EFFECTS OF LIGHT AND TEMPERATURE ON THE NITROGEN METABOLISM OF TROPICAL RICE

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
MANLIO SILVESTRE FERNANDES
1974

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ABSTRACT

EFFECTS OF LIGHT AND TEMPERATURE ON THE NITROGEN METABOLISM OF TROPICAL RICE

By

Manlio Silvestre Fernandes

An experiment was conducted in a controlled environment (growth chambers) in order to study the effects of light, temperature, N rates, and N carriers on the metabolism of Come-Cru (C-C) a brazilian rice, and of IR-8.

Two levels of temperature (35°C and 24°C) and two levels of light supply (3,700 foot candles and 1,600 foot candles) were used in three combinations (high light-high temperature; low light-high temperature; low light-low temperature). To each of the three combinations, N was applied either as ammonium (NH_4^+) or as nitrate (NO_3^-) at 5 ppm, 20 ppm or 150 ppm rates.

Two week old rice plants were used and the plants were kept in the nutrient solution under the experimental conditions for a period of 10 days. After the 10 day period the plants were harvested and the shoots were weighed, extracted with ethanol, and determinations were made of the contents of free amino-N, ammonia (NH_3) N, NO_3^--N, non-structural sugars, and the total N of the oven-dry residue. Quantitative

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determinations of free amino acids were made on plants diverging widely in the content of the factors mentioned above.

The results showed a physiological superiority of the IR-8 over the C-C plants for the utilization of N.

The mechanisms for the metabolic regulation of the levels of reduced N in the tissues of the experimental plants seemed to differ in NO_3^- fed plants and NH_4^+ fed plants. In the NH_4^+ fed plants the mechanism seems to reside in the tying up of the reduced forms of inorganic N into organic forms, while in the NO_3^- fed plants the excess NO_3^- taken up seems to be taken off the metabolic pool, so that the nitrate reductase (NR) activity is kept down (as deduced from the contents of reduced N in the tissues) despite the accumulation of NO_3^- in the plants. The term "NO_3^- sequestration" is used to describe this mechanism, and it is concluded that photosynthetic efficiency is the prime factor in NO_3^- utilization by rice plants.

The data indicate that under conditions of low light and low temperature the plants studied reacted positively to N applications, either as NO_3^- or as NH_4^+, while conditions of high light and/or high temperature cause marked disturbances in the growth of plants. The data also show that under stress conditions caused by light, temperature, and N rate, NH_4^+ applied as fertilizer is far more harmful to plants at any rate of application than NO_3^-. It is suggested that NO_3^- is a better form of N fertilizer for the upland crop system of the humid tropics than is NH_4^+.

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It is also suggested that a knowledge of the fluxes of energy in the environment plays a fundamental role in the choice of when and how to apply N fertilizers to crops.

EFFECTS OF LIGHT AND TEMPERATURE ON THE NITROGEN METABOLISM OF TROPICAL RICE

By

Manlio Silvestre Fernandes

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1974

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ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major professor, Dr. B.D. Knezek, for his guidance and continuing interest during the course of my studies, and to Dr. A.R. Wolcott for his assistance with the preparation of this thesis.

Thanks are also due to Drs. D. Penner and E.C. Doll for their helpful discussions and suggestions, and to Dr. W.G. Bergen for his invaluable help with the amino acid analysis.

I would also like to express my gratitude to Drs. E.A. Erickson and D. White for allowing me to use their equipment, and to Dr. D. Christenson for his help with the statistical analysis.

I wish to extend my thanks to Mrs. Betsy Bricker for her assistance throughout this work, and for her patience in reading the manuscript of this thesis.

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INTRODUCTION

Rice (<u>Oriza sativa</u>) is grown in Asia mostly in "paddy" fields or lowlands, while in South America the upland or "dry land" system of culture is the most common. Brazil maintains approximately 80% of its rice acreage as upland rice and it is the largest producer of this cereal outside Asia.

Rice plants are unusual in their ability to grown under conditions of low oxygen supply to the root system and they are able to translocate oxygen from the shoots to the roots.

