THE RELATIONSHIP OF NITRATE REDUCTASE AND FORMS OF REDUCED NITROGEN TO THE DEVELOPMENT OF REPRODUCTIVE STRUCTURES IN NAVY BEANS, (PHASEOLUS VULGARIS L.)

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By

Mohsen A. Younes

AN ABSTRACT

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APPROVED 

ABSTRACT

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Experiments were conducted in the field to study nitrate reductase activity, nitrate levels and percent protein in bean leaves during the development of reproductive structures. Experiments were also conducted under controlled conditions to study factors affecting the enzyme activity.

These factors were plant parts, stage of development, light period, light intensity, temperature, nitrate concentration in plant tissue, and development of reproductive structures.

The distribution of enzyme activity varies greatly in the different parts of the plant. The leaves have higher activity than the stem and petioles, while roots show no activity. The pronounced peak in activity was associated primarily with rapidly expanding leaves. This kind of distribution of the enzyme activity was independent of the type of plant growth whether it was determinate (bush) or indeterminate (vine) growth type.

There was a continuous decrease in activity in leaves, flower pods and pod wall during maturation.

The enzyme activity was undetectable in the pod in the early post-fertilization stage and increased with pod enlargement and seed growth and development. The protein level was high in the pod in the early post-fertilization stage and decreased in the pod-wall, while it increased in the seed.

The protein level decreased as leaf age increased. The enzyme activity in general seemed to follow three characteristic trends throughout the period of sampling: (a) great fluctuation in the late vegetative stage and flower bud development; but was at still relatively higher levels than at later stages, (b) a general decrease during the blossoming period, (c) a temporary recovery in activity after blossoming and during fruit and seed development. Concentration of nitrate, in general, seemed to follow the same pattern of enzyme activity. The lowest activity was associated with the lowest nitrate concentration in plant leaves.

Protein content fluctuated throughout the sampling period. All lines showed a significant decrease in protein after blossoming. There was a continuous decrease in protein content during fruit development while nitrate and nitrate reductase fluctuated during this stage.

There was a highly significant loss in pods from opened flowers. The determinate growth habit lines showed a low percentage loss in new pods from opened flowers in comparison to indeterminate ones. Most of these losses were in the later stages of blossoming in both cases.

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INTRODUCTION

Yield of seed in the common bean, Phaseolus vulgaris, is the multiplicative product of the average number of pods per plant or per unit length of row, the average number of seeds per pod, and the average seed weight. These yield components completely determine yield in the sense that there can be no variation in yield that does not result from variation in one or more of the components. Granted that component values and inter-relationships account for yield variation as immediate (and morphological or structural) causes, the question is still germain"what are the more primary causes of yield variation"? The long-accepted answers are couched in semi-physiological terms, whether it be in respect to leaf area indexes, photosynthetic efficiency, ability in respect of nutrient uptake and utilization, respiration rates, etc.

Yield of seed would appear to be maximized for a given plant type and environment when the highest genetic potential for easier yield component could be simultaneously expressed. This condition implies a lack of interaction or interference between the components. In fact, it implies developmental independence. But the widespread findings that yield components show negative correlations inter se argues against developmental independence. The question is "what is (are) the cause (s) of negative associations - developmental inter-dependence-among seed yield components?"

A hypothesis of limited metabolic input has been advanced. This hypothesis states that the necessary physiological building or storage materials for the formation or development of reproductive structures are limited in amount at one or more critical stages during development. The corallary is that input would be limiting at one stage but not at another which could lead to negative correlations.

It therefore seemed worthwhile to try to measure at least one important input system to determine its pattern with respect to the pattern of

development of reproductive structures for an array of genotypes that differed in their component relationships.

One of several major possible input systems - the reduced nitrogen (NH_2^-) system - has been chosen for consideration in this experiment.

This system is not independent of other systems in vivo operation, but as it is not possible to study all systems simultaneously, the main emphasis as a starting point concerns the (NH_2^-) - input system. Furthermore, only the primary enzymatic step wherein nitrogen as nitrate (NO_3^-) is reduced to nitrite (NO_2^-) will be considered. The enzyme catalyzing this first step of nitrate reduction is called nitrate reductase. This enzyme and certain fractions of reduced nitrogen, mainly protein were investigated during the reproductive stages of the navy bean plants. Whether a given metabolite will follow a specific pathway, with or without a distinct pattern, will depend first upon whether the required enzyme is present. Therefore, consideration must be given to the factors that control the kind of a given enzyme which exists in plant tissues, the amount or the activity of that enzyme, the location of the enzyme, and the factors that control the enzyme activity.

Since our basic interest is in a metabolic input system that leads eventually to yields of beans, it may be worthwhile to follow the enzyme activity and one of the products in the input system (as protein), throughout the growing season of the bean plants under normal conditions. Major external environmental factors such as temperature, light, and precipitation should be taken into account so that any relationship which might occur could be explained.

Recording data on developmental reproductive stages may help explain the variations in the given metabolic system during these stages.

LITERATURE REVIEW

It is well established that nitrate is a major source of nitrogen absorbed and utilized by green plants. Nitrate must be converted to nitrite; further to ammonia or amino acid level for the ultimate synthesis of protein; nucleic acid or other nitrogen substances in the cell. This process of nitrate reduction is called nitrate assimilation or assimilatory nitrate reduction. The reduction and assimilation of nitrate belongs to the class of fundamental biochemical reactions in the plant. Hageman et al. (24) stated that a metabolic pathway was suggested by Mayer and Schultze in 1894 (38) to be:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow H_2N_2O_2 \longrightarrow HONH_2 \longrightarrow NH_3$$

and by Kessler 1964 (32) in a most general form as: $HNO_3 + 8H \longrightarrow NH_3 + 3H_2O$.

He also reported that the transformation of nitrogen from the oxidation number + 5 to -3 thus requires eight atoms of hydrogen or eight electrons which must be supplied by other metabolic processes of the cell. Fewson and Nicholas (23) gave the following sequence of reactions based upon biochemical studies with plants from several phyla:

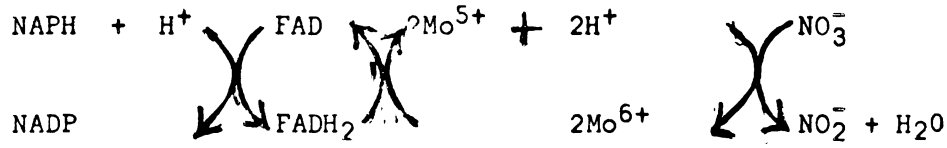
$$HNO_3 \longrightarrow HNO_2 \longrightarrow NO \longrightarrow (NOH) \longrightarrow NH_2OH$$

The large amount of earlier, mainly physiological work in this field has been reviewed by Burstron (10). In the course of the past twelve years, the problem of nitrate reduction has again received much attention. As a result of work in several laboratories, there has developed a clear understanding of both the enzymological basis and physiological relations of nitrate reduction in plants. This new work or some aspects of it has been summarized in a number

of reviews, (8, 19, 31, 44, 45, 49, 50, 51, 71). In most cases enzymology has received primary attention.

The present review considers solely the reduction of nitrate to nitrite as the primary step of nitrate reduction and assimilation. The enzyme responsible for this conversion is called nitrate reductase. It may exist in different forms, in association with different hydrogen donors, cofactors, and enzymic reaction sequences depending on the conditions of growth of the organism (46). This enzyme was first obtained by Evans, Nason and Nicholas from the fungus Neurospora. It is a metallo-flavo protein containing FAD, molybdenum, and active sulfhydryl groups, with $NADPH_2$ serving as hydrogen donor (21, 48, 52, 53, 54, 57, 58).

During nitrate reduction, the hydrogen or electron transport goes from $NADPH_2$ via FAD and Mo to nitrate. It involves a change of oxidation state of the molybdenum between +5 and +6 (59). Kessler (32) described the reaction by the following scheme:



Nitrate reductases have been shown to be present in green algae (62, 67), fungi (63, 67), bacteria (10, 27, 56, 61), and higher plants (1, 11, 18, 20, 22, 25, 55, 66, 68). In some cases $NADPH_2$ acts as the hydrogen donor, in others $NADH_2$ was effective or even essential.

No published information was found relating nitrate reductase activity to development of reproductive structures under normal condition. However, the reduction of nitrate and its assimilation to ammonia in higher plants depends on several factors including light (10, 11, 25), mineral elements

(9, 11, 28, 29), and source of nitrogen supply (11, 28).

Cell Development and Nitrate Reductase Activity:

Kessler (32) stated that work by Soeder, Muller and Ried (65) with synchronized cultures of Chlorella indicated that the nitrate reducing capacity of this alga strongly depends upon the developmental stage of the cells. Pronounced seasonal changes in the activity of nitrate and nitrite reduction have been observed in green algae by Kessler et al. (33). Hageman and Flesher (25) reported that the decrease in nitrate reductase with increase in age of corn plants grown in vermiculite was observed repeatedly under varied environmental conditions. Also, the loss of nitrate reductase activity with increased age of corn seedling was attributed, in part, to the proportionate increase in stem tissue. They mentioned that the expanded leaves had the highest level of nitrate reductase. Candela et al. (11) found that net nitrate reductase activity was maximal in cauliflower mature leaves and was markedly lower in both senescent and rapidly expanding young leaves. Also, they found that extracts from leaves possessed greater net activity than those from stem or petioles, and that the roots were extremely low. The specific activity per mg protein was much greater in petioles than in leaves or stems.

Nason (46) in his review indicated that the nitrate reductase responsible for the conversion of nitrate to nitrite exists in several different forms, in association with different hydrogen donors, cofactors, and enzymic reaction sequences depending on the organism and the conditions of growth.

Developmental Reproductive Stages in Plants:

Borthwick and Parker (7) have investigated the relation between the age of soybean plants concluding that the effectiveness of the photo-inductive treatment normally conducive to flowering appears to depend on the age of the plant. Also, they showed that young immature leaves play no part in photo-induction and only after the plant has achieved a certain amount of vegetative growth can the shift towards reproductive growth commence. Murneek (41) demonstrated that the fertilization of the flowers results in a growth stimulation beyond the fruit. Subsequently, Dearbon (14) observed a similar effect in the cucumber. Further observations on the general effects of flower and fruit development in the bean, pepper, and rudbeckia were presented by Murneek and Wittwer (43).

Wittwer and Murneek (74) also demonstrated that, as a result of sexual reproduction, the vegetative parts of a plant are vitally influenced. Furthermore, they stated that it seems reasonable to conclude that some catalyst or substance of hormone-like nature must be produced at the time the male and female gametes unite, resulting in increased vegetative development.

Nitrate reductase activity and protein:

Candela, et al. (11) reported that changes in nitrate reductase activity were not closely related to the total soluble protein content of the tissues. In contrast, Hageman and Flesher (25) reported that, in general, there was a positive correlation between the growth (fresh wt.) or protein content of the corn plant and nitrate reductase activity. Kostychev (34) found that the formation of proteins from nitrates in seed-plants is a photo-chemical process at least in the first stage.

Nason, Abraham, and Averbach (47) reported briefly that a partially purified soybean leaf protein, extracted by their method, contained an active nitrate reductase and could also catalyse ammonia formation from nitrite in the presence of reduced diphosphopyridine nucleotide (DPNH) and manganese ions.

Hewitt and Afridi (28) showed that an increase in the activity of the enzyme nitrate reductase greatly exceeds corresponding changes, if any, observed in soluble protein content in small fragments of leaves excised from cauliflower, and mustard plants.

Nitrate reductase activity and light:

The requirement of light for the reduction of nitrate by higher plants has been under investigation by several workers. Hageman (25) mentioned that Burstrom (10) showed evidence that wheat leaves would reduce nitrate in the light but not in the dark. His conclusion was that nitrate reduction was directly linked to a photochemical reaction, or that it occurred concomitantly with the fixation and reduction of carbon dioxide. In contrast, Delwiche (16) used isotopic nitrogen to demonstrate that tobacco plants metabolize nitrate or ammonia in the dark as well as in the light. Mendel, et al. (37) demonstrated that nitrate and hyponitrite are metabolized both in dark and light but that in light the rate is accelerated. Hageman (25) demonstrated that an increase in nitrate reductase activity was detectable within two hours after the plants were returned to full sunlight in the greenhouse after 65 hours of darkness. Also, the activity increased with time. He also stated that both nitrate and light are necessary for the formation of nitrate reductase in quantities required by plants for normal growth. Although the strong stimulating influence of light in nitrate reduction in green plants has been known for some time, the

explanation for this phenomenon has been highly controversial. The photochemical processes that may provide the hydrogen donors (i.e. reduced pyridine nucleotides) for nitrate reduction was shown by Evans and Nason (22) through the use of grana and purified nitrate reductase from soybean leaves. A similar concept by VanNiel et al. (69) was based on a competition between nitrate and carbon dioxide for reducing power. This interpretation was a result of their experiments with Chlorella. Warburg and Negelein (70) assumed that the light increased the permeability of the Chlorella cell for nitrate.

Kessler, 1964 (32) in his review, assumed an indirect effect of light, due simply to the ample production of carbon compounds by photosynthesis; in this case the carbohydrates would provide the hydrogen donors required for the reduction of nitrate just as they do in the dark. In addition, he reported that a more direct effect of light seems conceivable. Thus the photochemical processes might produce the cofactors necessary for the reduction of nitrate.

In favor for the latter possibility, evidence has been obtained by Evans and Nason (22) and Jagendorf (30). These authors were able to combine the known ability of chloroplasts to reduce NADP photochemically with the action of nitrate to nitrite. Delcamp et al. (15) recently demonstrated with spinach chloroplasts a light-dependent reduction of nitrate to nitrite which required the addition of benzyl viologen as an electron carrier. The work by VanNiel, Allen and Wright (69) with intact Chlorella indicated that nitrate and carbon dioxide competed for the photochemically produced hydrogen donors. Thus the existence of nitrate leads to a decreased rate of CO_2 reduction at low light intensities (26, 69). At light saturation, (high light), where the rate of

photosynthesis is limited by the capacity of the enzymes involved in the reduction of CO_2, the addition of nitrate leads to an increase of oxygen evolution (69).

Kessler (32) concluded from various lines of evidence that although the photochemical reduction of pyridine nucleotides is likely to be one of the important factors in the light enhanced reduction of nitrate, it cannot explain all the available experimental results. He believes that the action of light is a complicated phenomenon contributing to the supply of hydrogen donors, energy - rich phosphate bonds, and carbon compounds.

