on leaf fresh weight (in grams/plant) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	2,258.16
Chamber within treatment	2	319.95**
Subsample within chamber	20	65.67

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	34.34
Chamber within treatment	3	2,532.37*
Subsample within chamber	30	699.13

Appendix Table 58. Analysis of variance of the effect of carbon dioxide on fresh weight of pods (in g./5 plants) of the dry bean cultivar Porrillo Sintetico at 18 to 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	2,302.37
Chamber within treatment	3	306.03
Subsample within chamber	30	271.01

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	2,302.37**
Pooled error	33	274.19

Appendix Table 59. Analysis of variance of the effect of carbon dioxide on root dry weight (in g./5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	.0030
Chamber within treatment	2	.0037
Subsample within treatment	20	.0973

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	1.25
Chamber within treatment	3	1.04
Subsample within chamber	30	0.35

Appendix Table 60. Analysis of variance of the effect of carbon dioxide an organic nitrogen concentration of roots (mg./g. dry weight) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	0.88
Chamber within treatment	2	1.31
Subsample within chamber	20	1.10

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	0.43
Chamber within treatment	3	1.14
Subsample within chamber	30	0.02

Appendix Table 61. Analysis of variance of the effect of carbon dioxide an organic nitrogen content of roots (in mg./5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	2.02
Chamber within treatment	3	3.61
Subsample within chamber	20	23.75

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	217.22
Chamber within treatment	3	230.15
Subsample within chamber	30	83.59

Appendix Table 62. Analysis of variance of the effect of carbon dioxide on stem dry weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 36 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	.0037
Chamber within treatment	2	.3515
Subsample within chamber	20	.1613

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	51.13
Chamber within treatment	3	14.01
Subsample within chamber	30	4.95

Appendix Table 63. Analysis of variance of the effect of carbon dioxide on organic nitrogen concentration of stems (in mg/g dry weight) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p.(A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	4.94
Chamber within treatment	2	11.95
Subsample within chamber	20	8.46

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	32.67*
Chamber within treatment	3	3.30
Subsample within chamber	30	2.48

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	32.67**
Pooled error	33	2.55

Appendix Table 64. Analysis of variance of the effect of carbon dioxide on organic nitrogen content of stems (in mg/g dry weight) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	58.01
Chamber within treatment	2	19.97
Subsample within chamber	20	247.06

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	3,508.11
Chamber within treatment	2	4,847.25
Subsample within chamber	30	2,004.24

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	3,508.11
Pooled error	33	2,262.67

Appendix Table 65. Analysis of variance of the effect of carbon dioxide on leaf dry weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	2.14
Chamber within treatment	2	3.48
Subsample within chamber	20	2.14

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	97.49
Chamber within treatment	3	50.58*
Subsample within chamber	30	13.55

Appendix Table 66. Analysis of variance of the effect of carbon dioxide on organic nitrogen concentration of leaves (in mg/g dry weight) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	0.0067
Chamber within treatment	2	2.3223
Subsample within chamber	20	5.7330

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	54.85*
Chamber within treatment	3	2.79
Subsample within chamber	30	21.18

Appendix Table 67. Analysis of variance of the effect of carbon dioxide on organic nitrogen content of leaves (in mg/5 plants) of the dry bean cultivar Porrillo Sintetico for two time periods: 18 to 32 d.a.p. (A), and 18 to 46 d.a.p. (B).

A. 18 - 32 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	23	
Treatment	1	2,617.54
Chamber within treatment	2	7,428.16
Subsample within chamber	20	2,643.73

B. 18 - 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	174,379.94
Chamber within treatment	3	68,168.40*
Subsample within chamber	30	22,384.86

Appendix Table 68. Analysis of variance of the effect of carbon dioxide on pod dry weight (in grams/5 plants) of the dry bean cultivar Porrillo Sintetico at 18 to 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	45.23**
Chamber within treatment	3	0.52
Subsample within chamber	30	3.86

Appendix Table 69. Analysis of variance of the effect of carbon dioxide on organic nitrogen concentration of pods (in mg/g dry weight) of the dry bean cultivar Porrillo Sintetico at 18 to 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	175.71
Chamber within treatment	3	1.83
Subsample within chamber	30	6.51

Appendix Table 70. Analysis of variance of the effect of carbon dioxide on organic nitrogen content of pods (in mg./5 plants) of the dry bean cultivar Porrillo Sintetico at 18 to 46 d.a.p.

S.O.V.[1]	d.f.	M.S.
Total	35	
Treatment	2	41,063.35**
Chamber within treatment	3	1,583.37
Subsample within chamber	30	5,278.26

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