The plants have a rather low light saturation point, around 40 to 50 Kilolux which is equivalent to about half of the intensity of full sunlight. The photosynthetic ability of the rice plant (indica) is not affected by an increase in temperature in a range of 25°C to 35°C. Photosynthetic levels are, however, closely related to light supply and to the protein content of the leaves. Though Pmax (maximum photosynthetic activity) has been reported to be quite low for some varieties, averages of 40 mg dm^{-2} hr^{-1} of CO_2 fixation have been found under conditions of good light supply. This supply is much larger than those usually reported.

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shown to increase with an increase in the Leaf Area Index (LAI) and 5 to 6 was shown to be the otpimum LAI when N supply is good. Although the application of N can increase photosynthetic rates it also tends to reduce starch accumulation in rice plants and increases the loss of carbon through respiration. It was observed that the best rate of photosynthesis was obtained when N was fed to the plants at a 20 ppm rate.

In this experiment, we study the effect of different rates of N, supplied either as NO_3^- or as NH_4^+ to rice plants under the different levels of photosynthesis and respiration induced by the different combinations of light supply and temperature.

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LITERATURE REVIEW

The growing season in the tropics

Climatic conditions in the tropics are limited to a dry and a rainy season. Rice production occurs from January to June which is the rainy season while crops are not produced during the dry season (July to December).

Meterological data for the area of Belem in northern Brazil (01°28' S - 48°27' W) shows an average light energy of 0.50 cal/cm^2/min., and for the dry period 1.3, cal/cm^2/min., (Bastos and Sa, 1972).

The average temperatures at the city of Sao Luis (02°32' S - 45°16' W) and for the city of Barra do Corda (05°30' S - 45°16' W) are 26.7°C and 26°C, respectively. On the average, the maximum temperature for the Sao Luis area is 34.3°C and for the area of Barra do Corda it is 37.9°C (Ministerio da Agricultura, 1970).

The combination of rainfall and hours of insolation (Figure 1) shows the growing season as a combination of low light supply and high rainfall. The data on the maxima either of radiation or temperature show that periods of high light intensity or high temperature may occur during the rainy season.

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grown can be characterized meteorologically as having one growing (rainy) season per year with a favorable average temperature (26°C), low radiation (0.5 cal/cm^2/min) and high rainfall (1,952.8 mm). The data also indicate the possibility of periods of high light supply (1.43 cal/cm^2/min and high temperatures (34.3°C) during this season.

Nitrogen in tropical soils

The content of organic matter and N in tropical soils is thought to be very low (Jenny, 1941). However, Vine (1954) has challenged this pessimistic view as related to soil fertility in Africa and Birch and Friend (1956) reported higher levels of organic matter and N in the tropical soils of East Africa. These authors observed that the dark color of the soil was not related to organic matter content in the tropics as is the case in the temperate regions.

The existence of a seasonal "nitrate flush" in tropical soils has been reported by many workers (Greenland, 1958) and was related to several causes. Birch (1958) relates this flush to the youth physiological phase of microbial activity after a drying and wetting cycle. The solubilization of organic matter and the mineralization of N at the surface of the colloids increased as the length of time the soil was kept in a dry condition increased (Birch, 1959). Temperature of soils had an influence on the mineralization of N and high levels of mineral N were produced depending on the C content of the soils (Birch, 1960). Besides the biological activity,