The Flowering Stage

The flowering phase is usually characterized by a subsidence of anabolic activity as well as the inauguration of fundamental modifications (35), and redistribution of organic and inorganic nutrient components (4, 12, 17). Subsequent events vary considerably among species, some of which, for example, undergo no further elongation of the main axis following anthesis (4). The decline of anabolic activity associated with blossoming gradually gives way to what is usually the final resurgence of absorption of mineral nutrients and acceleration of organic synthesis in vegetative tissues. This anabolic stimulus is associated with the fusion of male and female nuclei in syngamy and the very early enlargement of young fruits (40, 42, 43, 72).

As stated, the onset of blossoming or anthesis is marked by an apparently simultaneous reduction in absorption by roots and an internal shift in water balance (4, 17, 64).

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The Fruiting Stage

The early stages of fruit enlargement are commonly associated with marked increments in absorption by roots and accelerated anabolism of the younger parts of the shoot (6, 13, 36, 41, 73). Gains in nitrogen and potassium become apparently higher (36). The origin of the systemic stimulus to accelerated activity following syngamy appears to be associated with increments in growth substances at reproduction loci and their translocation to adjacent tissues (3, 39). Seeds and fruits are highly selective in the elements which they accumulate from leaves and stems (2).

The work of Rankin (60) on staggered and late supplies of nitrogen in producing differential effects upon the number of spikes, florets per spike, weight and number of kernels per plant in wheat is a case in point. The effects of a heavy crop of fruit in depleting the nitrogen reserves of leaves and stem are known, as also are the cyclic renewals of vegetative activity when fruit abscission or maturation occurs (42).

Experimental Plant Materials in the Field

Twelve lines of the navy bean Phaseolus vulgaris L., six determinate types and six indeterminate types, were planted in the field at the Michigan State University farm, June 1, 1964. These twelve lines differ in maturity, yield and some other morphological characteristics. All lines were in the fifth selfed generation.

The main purpose of planting twelve different lines was to extend the scope of the experimental material to provide a broad basis for the formation of generalizations from the observed data. The experimental plan involved three replications in a randomized block design. The plots were three-twelve foot rows 32 inches apart with 3 inches between plants within rows. Seeds were treated just before planting with a combination of fungicide and insecticide. Fertilizer in the amount of 255 pounds of 5-20-20 plus two per cent manganese and one per cent zinc was side-dressed at planting time. The plants were thinned approximately a week after emergence, to get as uniform a stand as possible.

Fifteen plants were chosen at random in the center in each of the three replications and marked with small stakes. Data were taken from these plants concerning the development of reproductive structures.

The other two twelve-foot rows in each replication were used for leaf, stem, flower, bud, and pod samples for the determination of nitrate reductase activity, and forms and levels of reduced nitrogen. Also, two-foot samples were taken at intervals near the end of the season for data on dry weight and yield components.

Temperature was measured continuously by a recording thermograph located in the plot area. Precipitation was measured throughout the growing season.

Hemispheric solar radiation on a horizontal surface was calculated to the nearest 1/2 degree of latitude, on a daily and/or hourly basis and given in whole Langleys (gm. cal. 1 cm^2). This measurement was made continuously throughout the season at a point approximately half a mile from the experimental field by the Agricultural Engineering Department at Michigan State University.

Nitrate Reductase Assay in Navy Beans:

Leaves of seedlings, rapidly expanding growing leaves, mature leaves, senescent leaves, stems, roots, flower buds, flowers, or pods at different stages were used as source material for measuring the level of activity of the enzyme. Fully expanded leaves from the upper third of the plant were used as a source material for nitrate reductase activity and levels of forms of reduced nitrogen throughout the growing season. At noon, regularly every third day, sample materials were removed from 12 to 15 plants of each line in each of the 3 replications, and were composited to form a sample. Each sample was immersed immediately in cold (0°C. to 2°C.) deionized water in individual sample bottles and taken directly to the laboratory.

Nitrate reductase activity was determined by a modification of the method outlined by Hageman and Flesher (25).

The sample material (leaves or stems or pod wall) was blotted dry, a 5-gram sample weighed and cut into small pieces and ground in 20 ml. of extraction medium, composed of 0.1 M Tris (tris hydroxymethyl aminomethane) 0.01 M cysteine and 0.0003 MEDTA (ethylene diamine tetraacetic acid) in equal proportions and adjusted to pH 7.0 with dilute HCl. The samples were ground in an Omnimixer at maximum speed for 3 minutes with the cup

immersed in an ice bath. The homogenate was pressed through cheesecloth and centrifuged for 15 minutes at 20,000 gravity in a refrigerated centrifuge. The supernatant liquid was decanted through glass wool and assayed. The homogenates and extracts were kept cold (1-2°C) throughout the entire analysis. The assays were completed within about four hours from the time of sampling.

Nitrate Reductase Activity Determination:

Nitrate reductase activity was measured by further modification of the Evans and Nason method as reported by Hageman and Flesher (25). The assay mixture contained 1.0 ml. of 0.1 M potassium phosphate buffer solution at pH 7.5 and 0.2 ml. of 0.1 M KNO_3. To this mixture were added 0.5 ml. of 1.36×10^{-3} M DNPH, (reduced nicotin amide adenine dinucleotide) to initiate the assay first by DNPH and immediately thereafter 0.2 ml. of the enzyme extract. The mixture was incubated at 27°C. for 15 minutes and the reaction stopped by adding 1 ml. of 1% w/v sulfanamide in 1.5 NHCl. One ml. of 0.02% w/v (N-(1 napthyl) ethylene diamine hydrochloride was added and the contents mixed by inverting the tubes. The color was allowed to develop for 10 minutes before centrifuging at 20,000 G for 15 minutes to remove the turbidity. The absorbency was determined by reading each sample against a special constant line blank (complete except for DNPH) in a Beckman DU spectrophotometer at a wave length of 540 microns against a constant blank for all lines.

Concentrations of nitrate formed during the reaction were calculated by reference to standard curves made with potassium nitrate and the enzyme activity was expressed as μ gm N in 20 min. per 0.2 ml. extract.

Determination of Nitrate Reductase Activity Levels:

The modified method of Evan and Nason by Hageman (25) was used for drawing a standard curve. The standard curves were made from triple dilutions of KnO_2 in deionized 2 ml. of the diluted KnO_2 in deionized water. Two ml. of the diluted KnO_2 was added to 1 ml. of 1.0% w/v sulfanilamid in 1.5 NHcl. and 1 ml. of 0.2% w/v N-(1-napthylethylene diamine di-hydrochloride.) The solutions were mixed by transferring the solution into another tube three times. The color was developed for twenty minutes, and was read on the Beckman DU at 540 mμ slit 0.04 mm against its own blank of deionized water. The relation between the optical density readings on the Beckman DU and nitrogen amount expressed as μ gm. N in the sample is shown in Table I.

Samples	Optical density O.D.	gm N
1	0.003	0.025
2	0.057	0.050
3	0.064	0.072
4	0.089	0.105
5	0.132	0.148
6	0.173	0.216
7	0.223	0.260
8	0.266	0.328
9	0.357	0.424

Statistical correlation was made between the development of the color expressed in the reading of the optical density and amount of nitrogen in each sample. The correlation regression was 0.8338, and the correlation coefficient 0.996.

Protein Assay in Navy Beans

Rapidly expanding, growing leaves, fully-expanded leaves, stems, roots, flower buds, flowers, or pods at different stages were used as source material for measuring the level of total reduced nitrogen, mainly protein. Fully expanded leaves from the upper third of the plant were used as source material for protein determination throughout the sampling period.

Regularly, at noon, every third day, sample materials were removed from 12-15 plants of each of the three replications, and were composited to form a sample from each line. Each sample was immersed immediately in cold (0°C. to 2°C.) deionized water in individual sample bottles and taken directly to the laboratory. Each sample was dried in individual tightly locked jars for 48 hours in a Virtis machine under vacuum and at minus 60° centigrade, until it reached constant weight.

Each sample was ground using the Wiley-Mill machine which has a 1/40 inch hole screen. The samples were kept in tightly covered wax paper cups, all of which were kept under the same environmental conditions.

Protein Method Determination:

A 475 mg. sample of ground, fully-expanded leaves (dried under vacuum, under -60°C) was pipetted by automatic pipette with 50 ml. setting of 1000, of Reagent Dye Solution part No. SL-1210 for protein analysis made by UDY - Analyser Company*.

*Udy Analyser Co., P. O. Box 148, Boulder, Colorado, U.S.

Multiply Balance scale reading by 2.0 (sample weight used exactly 475 mg. in all cases) to get a correct Pipette scale setting (range 950 was used). Placing all of the Reagent Dye solution in the reaction tube, the sample was added and reacted for 4.5 minutes in the React-R-Mill. The reacted assay solution was then transferred to a P/E filter bottle and measured by filtering 15 to 20 drops in the short-light-path Color Analyser's Curvette. The optical density (absorbency) was measured and readings were recorded.

Protein percentage was calculated by developing a standard curve by correlating the optical density (absorbency) in the colormetric method of UDY-Protein Analyser and the amount of protein calculated by the Micro-Kjeldahl nitrogen analysis method.

Six samples of the ground leaf material that varied in protein amounts were used for this test.

Table II. The relation between optical density (absorbency) and the percentage of protein in the samples calculated by Micro-Kjeldahl-Nitrogen analysis.

Sample No.	Optical density* (absorbency)	% protein**
1	74.20	28.47
2	73.85	28.82
3	69.65	26.96
4	47.90	20.11
5	42.45	18.65
6	30.70	14.61

*Average of four samples. **Average of two samples.

The Micro Kjeldahl-Method determined the ml. eg. N in the samples. Then the amount of nitrogen in the samples was calculated, using the factor 6.25 to get the amount of protein. The O.D. and % protein in the samples correlated well statistically. The a = A constant which fixed the position of the regression line = - 13.76 and b = the regression coefficient of y on x which represents the slope of the line = 3.066. From this relation the following equation was developed to give the percentage of protein:

$$\% \text{ protein} = \frac{\text{O.D. reading}}{3.066} + 4.5$$

The percentage of protein in the sample material is shown in different tables and was obtained by this correlation.

Data on Plant Component and Reproductive Structures Development

Flowering Notes: Data on flower development were recorded daily at two distinct stages of flowering. The first was at the first appearance of visible flower buds. After flower bud initiation and differentiation the number of flower bud clusters was recorded. At the second flowering stage the number of newly opened flowers, (i.e. flowers at pollination and fertilization stage) was recorded. Data on each line were taken at the same time every day.

Pod Development Data: The newly formed pods on the marked plants in each replication within each line were counted as soon as the corolla had abscissed so as to clearly reveal the developing pod.

Differentiated Tissues, Plant and Yield Component Information From the Field

Near the end of the growing season, uniform sections of row, each two feet in length were pulled and put immediately in paper bags. After the samples were enclosed each in a separate paper bag, they were weighed for fresh weight. They were then put in a dryer for 8 to 10 days until they had reached an approximately constant dry weight, which was then recorded. Each sample was then separated into component parts - leaves, roots, stems and pods and the dry weight recorded.

EXPERIMENTAL RESULTS

1. Nitrate reductase activity and levels of protein in different parts of the plant.

   (a) Nitrate reductase location in the plant.

Sample materials were taken from different parts of the same bean plants grown in the field.

Table III. The nitrate reductase activity* in different parts of lines 8 and 12 in plants eight weeks old.

	SAMPLE MATERIALS			
Lines	Full size green leaves	Stem and petioles	Pod-wall of normal full-size pods	Roots
Line 8	0.022	0.005	0.056	0.000
Line 12	0.077	0.007	0.022	0.000

* μ gm. N in 20 min. per 0.02 ml. extract, (average of two samples)

The results from Table III show that the fully-expanded-leaves and the pod-wall of normal full sized green pods have more activity than stems, petioles or roots. The activity in roots was undetectable as was shown by

the samples from the two lines in the above table. A specified regional study at different stages of root growth is needed since the above results were done with roots of plants eight weeks of age. A similar study is needed in stem and petioles since the main interest in this paper was in leaves and fruits.

The enzyme activity at different stages of different parts of bean plants is found in Table IV.

Table IV. Nitrate reductase activity* at different stages of bean leaves (August 12, 1962).

Lines of beans	Rapidly expanding leaves, aged 1-2 weeks	Young full-size leaves, aged 3-4 weeks	Mature leaves (full-size green) aged 5-6 weeks	Senescent leaves 9-10 weeks
Line 4	0.098	0.032	0.016	0.004
Line 7	0.034	0.019	0.011	0.000
Line 11	0.075	0.028	0.013	0.000

*Average of two samples in μ gm. N in 20 min. per 0.02 ml. extract.

The data in Table IV shows that the activity of nitrate reductase in bean plants varies with growth stage of leaves. The pronounced peak in activity associated with rapidly growing leaves but not with fully expanded or senescent leaves of normal plants is noteworthy. There was a continuous decrease or loss in the activity with increasing age of the plant. There is a relatively rapid reduction between rapidly growing leaves and young fully expanded leaves. No adequate answer or explanation can be given for the continuous loss in activity with increasing age.

The results in table III indicate that the enzyme nitrate reductase is

unstable. There is a loss of activity even in the absence of cell growth. This may be due to "spontaneous" inactivation of the enzyme in the absence of a substrate [as will be shown in other places in this paper], or perhaps to a metabolic destruction since the loss or decrease in activity occurs in the leaves at different stages on the same plants at the same time. However, the rapid decrease or loss in enzyme activity in the early rapidly growing young and in young fully expanded leaves may be due to the fact that the induced enzyme is "diluted out" as the cells grow, and the rate of reversion depends on the speed of leaf-growth. The undetectable or trace amount of the enzyme activity in senescent leaves, beside the above possibilities in the enzyme activity, suggests that chlorophyll may have some effect on the activity of the enzyme.

Nitrate reductase activity in bean pods at different stages of growth is shown in the following table.

Table V. Nitrate reductase activity* at different stages of pod development of bean plants. (August 12, 1964).

Lines	Early post-fertilization stage	Early seed growth and development stage	Pod-wall of young, normal full-size green pods	Pod-wall of old, normal full-size yellowish pods	Mature pod wall
Line 7	0.000	0.002	0.015	0.003	0.000
Line 11	0.000	0.001	0.019	0.002	0.000

*Average of two samples, in μ gm. N in 20 min. per 0.2 ml. extract.

The data of Table V shows that activity could be measured, in the early stages of pod and seed development, a very few days after fertilization. The activity increased with the increase in pod and seed-size during the rapidly growing stage of the pod and seed. The maximum activity was

found in the pod-wall of young full-size pods during seed growth. A decrease in the activity was found in the pod-wall of full-size pods when they started to yellow and mature and there was no detectable activity in the mature pod wall.

The activity shown in the pod may be due to a stimulating effect of the seed. No adequate explanation can be given as to why there was no activity detected in the early post fertilization stage, whereas the loss in activity during maturity may be due to the first two possibilities given under the enzyme activity in leaves.

Table VI. Nitrate reductase activity* in young flower buds and recently opened flowers of bean plants in summer, 1964.

Lines used	Samples materials	Young flower bud July 7	Recently opened flowers July 17
Line 1		0.022	0.001
2		0.016	0.000
3		0.030	0.000
5		0.025	0.013
6		0.012	0.002
7		0.018	0.009
8		0.038	0.007
9		0.028	0.009
12		0.029	0.008

*Average of two samples of nitrate reductase activity level in μ gm N/0.2 ml. extract.

It seems that the levels of activity of nitrate reductase was higher in the young flower bud, while there is very little if any activity at blossoming. However, the activity level in the young flower bud is lower than the level in young rapidly expanding leaves. There were differences in the levels of activity among different lines. There is no adequate explanation for the loss in activity level with increasing flower age and

organization. The possibilities for this loss or decrease in the enzyme activity might be due to the same possibilities given under the loss in activity in leaves with increasing age.

Table VII. The activity* levels in bean pods at different stages of development and effect of time trend.

	Early pod development stage (post-fertilization)		Pod-wall of full sized pod	
Lines	July 31	August 5	July 31	August 5
Line 1	0.000	0.000	0.008	0.048
2	0.000	0.000	0.004	0.030
3	0.000	0.000	0.006	0.054
4	0.000	0.000	0.005	0.061
5	0.000	0.000	0.003	0.022
6	0.000	0.000	0.040	0.033
7	0.000	0.000	0.001	0.011
8	0.000	0.000	0.018	0.039
9	0.000	0.000	0.017	0.083
10	0.000	0.000	0.015	0.098
11	0.000	0.000	0.019	0.135
12	0.000	0.000	0.027	0.030

*Average of two samples in μ gm N. in 20 min. per 0.2 ml. extract.

The results shown in Table VII indicate that there is no detectable activity in very early pod development, while activity was detectable when seed starts rapid growth. The samples from the full-sized pod wall show that activity was relatively lower in samples July 31 than in samples taken on August 5, except in line 6 which had the highest activity in samples of July 31. Line 6 is the earliest in maturity and on July 31, the seed had almost reached the full normal-size, and therefore, the activity decreased on August 5 as the seed matured. Line 7 had the lowest activity in the samples of July 31, because this line is the latest one in flowering and maturity and at this sampling date the pods were in the very

early-seed growth stage, and the activity increased with the development and growth of seeds. The same pattern was shown in the other lines with higher activity because they were in an advanced stage of seed growth and development. This may suggest that seed growth and development in the pod, stimulates nitrate reductase activity in the pod-wall, since the activity increased with increase in seed growth and development and decreased when the seeds reach the full normal size and start to mature.

(b) Total protein levels in bean plant parts at different stages of growth and development.

Table VIII. The total protein percentages and nitrate reductase activity in leaves at different stages of development (samples taken August 4, 1964).

		Rapidly expanding leaves, aged one week	Fully-expanded green leaves, aged 4-5 weeks	Senescent leaves aged 8-9 weeks
Line 4	% protein**	24.00	19.89	13.78
	Nitrate reductase activity*	0.117	0.024	0.005
Line 11	% protein**	25.99	22.74	11.99
	Nitrate reductase activity*	0.131	0.028	0.004

*Average of two samples in μ gm N in 20 min. per 0.2 ml. extract.

**Average of four samples.

The results from Table VIII show that the rapidly-expanding leaves have a higher level of total protein as well as nitrate reductase than the later stage of leaf development. However, enzyme activity dropped relatively faster than the total protein level. This may be due to the

rapid sensitivity of the enzyme to environmental factors as will be shown in other places in this thesis.

Table IX. Total protein levels in bean pods at different stages of development.

The following table shows the percentage of total protein* content in pod parts of a bean plant.

Sample materials	% protein	Nitrate reductase activity**
Post-fertilization pod development stage (aged, less than a week)	23.55	0.000
Early seed-growth and development stage (aged approximately two weeks)	15.33	0.010
Pod-wall of young full-size pod (normal size)	11.99	0.034
Whole pod with seed (normal size green pod)	12.96	-----
Seed only from normal size green pod	23.90	-----

* Average of four samples.

** μ gm N in 20 min. per 0.2 ml. extract.

The results in the above table IX indicate that the pod wall in the post-fertilization stage has the highest amount of protein and it decreases during the course of seed growth and development in the pod. This early stage in pod development has no detectable activity of nitrate reductase while it has a relatively high amount of protein. This may suggest that nitrate reductase and total protein are independent in some stages, since the amount of protein falls as the enzyme activity decrease in later stages of pod development as well as in leaves.

This high amount of protein and rapid decrease with seed development may suggest that the pod-wall in early stages probably plays a part in seed growth and development by supplying form (s) of reduced nitrogen.

2. Effect of Light Intensity and Temperature on Nitrate Reductase Activity in Bean Seedlings

Seeds of two different varieties of navy beans, Saginaw and Sanilac, were planted in small pots of fertile soil. These were placed in a growth chamber at room temperature and with a photoperiod of twelve hours daily. Four plants were maintained in each pot. Adequate soil moisture was maintained and an equal and adequate amount of nitrate (KNO_3) was added at planting time.

During the dark period of the 21st day after planting, half of the plants from each variety were placed under one of two different temperatues of 20°C. or 30°C. After 12 hours of complete darkness under one of the above temperatures, the first composite sample of seedling trifoliate leaves was collected from twenty plants of five pots under the same temperature treatment. These samples were immersed immediately in cold (0°C. to 2°C.) deionized water in individual sample bottles and nitrate reductase activity determined. Directly after this sampling, one fourth of the remaining material of each variety was put under one of the following combined treatments of light intensity and temperature:

(1) Relatively high temperature (30°C.) and low light intensity (500 ft. candles).

(2) High temperature (30°C.) and high light intensity (2200 ft. candle).

(3) Low temperature (20°C.) and high light intensity (2200 ft. candle).

(4) Low temperature (20°C.) and low light intensity (500 ft. candle).

The light intensity was controlled in the growth chamber by adding several cheese-cloth layers on the sky-liner of each room of the low light intensity. This was checked and measured by a light meter until the light intensity was as desired in each treatment before the beginning of the dark period the day previous to the sampling.

Two hours later under the combined effects of light and temperature, the second sampling was made. Trifoliate leaves of twenty plants from the pots under each treatment, were gathered to form a composite sampling. These leaves were immersed immediately in iced water (0°C. to 2°C.). A few minutes later they were assayed for nitrate reductase activity.

The third sampling was made after the effects of four hours of the temperature and light treatment from the same number of plants as the previous sampling in each treatment.

The results were as shown in Tables X and XI.

Table X. Effect of temperature, light intensity and time on nitrate reductase activity*, in trifoliate leaves of bean seedling after a period of 12 hours darkness, (Phaseolus vulgaris var. Saginaw)

Treatments	Period under light	0.00 hrs.	2.00 hrs.	4.00 hrs.
30°C.	500 f.c.	0.011	0.007	0.020
30°C.	2200 f.c.	0.015	0.020	0.034
20°C.	2200 f.c.	0.050	0.050	0.103
20°C.	2200 f.c.	0.052	0.032	0.086

*Average of two replications of nitrate reductase activity in μ gm. N in 20 min./.02 ml. extract.

Table XI. Effect of temperature, light intensity and time on nitrate reductase activity*, in trifoliate leaves of bean seedlings after a period of 12 hrs. darkness (in variety Sanilac).

Treatments	Period under light	0.00 hrs.	2.00 hrs.	4.00 hrs.
30°C.	500 f.c.	0.018	0.012	0.022
30°C.	2200 f.c.	0.021	0.041	0.070
20°C.	2200 f.c.	0.081	0.061	0.084
20°C.	500 f.c.	0.082	0.054	0.080

*Average of two replications in μ gm. N in 20.0 min./0.02 ml. extract.

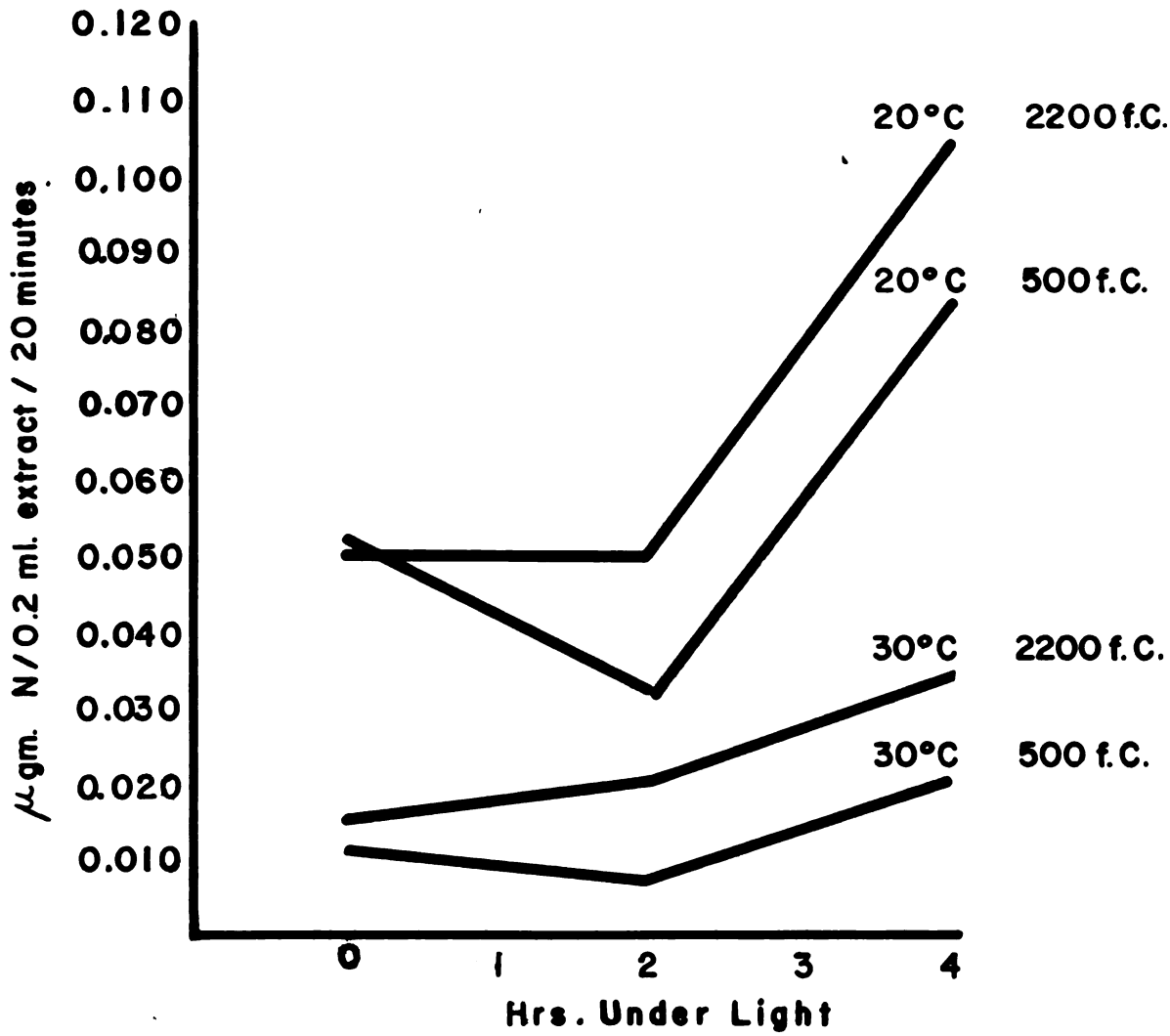

Figure I. Levels of nitrate reductase activity (µgm. N/0.2 ml. extract/20 minutes) in trifoliate leaves of bean, variety Saginaw, seedlings under the effect of temperature and light intensity after a period of darkness.

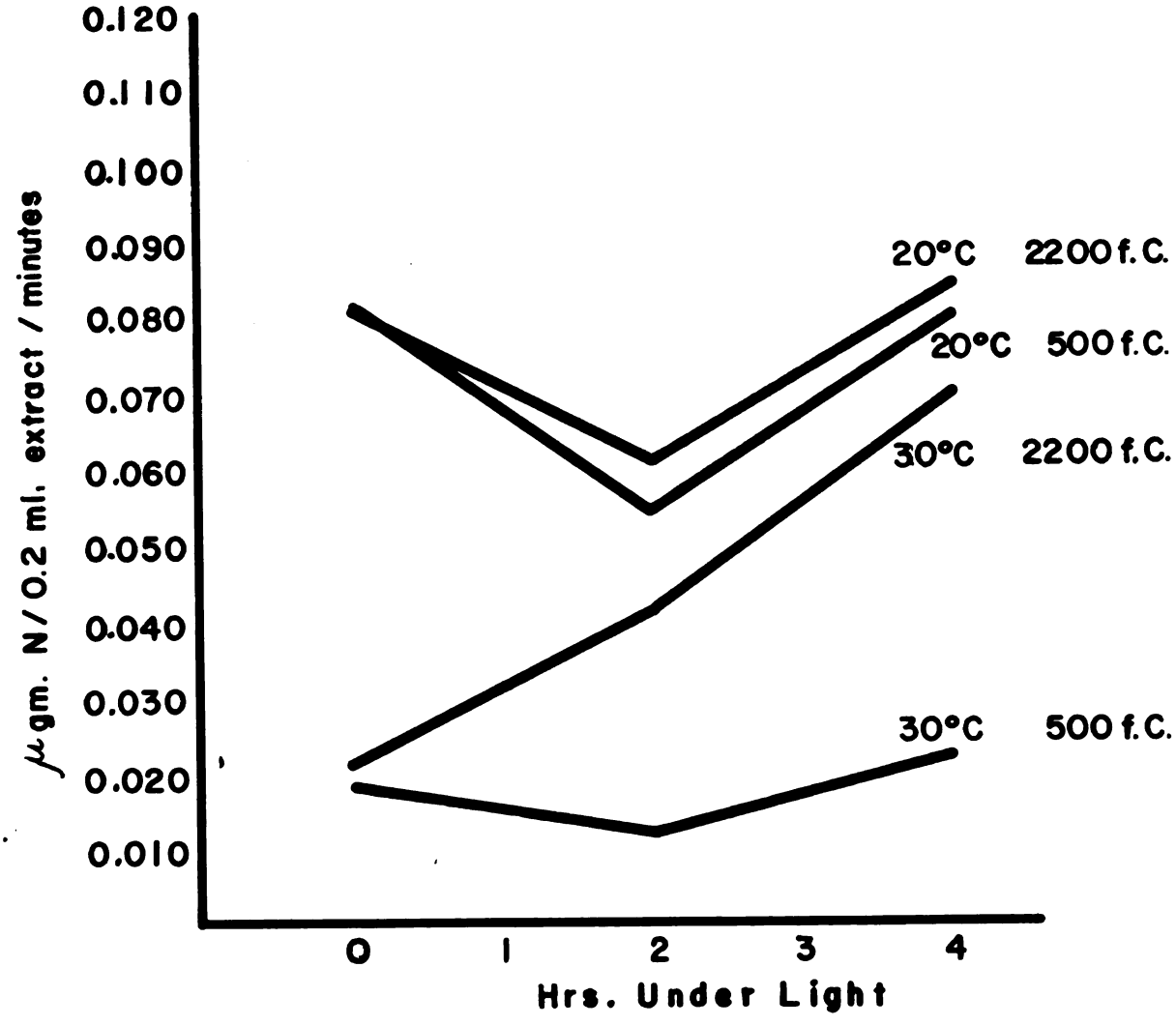

Figure II. Levels of nitrate reductase activity (μgm N/ 0.2 ml. extract/20 minutes) in trifoliate leaves of bean, variety Sanilac, seedlings under the effect of temperature and light intensity after a period of darkness.