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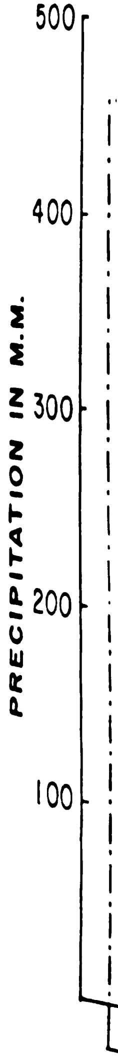
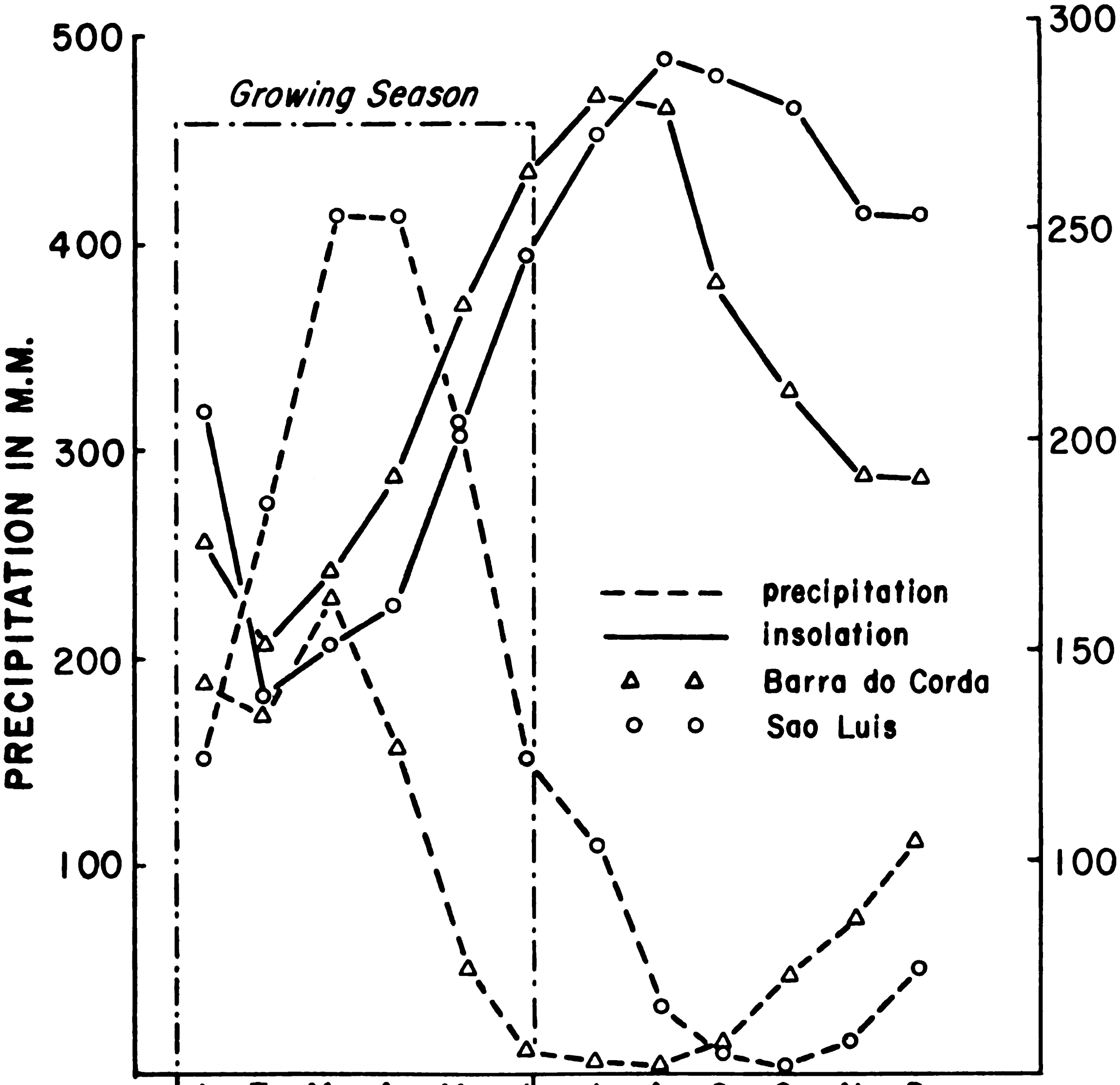
Wetselaar (1960) demonstrated the migration toward the surface, and the accumulation there, of NO_3^- during the dry season in the tropics. Levels of NO_3^- ranged from 38 ppm to 229 ppm in the upper inch of soil. Using Cl^- as well as NO_3^- in his studies, Wetselaar (1961, 1962) was able to show the migration and accumulation of both anions at the surface of the soil. Besides the physical factors, some biological production of NO_3^- was also observed. Water deficit was shown to be responsible for the lack of nitrification in the upper layers of these soils and rainfall was responsible for the removal of NO_3^- from the soil surface. Similar results were reported by Robinson and Gacoka (1962) for the Kikuyu red loam coffee soil. These results show that under the conditions of tropical climates, especially where there are two seasons with a marked period of dryness, NO_3^- accumulates at the surface upon moistening. This accumulated NO_3^- and the NO_3^- produced by biological activity produces the "flush" of NO_3^- with NO_3^- levels rising as high as 300 ppm in the soil solution. With the rains the NO_3^- is leached into the soil profile so that the levels of NO_3^--N are kept low throughout most of the growing season. The presence of NH_4^+-N under conditions of good drainage is very small. However, the NH_4^+ content can increase if excess water creates a reducing environment in the soils.