Figures I and II show the activity of nitrate reductase levels in young trifoliate leaves of bean seedlings grown under different temperatures and light intensity treatment after a period of 12 hrs. darkness.

The enzyme activity level was higher at the first sample time, namely, directly at the end of the 12 hrs. dark period in the seedlings under 20°C. than those under 30°C. treatment. The 20°C. may be more optimal for enzyme activity or the 30°C. may inactivate the enzyme during dark. However, the variety, Sanilac, has a higher level of nitrate reductase activity than that of Saginaw, at the first sample time. This might be due to varietal response to night temperature.

In general, there was a decrease or loss in levels of the enzyme activity or only a small increase after a two hour period of exposing the plants to light. No adequate explanation at the present time can be given for this loss or decrease in the level of enzyme activity after two hours of light. The 30°C. and 2200 f.c. treatment was the only treatment of the two varieties that showed a detectable increase after 2 hrs. of exposing the plants to light. A significant increase occurred in the enzyme activity after four hours under light in comparison to the levels after two hours.

The variety, Sanilac, had a higher activity at any time under any treatment than the variety Saginaw. This may be due to varietal response to light and temperature.

The variety, Saginaw, had a significant increase in enzyme activity after four hours compared to that of the starting samples and those after two hours under light. It had an 81.81% increase over the starting sample under 30°C. and 500 f.c. treatment, 126.67% increase under 30°C. and

2200 f.c., 106.00% increase under 20°C. and 2200 f.c., and 65.38% increase under 20°C. and 500 f.c. treatment.

While the levels of the activity of nitrate reductase increased 44.44% after four hours in light, under 30°C. and 500 f.c., and 138.09% increase under 30°C. and 2200 f.c. and 65.38% increase after four hours of light under 20°C., and 500 f.c., there was a decrease in the activity of 2.44% under 20°C. and 500 f.c. All these comparisons were between the levels of the enzyme activity at the first sample, immediately before putting the plants under light and after four hours under light. Also, the activity level of nitrate reductase was always higher under 20°C. treatments than 30°C. Furthermore, the activity was always higher under 20°C. and 2200 f.c. than under any other treatment of the two varieties.

The order of enzyme activity levels under the different treatments was:

20°C. > 2200 f.c. > 20°C. and 500 f.c. > 30°C. 2200 f.c. > 30°C. and 500 f.c.

in the two varieties, except at the first sample in the first two treatments. So, the high light intensity had a higher activity level than the low light intensity, and the 20°C. had a higher activity level than 30°C. in all cases.

3.- Nitrate Reductase Activity During Reproductive Structure Stages

Table XII and Figures III, IV and V show nitrate reductase activity behavior in fully-expanded bean leaves during the period of growth of reproductive structures of the plant. Enzyme activity fluctuated during the period of sampling. The activity fluctuation had a distinct pattern

in all of the bean lines used. The pattern of activity fluctuation is in general a damping pattern type. Enzyme activity was higher and fluctuated greatly before any marked blossoming in all the twelve bean lines. Otherwise, in general, the activity decreased and reached a very low point at maximum blossoming. There was temporary stimulation in enzyme activity a few days immediately after maximal blossoming. This may suggest that an anabolic stimulus is associated with the fusion of male and female nuclei in syngamy and the very early enlargement of young fruits. At maximal blossoming or anthesis there is a marked simultaneous reduction in enzyme activity. The lowest activity level during blossoming occurred in sampling dates on July 21-24. This may indicate that an external factor may play a role. However, as will be shown later the lowest level of nitrate (NO_3^-) was found during this period of sampling on July 21-24. It is known that nitrate (NO_3^-) is the substrate for nitrate reductase, so, it is not surprising if it may act as a limiting factor for enzyme activity during the blossoming stage. It was found, too, that a high level of nitreate (NO_3^-) was found in the leaves during the period of stimulation in enzyme activity immediately after blossoming and during young fruit enlargement. This marked reduction in enzyme activity and nitrate levels in the plants during blossoming and their simultaneous increase after fertilization and during young fruit enlargement, may indicate that the flowering phase is usually characterized by a subsidence of anabolic activity as well as the inaguration of fundamental modifications (62), and redistribution of organic and inorganic nutrient components(63, 64, 65).

There was a continuous decrease in enzyme activity with little fluctuation as the plant advanced in maturity.

Temperature to which plants were exposed in the field varied considerably from the first to the second half of July and to a lesser extent in August during the course of sampling as shown in Table XXII. During the second week of July the temperature was relatively low. The activity in general was high when temperature was low. This agrees with the results of laboratory experiments. However, a specified study of temperature is needed to indicate the range at which the enzyme functions. In bean lines six and twelve blossoming occurred during this period of low temperature, the activity was high and nitrate (NO_3^-) level was high.

Temperature may affect activity greatly during the vegetative stage. Temperature seemed to have little effect during the blossoming and later stages.

Precipitation (Table XXIII) shows no detectable effect on nitrate reductase activity or nitrate levels. This may indicate that soil moisture and nitrate were not limiting factors.

Summarizing, the activity of the enzyme seemed to follow three characteristic trends:

(a) At the late vegetative growth period, the enzyme has higher activity, relatively, than at any later stage in the same tissues (fully-expanded leaves), it fluctuates greatly and the lowest activity was higher than the level at the blossoming stage.

(b) A general decrease during marked blossoming of the plants.

(c) A general simultaneous increase in a very few days after maximal blossoming and during the rapid increase in size of the fruits, followed by a decrease in activity with little fluctuation. The state of health of the plant probably has some effect, since bean line 10 (irradiated by radioactive material) was still green and healthy even during maturity and activity was higher than in any other bean line and even during the period of maturity, enzyme activity fluctuated in a relatively great amount.

Determination of Nitrate:

The modification of the method of Nelson, Kurtz, and Bray made by Woodley, Hicks and Hageman (76) was used for drawing a standard curve. For determination of nitrate 0.1 gm. of dried sample was diluted in 20 ml. of deionized water. One ml. of this solution was diluted in 9 ml. of 20% acetic acid with 2 ppm of copper sulfate. Between 0.6 - 1.0 gm. of nitrate powders, (100 gm. barium sulfate, 75 gm. acetic acid, 10 gm. manganous sulfate dihydride, 4 gm. of sulfanilic acid, 2 gm. powdered zinc, and 2 gm. of 1-naphthlylamine), was then added. The samples were shaken three times for fifteen seconds with about six minutes between shakings. The color was allowed to develop for two hours and then the samples were centrifuged at 1500 G for 15 minutes. The light absorbence was measured on the Beckman DU at a wave length of 540 m microns. The sample was read against its own blank. The relation between absorbency and nitrate was correlated and a standard curve drawn. The amount of nitrate was calculated from the standard curve and expressed in μ gm N in two hours per 0.1 gm. dry material.

Table XII. Average of two samples of total activity* of nitrate reductase in full expanded green leaves of bean plants, during period of development of reproductive structures in summer of 1964.

Date of Sampling	BEAN LINES											
	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8	Line 9	Line 10	Line 11	Line 12
July 3	.031	0.072	-----	----	-----	-----	0.019	.048	----	----	----	-----
6	0.012	0.032	0.026	.047	0.114	0.089	0.026	0.28	0.026	0.109	0.022	0.102
9	0.019	0.083	0.017	.028	0.015	0.024	0.058	0.016	0.012	0.033	0.016	0.028
12	0.039	0.036	0.039	.046	0.025	0.087	0.042	0.085	0.078	0.072	0.147	0.073
15	0.021	0.024	0.024	.032	0.027	0.42	0.026	0.081	0.067	0.065	0.046	0.160
18	0.014	0.026	0.006	.032	0.012	0.027	0.013	0.036	0.089	0.037	0.042	0.077
21	0.003	0.007	0.003	.003	0.002	0.010	0.005	0.012	0.020	0.015	0.010	0.135
24	0.001	0.010	0.006	.010	0.005	0.012	0.003	0.014	0.013	0.008	0.006	0.010
27	0.015	0.017	0.012	.023	0.007	0.020	0.016	0.043	0.034	0.035	0.016	0.043
30	0.033	0.013	0.009	.030	0.012	0.010	0.014	0.009	0.024	0.015	0.016	0.016
Aug. 3	0.010	0.006	0.004	.006	0.003	0.005	0.012	0.011	0.013	0.028	0.047	0.018
6	0.014	0.008	0.006	.014	0.005	0.008	0.025	0.006	0.017	0.050	0.012	0.006
10	0.006	0.007	0.002	.012	0.000	0.001	0.010	0.003	0.025	0.005	0.030	0.003

*M gm. N in 20 minutes per 0.2 ml. extract.

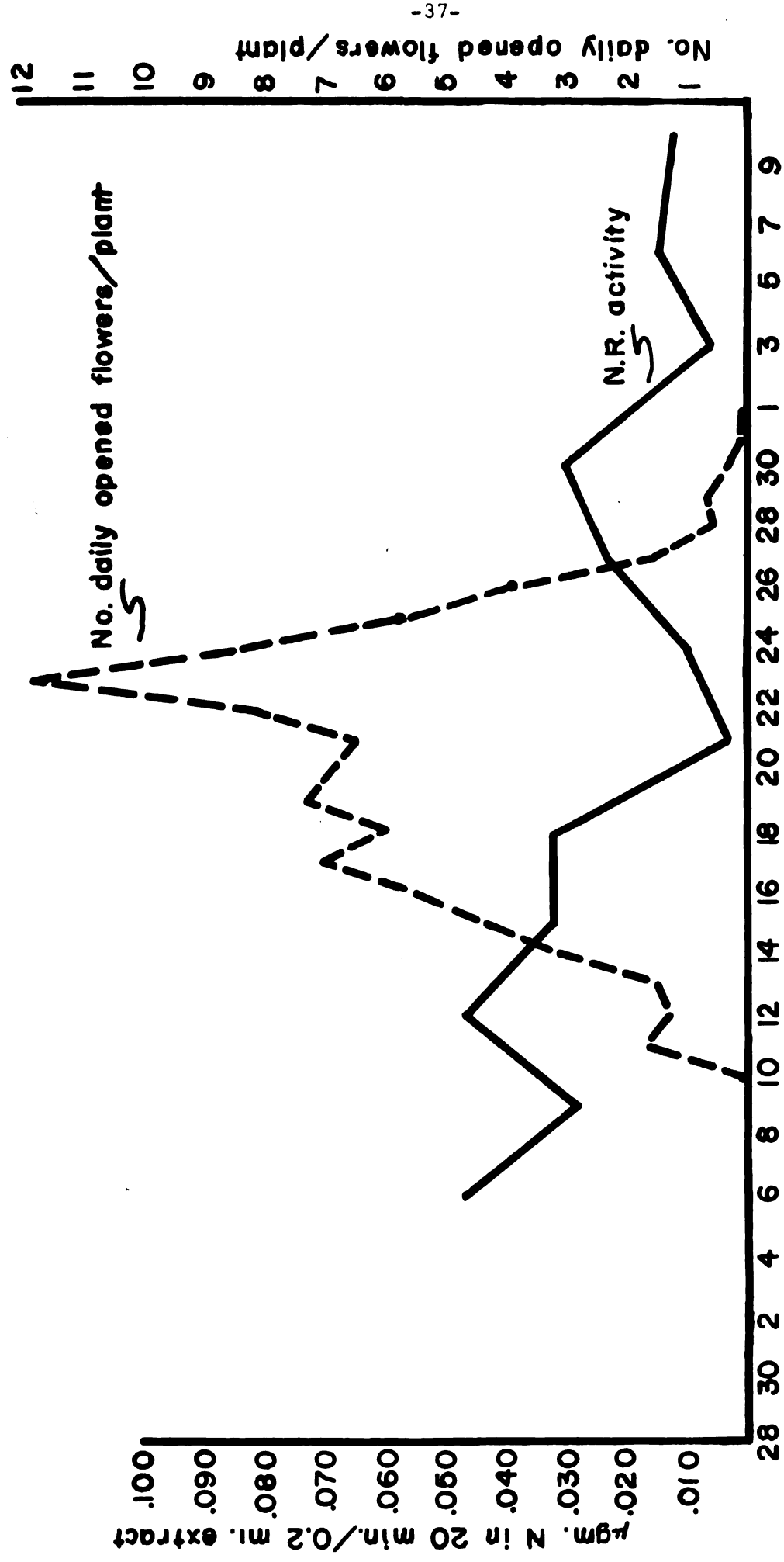

Figure IV. Relationship of nitrate reductase activity in bean leaves and daily opened flowers in bean line 4.

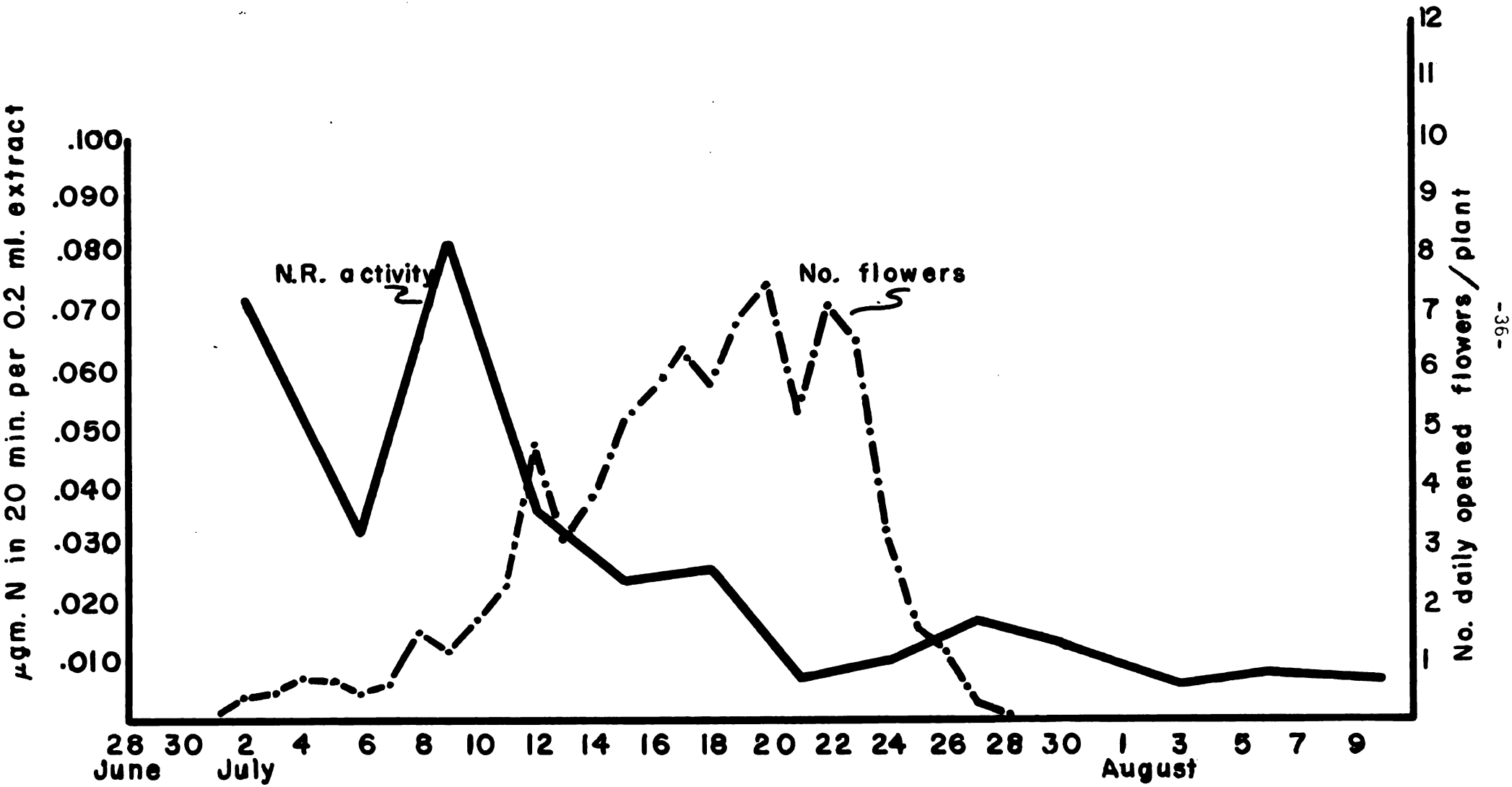

Figure III. Relationship between nitrate reductase in bean leaves (line 2), and no. of daily opened flowers.

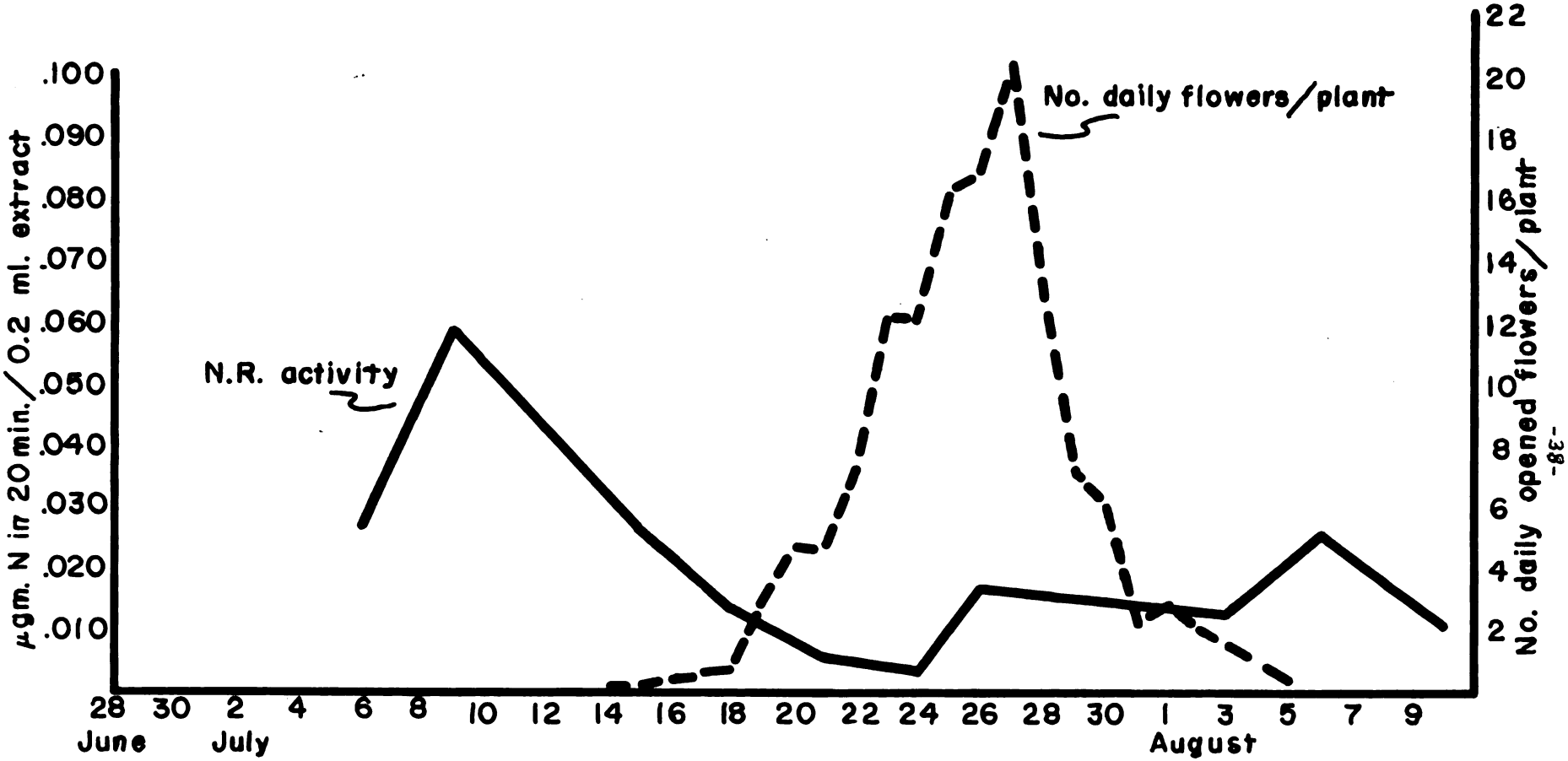

Figure V. Relationship of nitrate reductase expressed in µgm N. in fully-expanded leaves and daily opened flowers in bean line 7.

Nitrate (NO_3^-) Levels: Determination in Fully Expanded Leaves During Reproductive Structures in Beans:

One tenth gram of the dried material was diluted in 20 ml. of deionized water and nitrate determined as previously described.

Figures VI and VII shows the relation between the optical density (absorbency) and the amount of μ gm N in the sample.

Using samples of the dried material previously stored under vacuum and -60°C. nitrate levels were determined in four lines that differ in rate of development of reproductive structures and length of maturity stage (period).

Table XIII. Levels of Nitrate* in Full Expanded Leaves During Development of Reproductive Structures.

Time of Sampling	LINES OF BEANS					
	Line 1	Line 4	Line 6	Line 7	Line 9	Line 10
June 6	475.0	768.0	900.4	1240.0	1464.2	193.7
9	525.0	1056.2	-----	1224.2	1064.5	356.2
12	787.5	896.0	1240.0	1288.4	740.6	312.5
15	187.5	536.3	1025.5	476.5	1472.2	262.5
18	131.2	810.0	836.4	668.5	512.4	131.2
21	137.5	440.0	224.2	500.0	688.0	
24	----	480.0	388.2	1800.0	472.0	
27	237.5	604.4	152.4	512.3	488.2	
30	62.5	468.2	124.4	268.4	580.2	
Aug. 3	237.5	660.0	208.5	380.0	408.0	
6	250.0	116.2	88.5	148.0	124.0	
10	----	188.5	-----		-----	
11	----	348.1	-----		240.0	
14	----	-----	-----		256.2	

* μ gm N in two hours per 0.0 gm. dry sample

Nitrate Levels During Reproductive Structures in Bean Plant:

Concentrations of nitrate as shown in Table XIII were high in all bean lines studied in the period before blossoming. The nitrate level in the plant leaves fluctuated during the course of sampling. A general sharp decrease in nitrate level in bean leaves occurred with marked blossoming. In general, there is a similar pattern of behavior in nitrate concentration and nitrate reductase activity in plants during marked blossoming and rapid development of fruits. Differences in nitrate levels among bean lines exist. The lowest nitrate level was associated with the lowest activity of the enzyme at blossoming in samples of July 21 and 24. The recovery of the enzyme activity at early pod and seed development was associated with an increase in nitrate level in the same sample materials. Lines that had high nitrate reductase activity during part of the blossoming stage (as in line 6) show a high level of nitrate at this time in the same material.

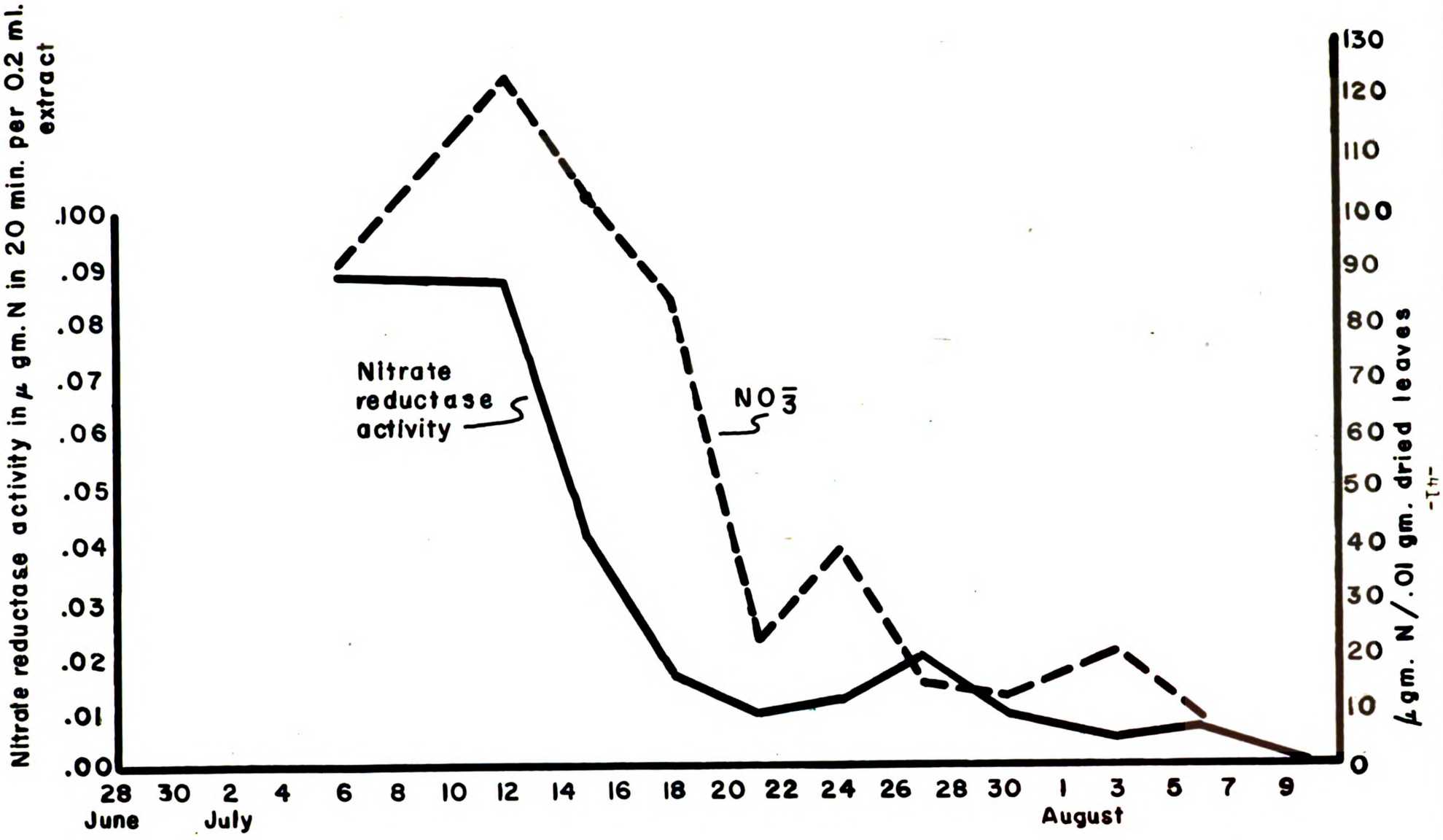

Figure VI. The relationship of nitrate reductase activity levels and levels of nitrate (NO_3^-) in fully expanded bean leaves during reproductive stages under the field condition, in the bean line 6.

Figure VII. Nitrate (NO_3^-) and nitrate reductase levels, expressed in µgm N/0.1 gm. dried sample, of full expanded leaves during reproductive structure period in line 4 grown under field conditions.