Plant uptake of N

Rice plants are able to take up N from the soil solution either as NH_4^+ or as NO_3^-. The process of uptake is energy

Figure 1. Meteorological data (precipitation and insolation) in two locations in the humid tropics: Sao Luis (02°32' S, 44°17' W) and Barra do Corda (05°30' S, 45°16' W).

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dependent for both ionic forms (Epstein, 1972; Hodges, 1973) and the uptake of NO_3^- has been reported to be enhanced by light (Beevers, et al., 1965). The uptake of NO_3^- and NH_4^+ is influenced by the pH of the nutrient solution and NO_3^- is taken up at a faster rate when the pH of the bathing solution is low. The opposite is true for NH_4^+. Fried, Zsoldos, Vose and Shatoklin (1965) using ^{15}N either as NO_3^- or as NH_4^+, observed a greater uptake of NH_4^+ by rice roots as the pH increased with the highest uptake at pH 8.5. The reverse was observed for NO_3^- where the higher uptake occurred at pH 4.0. However, at each pH level, NH_4^+ uptake was always higher than the NO_3^- uptake. For instance, at the intermediate pH of 5.5, the rice roots took up 300 ug N/g dry weight when supplied with NH_4^+ but only 68 ug N/g dry weight when NO_3^--N was used. Even at pH 4.0, where NO_3^- uptake was greatest, rice plants took up 5 to 10 times more N as NH_4^+ than as NO_3^-. In this same work it was shown that NH_4^+ can inhibit the uptake of NO_3^- whereas NO_3^-, even at high concentrations, did not inhibit the uptake of NH_4^+. In apple roots Frith (1972) observed that when NH_4^+ was present in the nutrient solution in amounts as low as 14 ppm and NO_3^- was present in the same solution in amounts as high as 98 ppm, nitrate reductase (NR) activity was reduced by half. When 56 ppm of each form of N was used in the nutrient solution, NR activity was reduced to one-sixth of that for apple roots grown in NO_3^- alone. Pate (1968) has shown that NO_3^- usually does not accumulate in roots, but moves up to the shoots.

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Plant assimilation of N

Plants do not assimilate N in a high oxidation state. So, the NO_3^--N has to be reduced to the NH_3 form before it can be incorporated into organic compounds. The reduction of NO_3^- in plants is catalyzed by a group of enzymes of which NR is involved in the first step. Nitrate reductase (NR) can be induced by the presence of NO_3^- (Bandurski, 1965). The induction of NR by NO_3^- requires the presence of Mo and is enhanced by light and reduced in the dark (Hageman and Flesher, 1960).

The increase in the induction of NR activity by NO_3^- was also demonstrated by Beevers, et al. (1965) in excised corn seedlings. The increase caused by light in this experiment (4-fold) was thought to be indirect and caused by an increase in NO_3^- uptake. George, Rhykerd and Noller (1971) found an accumulation of NO_3^- in grasses with increasing temperatures and low light intensity. Beevers et al.(1965) have also shown a temperature dependent increase in NR activity. At 38°C and in the presence of NO_3^-, NR activity increased in the same pattern as shown for light. From these investigations it can be concluded that both light and temperature are able to independently increase NR activity and that the effect on the enzyme activity is indirect and due to an increase in the uptake of NO_3^-.

Zielker and Filner (1971) demonstrated that induction of NR activity by NO_3^- in cultured tobacco cells was through *de novo* synthesis, and there was a rapid degradation and resynthesis of the enzyme. Heimer and Filner (1971) used tungstate to create a non-functional NR, and they were able to isolate the NO_3^- uptake system from the NR system. The tobacco XD cells used in that experiment accumulated 80 times the external concentration of NO_3^-. The NO_3^- uptake was shown to be subject to end product regulation by amino acids.