Protein Levels During Development of Reproductive Structures in Bean Plants.

Samples of fully-expanded leaves were used as source materials for total protein during the development of reproductive structures of the bean plants. Table XIV shows the pattern and behavior during this period of sampling. The protein levels fluctuate during the period of sampling, but to a less extent in comparison to nitrate reductase activity.

The protein level was relatively high during the blossoming period, then it started to decrease after maximum blossoming. This was a general observation as it is shown in the data in Table XIV in all bean lines.

Line six, which has a short blossoming and maturity period, shows a rapid sharp decrease in protein level in the sample materials (leaves) after blossoming. It also has a relatively continuous sharp decrease in protein level during the pod and seed development stage, and has the lowest protein percentage of any line as shown in the data of August 6th.

Line seven, which is later and has a little longer flowering and maturity period, shows a relatively higher percentage of protein later and starts to decrease after maximal blossoming. It shows Figures VIII and IX, a continuous decrease during pod and seed growth and development. While line six starts to decrease in the samples on July 24th, line seven starts to decrease in protein percentage on July 30th.

Different lines show different levels of protein percentage. The decrease or loss in protein level after maximum blossoming may suggest that the bean leaves play a major role in supplying the pod and seed for growth and development. More study is needed of protein percentage in stems and petioles during the development of reproductive structures.

Table XIV. Percentages of Total Protein in Fully Expanded Green Leaves of Bean Plants, During Period of Development of Reproductive Structure Under Field Conditions.

Date of Sampling	1	2	3	4	5	6	7	8	9	10	11	12
July 6	27.78	29.91	30.00	28.16	30.14	30.23	30.95	28.53	30.46	----	28.9	30.16
9	26.62	28.71	28.80	29.57	29.58	27.69	26.60	26.35	28.28	29.74	28.60	28.34
12	28.29	28.44	29.78	26.73	29.65	----	26.98	28.57	29.60	28.70	29.30	28.15
15	28.22	28.47	28.47	29.42	26.98	26.58	28.70	28.08	30.30	----	30.40	28.70
18	30.56	29.66	34.40	30.56	30.56	27.69	31.14	30.07	32.02	27.60	31.20	30.00
21	30.97	30.74	33.16	31.05	----	31.05	30.23	30.88	32.20	28.50	32.60	31.63
24	29.59	29.91	32.60	31.14	31.54	22.15	32.35	28.93	29.45	27.00	34.90	30.60
27	29.32	27.66	30.69	30.40	26.32	21.44	30.88	24.37	28.60	25.00	31.10	29.70
30	24.20	----	27.79	26.08	25.84	20.46	29.09	23.23	30.34	24.00	28.64	29.80
Aug. 3	21.6	23.39	----	19.93	19.00	15.57	25.84	21.28	25.34	22.60	23.40	20.79
6	18.5	20.62	21.28	18.10	19.00	14.60	23.00	15.31	19.97	19.70	----	20.30
8	----	----	----	19.03	----	----	16.71	-----	19.15	----	----	----
14	----	----	----	19.12	----	----	16.68	-----	20.74	18.34	22.96	----

*Average of four samples.

Table XIV. Percentages of Total Protein in Fully Expanded Green Leaves of Bean Plants, During Period of Development of Reproductive Structure Under Field Conditions.

Date of Sampling	1	2	3	4	5	6	7	8	9	10	11	12
July 6	27.78	29.91	30.00	28.16	30.14	30.23	30.95	28.53	30.46	----	28.9	30.16
9	26.62	28.71	28.80	29.57	29.58	27.69	26.60	26.35	28.28	29.74	28.60	28.34
12	28.29	28.44	29.78	26.73	29.65	----	26.98	28.57	29.60	28.70	29.30	28.15
15	28.22	28.47	28.47	29.42	26.98	26.58	28.70	28.08	30.30	----	30.40	28.70
18	30.56	29.66	34.40	30.56	30.56	27.69	31.14	30.07	32.02	27.60	31.20	30.00
21	30.97	30.74	33.16	31.05	----	31.05	30.23	30.88	32.20	28.50	32.60	31.63
24	29.59	29.91	32.60	31.14	31.54	22.15	32.35	28.93	29.45	27.00	34.90	30.60
27	29.32	27.66	30.69	30.40	26.32	21.44	30.88	24.37	28.60	25.00	31.10	29.70
30	24.20	----	27.79	26.08	25.84	20.46	29.09	23.23	30.34	24.00	28.64	29.80
Aug. 3	21.6	23.39	----	19.93	19.00	15.57	25.84	21.28	25.34	22.60	23.40	20.79
6	18.5	20.62	21.28	18.10	19.00	14.60	23.00	15.31	19.97	19.70	----	20.30
8	----	----	----	19.03	----	----	16.71	-----	19.15	----	----	----
14	----	----	----	19.12	----	----	16.68	-----	20.74	18.34	22.96	----

*Average of four samples.

Table XIV. Percentages of Total Protein in Fully Expanded Green Leaves of Bean Plants, During Period of Development of Reproductive Structure Under Field Conditions.

Date of Sampling	1	2	3	4	5	6	7	8	9	10	11	12
July 6	27.78	29.91	30.00	28.16	30.14	30.23	30.95	28.53	30.46	----	28.9	30.16
9	26.62	28.71	28.80	29.57	29.58	27.69	26.60	26.35	28.28	29.74	28.60	28.34
12	28.29	28.44	29.78	26.73	29.65	----	26.98	28.57	29.60	28.70	29.30	28.15
15	28.22	28.47	28.47	29.42	26.98	26.58	28.70	28.08	30.30	----	30.40	28.70
18	30.56	29.66	34.40	30.56	30.56	27.69	31.14	30.07	32.02	27.60	31.20	30.00
21	30.97	30.74	33.16	31.05	----	31.05	30.23	30.88	32.20	28.50	32.60	31.63
24	29.59	29.91	32.60	31.14	31.54	22.15	32.35	28.93	29.45	27.00	34.90	30.60
27	29.32	27.66	30.69	30.40	26.32	21.44	30.88	24.37	28.60	25.00	31.10	29.70
30	24.20	----	27.79	26.08	25.84	20.46	29.09	23.23	30.34	24.00	28.64	29.80
Aug. 3	21.6	23.39	----	19.93	19.00	15.57	25.84	21.28	25.34	22.60	23.40	20.79
6	18.5	20.62	21.28	18.10	19.00	14.60	23.00	15.31	19.97	19.70	----	20.30
8	----	----	----	19.03	----	----	16.71	-----	19.15	----	----	----
14	----	----	----	19.12	----	----	16.68	-----	20.74	18.34	22.96	----

*Average of four samples.

While protein percentage was stable and relatively high during blossoming, nitrate reductase in general was at a low activity level. Also, the protein level started to decrease directly at or after maximum blossoming, while the nitrate reductase activity and nitrate level increased temporarily during the period of pod and seed growth and development in a fluctuating pattern. This may suggest more study in other steps of nitrate assimilation and protein synthesis in regard to yield components. This also may show that nitrate reductase activity and level of protein may be independent during reproductive stages. Since this conclusion is based upon fully-expanded leaves, only a study of rapidly expanding growing leaves may give some pertinent information.

Nitrate reductase activity and percentage of protein have the same behavior and pattern in young and old leaves as was shown previously in the study of their behavior in the tissues at different stages of development. This may suggest that nitrate reductase activity and total protein percentage are not completely independent in all stages. There was a continuous decrease in protein percentage after blossoming and rainfall seemed to have no effect on increasing protein percentage. This may indicate that soil moisture was adequate at sampling time.

Since, protein percentage continued to decrease during maturity stages while temperature fluctuated, the protein level may have been influenced by other major factor(s), in the later stages following the blossoming stage.

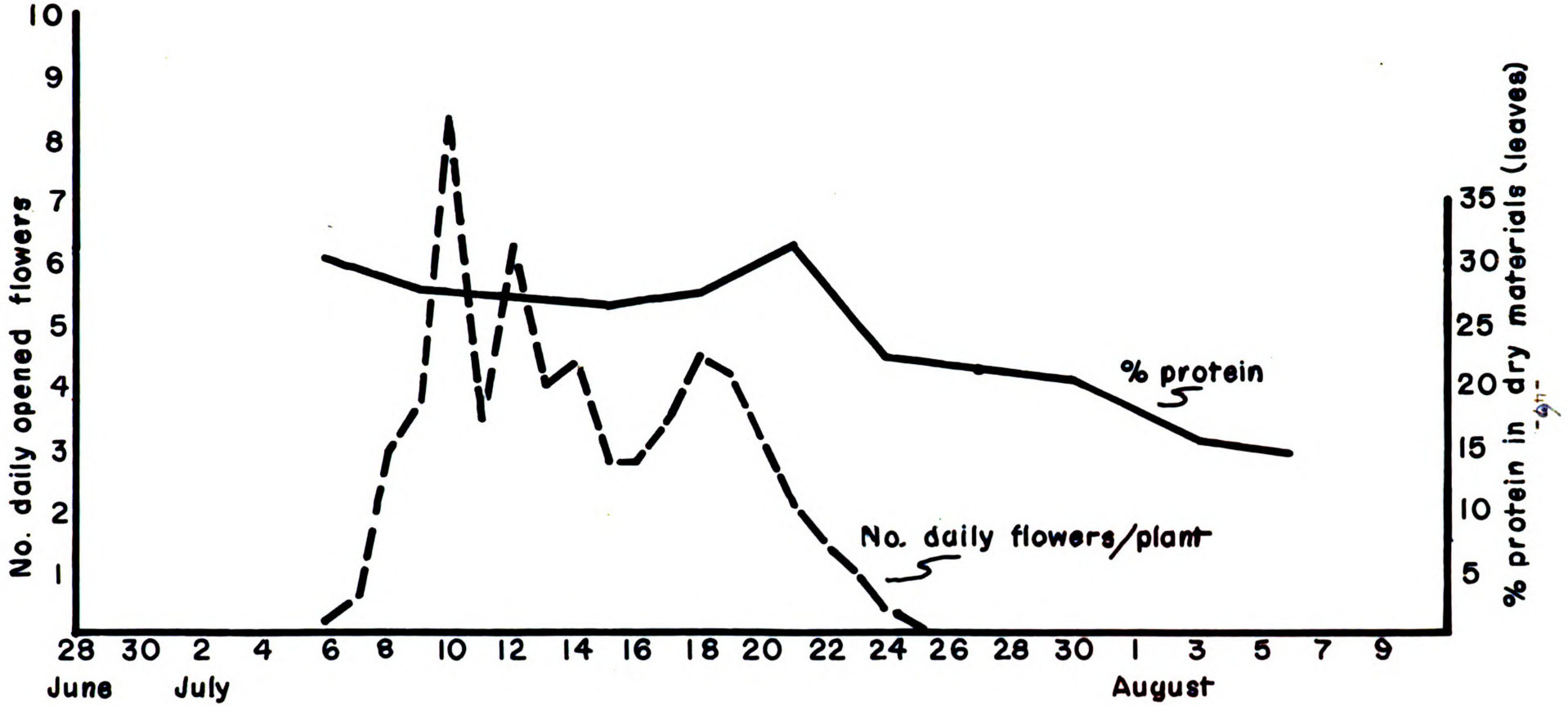

Figure VIII. Protein level during reproductive structure stages in leaves of bean line 6.

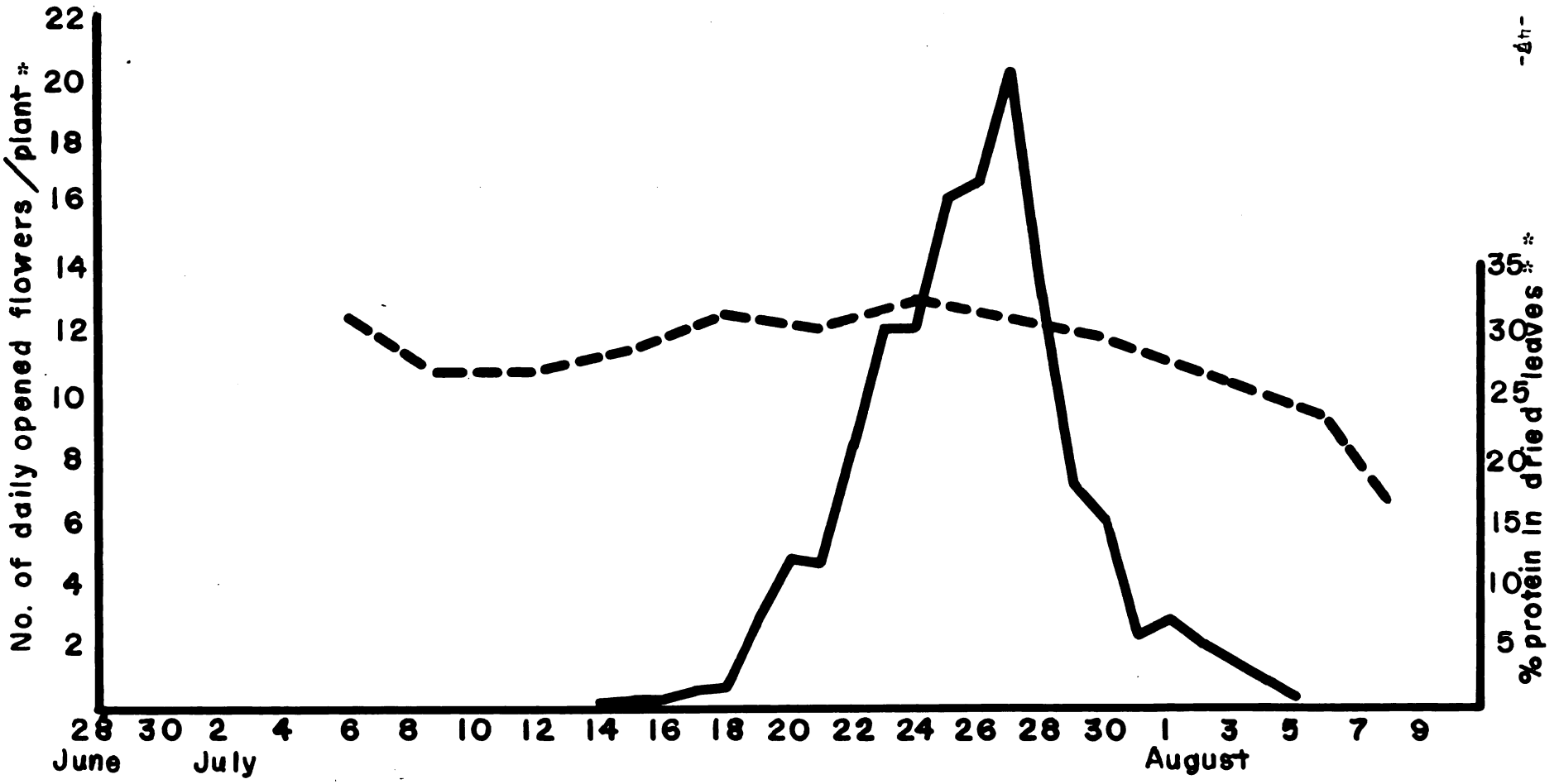

Figure IX. Percentage protein, and no. of daily opened flowers relationship in bean line 7.

*Average of 45 plants
**Average of four samples

Dry Weight of Plant Components as an Indication of Growth of Plant Parts During Seed Development and Maturity Stages

Two-foot samples were taken at intervals, total dry weight was recorded and plants were separated into: pods, leaves, stems, and roots. Tables XV, XVI, XVII and XVIII show this relationship during the period of sampling.

In general, total dry weight continuously increased and was greatly affected in the period between sampling on July 27th, and July 30 or August 3rd, with respect to different bean lines. This period was after blossoming and in early fruit and seed development and was associated with or immediately after the recovery in nitrate reductase activity. Protein level of leaves sharply decreased during this period of sampling. This may suggest that nitrate reductase and protein not only function in vegetative growth but also supply the seed with nutrient in its developmental course. In samples on August 6, and later stages there was a marked decrease in dry leaf-weight while dry weight of stems showed little decrease. During these later stages increase in total dry weight was associated similarly with increase in pod weight (Figure X).

Line ten that was green, even at maturity, showed a continuous increase in total dry weight of pods, leaves, stems, during the course up to maturity. It also showed a higher nitrate reductase level than any other line in later stages and less loss in protein level. However, this line has a very low number of pods per plant.

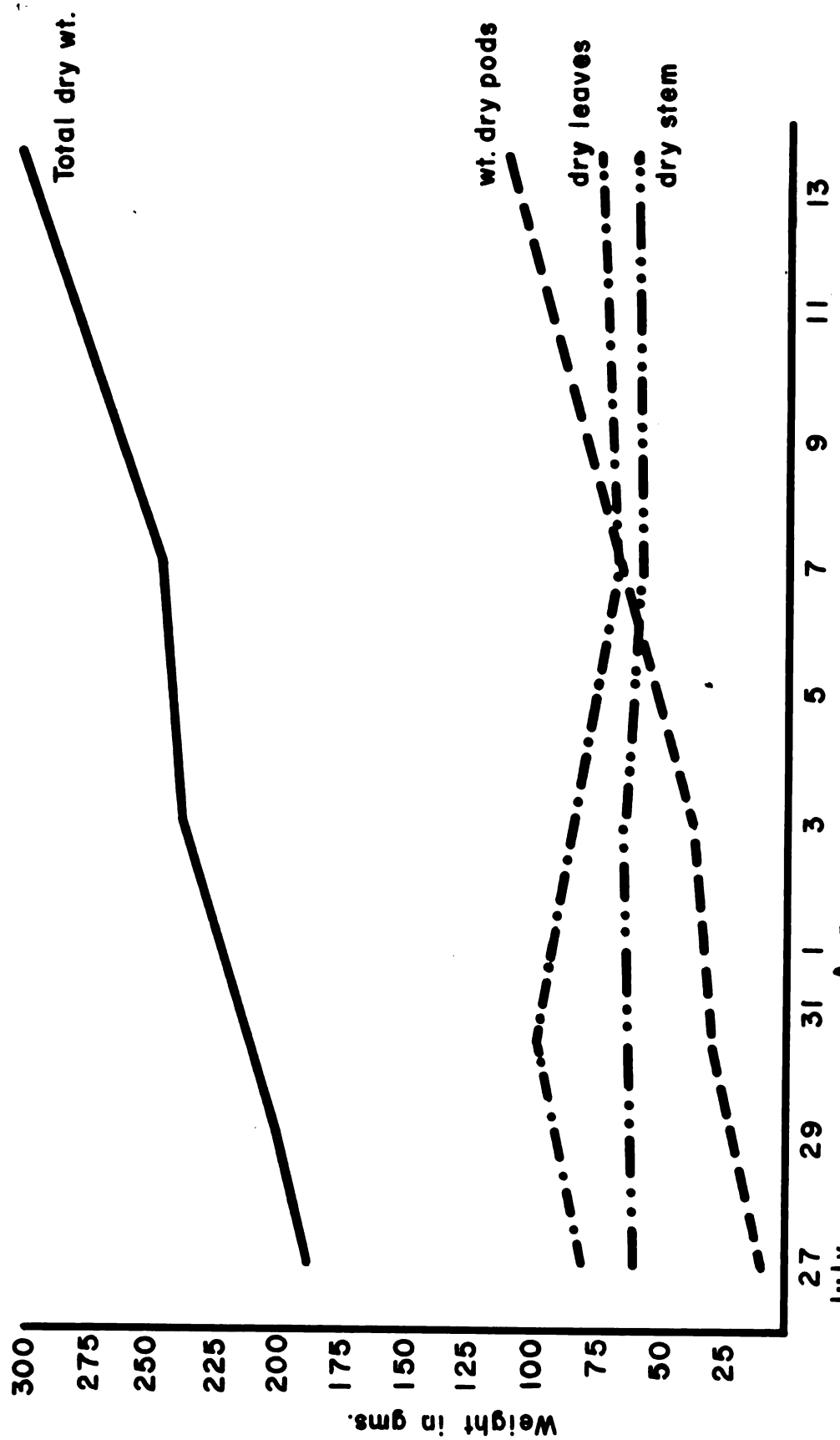

Figure X. Relationship between plant component during seed and fruit development in bean line 7, (average of three-two foot samples).

Table XV. Average Total Dry Weight of Three - Two Foot Samples of Bean Plants in Grams, at Several Dates.

No. of Line	Date of Sampling				
	July 27	July 30	Aug. 3	Aug. 7	Aug. 14
1	228.7	298.3	359.7	381.7	299.0
2	214.0	242.0	246.5	275.3	276.0
3	199.0	246.0	246.0	253.0	305.3
4	169.0	239.5	244.5	253.0	274.5
5	230.0	272.5	278.5	289.7	321.0
6	230.3	273.3	259.7	235.3	268.7
7	187.7	216.0	226.0	246.3	303.5
8	196.0	260.7	254.0	239.7	262.5
9	239.0	254.0	269.0	287.7	314.0
10	195.7	235.7	228.7	218.7	207.3
11	218.0	250.5	249.5	247.0	289.0
12	211.7	267.3	277.3	298.7	348.5

Table XVI. Average Dry Weight of Three - Two Foot Samples of Bean Dry Pods in Grams at Several Dates.

No. of Line	Time of Sampling				
	July 27	July 30	Aug. 3	Aug. 7	Aug. 14
1	44.0	71.7	113.3	119.7	136.5
2	62.7	78.5	92.5	141.7	142.0
3	48.0	57.3	84.0	116.3	141.0
4	46.0	55.3	83.5	102.3	126.5
5	52.7	66.5	91.5	143.3	157.5
6	70.7	100.7	133.7	132.7	145.3
7	8.7	21.3	39.7	64.7	112.5
8	45.3	52.7	104.0	114.3	136.5
9	25.0	59.0	67.0	112.7	122.0
10	14.3	17.3	20.7	29.3	41.0
11	25.7	56.0	63.0	90.7	130.5
12	56.0	62.3	83.7	109.0	169.0

Table XVII. Average Dry Weight of Three - Two Foot Samples of Bean Leaves in Grams, at Several Dates.

No. of Line	Date of Sampling				
	July 27	July 30	Aug. 3	Aug. 7	Aug. 14
1	69.0	80.0	88.3	62.3	57.3
2	57.5	64.0	69.0	50.3	45.0
3	66.3	74.3	76.0	53.7	64.3
4	52.5	75.5	70.5	56.7	45.5
5	79.0	79.0	48.5	59.0	56.5
6	56.0	62.0	69.9	32.0	40.0
7	79.7	98.3	85.0	66.0	75.5
8	59.0	62.3	68.7	47.7	37.0
9	83.0	91.0	89.7	69.3	71.0
10	85.0	92.7	100.7	88.3	105.0
11	72.0	81.3	86.5	62.7	56.0
12	66.3	66.7	87.0	76.0	67.5

Table XVIII. Average Dry Weight of Three - Two Foot Samples of Bean Stems in Grams, at Several Dates, in Summer 1964.

No. of Line \ Time	Date of Sampling				
	July 27	July 30	Aug. 3	Aug. 7	Aug. 14
1	59.67	55.0	63.00	60.67	59.50
2	49.50	50.00	50.50	46.33	45.00
3	51.00	47.33	53.33	47.00	53.33
4	46.00	58.33	55.50	54.67	53.00
5	56.00	60.00	65.00	55.67	57.00
6	43.67	42.67	47.33	35.00	37.33
7	60.00	63.00	64.67	58.67	61.50
8	46.33	44.67	50.00	40.67	42.50
9	67.33	60.67	65.67	59.33	60.00
10	60.00	61.33	71.67	64.67	76.00
11	62.00	58.33	67.00	58.67	53.00
12	52.67	49.67	64.67	75.00	60.00

Relationship of Blossoming, Newly Developed Pods and Mature Pods

These data were recorded on the marked forty-five plants in three replications. At harvesting time, two-foot samples were taken in two samples from each replication (duplicating). Data was taken on number of plants, number of pods, number of seeds, and seed weight.

Table XIX shows the relationship between total opened flowers and pod development stages throughout the maturity stage. The results show that there is a loss in number of newly developed pods from total opened flowers throughout the plant life cycle.

The proportion of loss in the developed pods from opened flowers range from 11.49% in line six to 41.97 in line seven. However, line six was the earliest in blossoming and maturity while line seven was the latest. It was noticed that many opened flowers drop after windy days or nights. The lines that have a longer blossoming period have a relatively higher loss, as well as those lines that flower heavily in a relatively short time. This is shown in line three and seven respectively. The determinate lines (bush type) 2, 4, 6, 8, 10, 12 have a low percentage loss in new pod numbers that developed from opened flowers, and the low ranges from 11.49% to 22.2%. In the indeterminate lines (vine type) 1, 3, 5, 7, 9, 11 have a high percentage loss in number of newly developed pods from opened flowers. The loss in this case ranges from 14.48% to 41.97%. Competition for nutrition between vegetative growth and development of reproductive structures in indeterminate lines may be involved.

The Table XIX shows also the percentage of losses in the number of mature pods from newly developed pods. The percentage was relatively high in all lines and ranged from 51.12% to 64.97%. It was observed during daily recording that most of the loss occurred in the later portion of newly developed pods from the latest blossoming, since the pods turned yellowish in color and stopped growth. Competition for nutrients among older pods developing seed and newly developed pods may be a possible cause. However, since some lines as line ten has a very low fruit load and a relatively high loss, other factors may be involved and need more specified studies.

Table XIX. The Relationship of Total Opened Flowers (blossoming), Newly Developed Pods and Mature Pods Per Plant of Bean Plants Grown Under Normal Field Conditions in Summer 1964.

	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8	Line 9	Line 10	Line 11	Line 12
Total opened flowers* and no. per plant during life cycle	69.04	84.49	90.09	93.38	86.20	59.96	130.42	89.97	82.85	17.17	80.70	77.56
Total No. of newly developed pods** per plant during life cycle	47.76	67.42	56.48	69.55	73.71	53.07	75.67	76.58	59.61	14.45	57.13	67.01
Total no. of harvested mature pods***per plant	22.18	25.86	24.07	25.15	30.01	25.94	26.52	28.82	20.88	6.47	23.35	32.05
%Total loss in opened flowers to develop newly pods	30.82	20.20	37.30	22.79	14.48	11.49	41.97	14.87	28.05	15.84	29.20	13.60
% Total loss in newly developed pods to reach maturity per plant	53.55	61.64	57.38	63.83	59.28	51.12	64.95	62.36	64.97	55.22	59.12	52.17

* and

**Average of 45 plants in 3 replications. ***Average of minimum 80 plants except in line 7 average of 60 plants, and in line 10, average of 50 plants. All cases had three replications.

Table XX. Average per day of recently (daily) opened flowers of the marked forty-five bean plants on each of the twelve lines.

Date of Counting	BEAN LINES					
	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
June 30		0.03				
July 1		0.07	0.13			
2		0.43	0.33			
3		0.53	0.30			
4	0.1	0.73	0.33			
5	0.15	0.67	0.23			
6	0.22	0.50	0.30			0.20
7	0.16	0.63	0.37			0.60
8	0.77	1.50	0.90		0.03	2.90
9	0.88	1.20	1.37	0.3	0.20	3.67
10	1.40	1.70	1.30	0.63	0.53	8.23
11	1.94	2.30	3.17	1.66	1.27	3.47
12	2.54	4.47	3.60	1.33	1.90	6.23
13	2.40	3.13	3.57	1.53	2.13	4.01
14	2.53	3.83	3.80	3.13	2.19	4.40
15	3.00	5.10	3.90	4.40	3.53	2.82
16	3.77	5.70	4.93	5.50	4.90	2.83
17	4.60	6.37	5.57	7.03	6.60	3.50
18	4.40	5.80	4.67	5.93	6.90	4.57
19	4.93	6.93	7.67	7.30	10.23	4.20
20	5.80	7.57	6.97	6.77	9.20	3.10
21	6.20	5.20	7.10	6.47	9.00	2.10
22	4.67	6.97	6.63	8.07	9.00	1.50
23	5.63	6.50	6.70	11.77	8.43	0.97
24	3.93	3.33	3.50	8.47	3.83	0.47
25	2.50	1.63	1.77	5.67	2.67	0.12
26	2.07	1.17	1.67	4.07	2.23	0.03
27	1.40	0.37	1.40	1.60	1.00	0.03
28	1.10	0.10	0.17	0.60	0.33	
29	0.89	0.03	0.07	0.67	0.10	
30	0.40			0.33		
31	0.43			0.12		
Aug. 1	0.15			0.03		
2	0.06					
3	0.02					
4						
5						

Table XX. Continued

Date of Counting	BEAN LINES Line 7	Line 8	Line 9	Line 10	Line 11	Line 12
June 30						
July 1					0.03	
2					0.07	
3					0.10	
4					0.13	0.03
5			0.03		0.10	0.06
6			0.03		0.13	0.13
7			0.13		0.33	0.30
8		0.03	0.43		0.67	1.77
9		0.13	0.47		0.40	2.70
10		0.20	0.83	0.03	0.90	4.57
11		0.90	1.47	0.03	0.97	2.83
12		3.33	1.50	0.63	1.70	3.90
13		3.80	1.23	0.73	1.47	2.83
14	0.03	4.73	2.50	1.70	3.00	2.80
15	0.13	6.57	3.20	2.53	3.30	2.67
16	0.27	6.67	3.53	2.07	5.33	3.50
17	0.70	6.43	3.63	1.67	5.43	4.27
18	0.59	6.40	4.90	1.60	5.80	4.10
19	2.87	8.23	4.63	1.10	6.23	5.07
20	4.60	8.07	4.87	0.67	5.37	4.83
21	4.57	8.30	5.63	0.43	5.37	3.10
22	7.10	7.37	6.60	0.53	6.90	3.87
23	12.00	9.47	8.60	0.67	7.47	5.33
24	11.87	4.47	6.53	0.56	5.47	4.93
25	16.10	2.00	6.87	0.50	4.07	4.23
26	16.63	1.60	5.70	0.37	3.93	4.57
27	20.30	0.93	5.07	0.26	2.30	2.90
28	13.03	0.27	2.47	0.33	1.13	1.10
29	6.90	0.07	1.20	0.30	0.87	0.63
30	3.93		0.30	0.20	0.57	0.37
31	2.17		0.33	0.10	0.33	0.17
Aug. 1	2.73		0.17	0.10	0.23	
2	2.00			0.03	0.50	
3	1.40			0.00	0.10	
4	0.37			0.03		
5	0.13					

Table XXI. Average per day of newly developed pods of bean plants on each of the twelve lines in the field average of forty-five plants.

Date	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
July 4		0.30				
5		0.17				
6		0.33	0.20			
7	0.10	0.20	0.15			
8	0.12	0.33	0.20			.1
9	0.11	0.20	0.20			.60
10	0.23	0.30	0.20	0.59		.9
11	0.43	1.04	0.30	0.9	.40	2.0
12	0.66	3.03	0.80	0.79	.70	3.0
13	0.53	2.00	1.20	1.81	1.40	5.5
14	0.95	2.50	1.90	2.00	1.7	6.3
15	1.68	3.76	3.50	2.44	1.4	6.07
16	2.20	3.50	3.80	4.66	1.94	4.5
17	2.27	3.24	3.90	4.00	2.1	2.9
18	2.86	4.9	3.50	5.00	4.0	2.43
19	2.76	4.20	4.00	4.20	3.3	3.60
20	4.00	5.51	5.00	4.00	6.84	4.5
21	4.28	6.00	5.60	4.00	10.0	4.10
22	3.24	5.59	6.40	5.09	9.0	2.5
23	6.75	6.81	6.00	6.91	8.86	2.5
24	3.00	7.00	4.00	4.20	6.14	1.3
25	3.17	4.00	2.50	5.26	4.63	0.96
26	2.51	1.50	1.7	8.00	3.3	.19
27	1.77	0.22	1.0	2.90	2.5	.1
28	0.90	0.28	0.3	2.00	2.0	.02
29	0.64	0.40	0.1	.50	2.0	
30	0.96	0.10	0.3	.20	1.10	
31	0.81	0.01		.1	0.3	
Aug. 1	0.39				.10	
2	0.34					
3	0.26					
4	0.10					
5						

Table XXI. Continued

Date	Line 7	Line 8	Line 9	Line 10	Line 11	Line 12
July 4						
5						
6						
7						0.08
8			0.06			0.14
9			0.04			0.32
10		0.08	0.12			1.81
11		0.17	0.38			2.47
12		0.23	0.43			3.40
13		1.07	0.80	0.03		3.07
14		3.04	1.40	0.20	0.05	2.50
15		3.37	1.37	0.62	1.40	3.43
16		4.43	1.23	1.38	2.33	2.67
17	0.20	4.74	2.10	2.00	2.27	1.90
18	0.60	6.30	2.63	1.50	3.80	3.50
19	1.04	6.93	3.37	1.49	5.69	4.49
20	1.5	7.17	4.00	1.34	4.21	4.11
21	1.9	6.16	4.54	1.17	6.30	5.00
22	6.7	8.00	4.46	0.86	5.15	5.10
23	4.8	6.00	4.90	0.54	5.85	5.03
24	6.00	5.00	5.00	0.50	5.60	4.50
25	9.26	5.63	4.70	0.43	4.49	4.79
26	7.85	3.77	3.80	0.40	3.19	3.21
27	8.87	2.20	3.30	0.47	2.1	2.00
28	9.0	1.15	4.70	0.53	1.5	1.50
29	4.4	0.94	3.06	0.33	1.47	0.91
30	3.57	0.20	2.00	0.20	0.90	0.59
31	3.93	0.01	0.9	0.20	0.40	0.19
Aug. 1	3.07		0.30	0.13	0.30	0.15
2	1.5		0.02	0.10	0.10	0.10
3	0.9			0.03	0.03	0.05
4	0.28					
5	0.10					

Table XXII. Hemispheric solar radiation on a horizontal surface, during period of sampling, expressed in Langleys (gm. cal./cm^2).

Date of reading	Daily total	Eight hr. period every day, from 5 a.m. to noon (Total 8 hours)	Average per hour during the eight hour period
July 1	584.9	342.5	42.81
2	439.2	215.3	26.91
3	473.3	296.4	37.05
4	807.7	483.4	60.42
5	783.3	479.7	59.96
6	354.0	391.9	48.98
7	393.2	367.3	45.91
8	252.0	122.1	15.26
9	591.5	317.7	39.71
10	730.4	445.7	55.71
11	533.4	401.3	50.16
12	338.3	231.4	29.05
13	220.9	158.0	19.75
14	424.5	222.0	27.75
15	601.3	369.9	46.23
16	666.5	402.2	50.27
17	708.5	428.9	53.61
18	667.5	387.7	48.46
19	701.1	424.8	53.1
20	448.9	367.7	45.96
21	510.3	272.1	34.36
22	704.8	434.9	54.36
23	715.1	433.2	54.15
24	667.4	412.2	51.52
25	452.7	322.7	40.33
26	717.5	429.7	53.71
27	699.3	440.0	55.00
28	637.7	411.7	51.46
29	718.5	440.0	55.00
30	592.5	421.4	52.67
31	499.2	291.7	36.46
Aug. 1	405.4	176.6	22.07
2	631.0	367.5	45.93
3	584.0	339.8	48.47
4	543.1	349.2	43.65
5	663.2	391.6	48.95
6	657.0	447.5	55.93
7	593.5	358.0	44.75
8	580.0	347.2	43.4
9	672.0	443.3	55.41
10	383.1	190.0	27.27
11	182.3	25.3	3.16
12	221.0	148.5	18.56
13	422.3	286.0	35.75
14	648.1	405.9	50.73
15	590.8	394.6	44.32

Table XXIII. Precipitation during June-August in inches at M.S.U. Farm.

	June	July	August
1			
2		.08	
3	.09		.60
4			
5			
6			
7	.09		
8	T*	1.13	
9			
10	.39		
11			T*
12			.71
13		.15	
14		.50	.05
15	1.15		
16			
17			
18	.36		
19			
20			.34
21		.15	1.00
22		.11	.760
23	.09		.29
24	T*		
25			
26			
27			
28			T*
29			.45
30			
31			

T* - means trace amount of rainfall

DISCUSSION AND CONCLUSION

The total activity of nitrate reductase in bean plants is determined by several factors. These factors are cell age and developmental stage, light period and intensity, temperature, nitrate concentration in plant tissue and development of reproductive structure.

The distribution of the enzyme activity varies greatly with the type of differentiated tissue in the plant parts. The leaves at different stages of development have higher activity than does the stem, and petioles, while the roots show no activity.

The pronounced peak in activity was associated with rapidly expanding leaves but was not as great with mature or senescent leaves of the normal plants. These results agreed with maximum activity in soybean leaves obtained by Evans and Nason (22) and are in contrast to maximum activity associated with mature leaves of cauliflower obtained by Candela, et al. (11).

This kind of distribution of enzyme activity was independent of the type of plant growth whether it is determinate (bush) or indeterminate (vine) growth type.

There was a continuous decrease or loss in activity with increasing age of the plant even in the absence of cell growth. No adequate explanation can be given for this continuous loss in activity with age and cell development. However, this may be due to spontaneous inactivation of the enzyme in the absence of a substrate or metabolic destruction since decrease in activity occurs in the leaves at different stages of the same plant at the same time. Furthermore, the rapid decrease or loss

in enzyme activity from early stages of primary and rapidly expanding leaves to young fully-expanded leaves may be due to the induced enzyme being diluted out as the cell grows. The rate of reversion depends on the speed of leaf growth in this case.

The undetectable or trace amount of enzyme activity in all senescent leaves, besides the above possibilities, may suggest that chlorophyll may have some effect upon the activity of the enzyme.

The results from laboratory experiments show that increasing the period of light and light intensity produce an increase in enzyme activity. The activity varies greatly during pod and seed development stages. The peak of enzyme activity was associated with the full size green pod wall while there is no detectable activity in early post-fertilization stage of pod development. However, activity increases as the pod and seed grow to full size. Enzyme activity declines rapidly as the pod matures and turns yellowish. This may also suggest that chlorophyll has a relation to the enzyme activity. Furthermore, the loss in activity may be due to the first two possibilities given under enzyme activity in leaves.

The young flower buds always have higher enzyme activity than at blossoming stages. Therefore, the enzyme activity in bean plants is unstable and showed wide variation during period of sampling.

The patterns of distribution of the total extractable leaf protein and nitrate reductase activity are relatively independent in some stages of plant growth, although in aging plants total activity fell as leaf protein decreased. These results coincide with those obtained in cauliflower by Candela, et al. (11). Temperature to which plants were exposed in the field

varied considerably during the period of sampling. The activity was relatively high during period of low temperature. These results agreed with our laboratory work which show higher activity under low temperature (20°C) and saturated light intensity (2200 f.c.) than those under 30°C. and 500 f.c. treatment. The effect of light on nitrate reduction in wheat leaves was known by Burström (10), who concluded that nitrate reduction was directly linked to a photochemical reaction of that it occurred concomitantly with the fixation and reduction of carbon dioxide. In contrast, Delwiche (16) demonstrated that tobacco plants metabolize nitrate or ammonia in dark as well as in the light. The activity of the enzyme in general seemed to follow three characteristic trends throughout the period of sampling: (a) great fluctuation in late vegetative stage and flower bud development but still relatively higher levels of activity than at later stages, (b) a general decrease during the blossoming period, (c) a temporary recovery in activity after blossoming and during fruit and seed development. This pattern of fluctuation was in general associated with nitrate (NO_3^-) levels in the sampled materials. Lower activity was associated with the lowest level in nitrate as shown in samples on July 18, or 21 or 24 with respect to different bean lines.

Concentration of nitrate (NO_3^-) seemed to fluctuate independently of nitrate reductase activity in some sampling dates, but to a pronounced effect on enzyme activity when the nitrate level was low.

Protein content, as determined by colormetric methods, fluctuated throughout the sampling period but to a less extent in comparison to nitrate reductase.

In general, all lines showed significant decreases in level of total protein in samples after the peak of blossoming, while nitrate content and

nitrate reductase activity increased during pod growth and seed development. Flowering phases were usually characterized by subsidence of anabolic activity (35) and redistribution of organic and inorganic nutrient components (4, 12, 17). This early period of fruit and seed development was associated with increased vegetative growth as shown in Tables XV, XVI, XVII and XVIII in the period from July 27 to August 3. This period was followed by a decline in dry leaf, stem, and total dry weight as shown in samples on August 6 to 14.

The decline of anabolic stimulus associated with blossoming gradually gives way to what is usually the final resurgence of absorption of mineral nutrients and acceleration of organic synthesis in vegetative tissues. This anabolic stimulus is associated with the fusion of male and female nuclei and very early enlargement of young fruits was also reported by several workers (40, 42, 43, 72). In general, blossoming was associated with high protein content and very low nitrate content and nitrate reductase activity. This may indicate that nitrate reductase is not the limiting factor in protein synthesis at least in some stages of development. The reduction in nitrate content and nitrate reductase activity may be explained on the basis of the findings of other workers (4, 17, 64). They found that the onset of blossoming or anthesis is marked by an apparently simultaneous reduction in absorption by roots and an internal shift in water balance. Immediately after blossming, and during early enlargement of young fruits and seed development there was a continuous decline in total protein in leaves even when both nitrate and nitrate reductase activity recovered after blossoming. However, it should be mentioned that external factors may have great effects since lowest activity occured in samples on July 21 and 24. More study is needed of effect from adding

nitrate at blossoming on enzyme activity. The continuous decline in total protein levels during early enlargement of young fruit and seed development suggests that bean leaves play a major role in supplying reduced nitrogen and seed growth and development. More study is needed of protein pattern and behavior in stem and other plant parts during the development of reproductive structures. The rate of protein decline seemed to be associated with rate of maturity. The early maturing line six showed a sharp decline in protein content while the late maturing line seven, showed less in decline.

The proportion of loss in the developed pods from opened flowers range from 11.49% in line six to 41.97% in line seven. The lines that have a longer blossoming period have relatively higher loss, as well as those lines that flower heavily in a relatively short time. This was shown in line three and seven respectively. The determinate lines (bush type) have a low percentage loss in new pod numbers that developed from opened flowers, and showed the low ranges from 11.49 to 22.2% loss. The indeterminate lines (vine types) have a high percentage loss in number of newly developed pods from opened flowers. The loss in this case ranges from 14.48% to 41.97%. This big loss and failure in number of flowers and developed pods to reach maturity may suggest an internal modification in beans for yield component. The proportion of losses in the newly developed pods to reach maturity was relatively higher than losses in opened flowers to developed pods. The proportion in losses ranged from 51.12% to 64.97%. Therefore, the known yield component expressed in number of pods per plant, number of seeds per pod and seed weight is not a simple relation. These losses in flowers and pods may be in part due to the effect of wind and other physical factors. More study is needed to find more factors that cause variation in reproductive structures and yield of seed. This finally may yield the primary cause (s) of negative relations among yield components of beans.

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