Nitrate reductase activity was reduced in the absence of an external supply of NO_3^- even with high cellular levels of NO_3^- (25 uMoles/g fresh weight), indicating the existence of distinct pools in the plants. The authors proposed the existence of an *inducing*, short-lived NO_3^- pool and an inactive, long-lived *substrate* pool. The plants could use the available NO_3^- for growth but the substrate pool could not replenish the inducing pool. In a more recent investigation Ferrari, Yoder and Filner (1973) demonstrated the existence of the two NO_3^- pools and were able to produce a leak from the non-metabolic to the metabolic pool using alcohols, dinitrophenol (DNP), and pyrazole.

Feedback inhibition mechanisms for NR have been suggested in *Candida fusca* by Losada et al., (1970) and in *Candida reinhardi* by Herrera et al., (1972). The inhibition in both cases was caused by NH_3. Ingle et al. (1966) found some repression of NR syntehsis by glutamate and asparagine, and Afridi and Hewitt (1962) reported that some sulfur amino acids markedly inhibited NR-activity.

Miflin (1967) proposed that both NO_3^- and NO_2^- reductases were particulate enzymes. In a later paper Miflin (1970 found that they were not associated with mitochondria. Since the synthesis of NO_3^- and NO_2^- reductases are regulated in different ways in higher plants, it seems that they are not located together in a "nitrosome" as proposed by Sims, Folkes and Bussey (1968) for yeasts. Syrett and Hipkin (1973), working with N-starved cells of algae, demonstrated the existence of NR as the constitutive enzyme in these organisms. The synthesis of the endogenous NR was repressed by the presence of NH_3.

Although NO_3^- is by far the most common form of N available to higher plants, they are also able to utilize NH_4^+-N. Some plants have been called "NH_4^+ plants" and they were thought to be unable to use NO_3^-, at least in some stages of growth (Bonner, 1946). Rice is perhaps the best known of the so-called NH_4^+ plants (Zsoldos, 1957). However, Tang and Wu (1957) were able to demonstrate the induction of NR in rice plants supplied with NO_3^- and Shen (1969) demonstrated the induction of NR in rice seedlings with the induction beginning 24 hours after germination. He observed the inhibition of NO_3^- reduction due to the presence of NH_4^+ but not the inhibition of NO_3^- uptake.

After NO_3^- is reduced to NH_3 or reduced N is taken up by plants, assimilation continues and glutamate is the route through which reduced inorganic N enters into organic compounds (Stadman, 1968). Direct reductive amination of pyruvate has also been proposed by Kretovich and Kasperek (1961) as a detoxification mechanism when plants are loaded with excessive amounts of NH_3. Other situations have also been

suggested under which alanine dehydrogenase activity can be enhanced (Tomkins and Yielding, 1961; Frieden, 1963). The enzyme responsible for the aminating (or deaminating) reaction is glutamate dehydrogenase (L-glutamate: DPN(oxidoreductase, EC 1.4.1.3.), GDH, which is in a polymeric association in animal and plant tissues (Frieden, 1963). The polymer has a molecular weight of about 1,000,000 and can be dissociated in monomers of molecular weight, 350,000. Aggregation was not necessary for the catalytic activity, but there are some metabolites like GTP that can induce the monomers to act as alanine dehydrogenase (Tomkins and Yielding, 1961; Frieden, 1963). The reaction has an equilibrium constant in favor of synthesis (Tomkins and Yielding, 1961; Frieden, 1963), but the Km for NH_3 was found to be quite low in beef liver (0.056 M).

In plants, only DPN-specific GDH was found (Frieden, 1965). A similar result was obtained by Thurmann et al. (1965) when they demonstrated the isoenzymic nature of GDH in higher plants. Working with *Vicia faba* and *Pisum sativum*, they were able to identify 6 to 7 isoenzymes in seeds, dormant shoots, and roots. As the plants aged the number of isoenzymes was reduced, until at a growth stage of 21 days only one of the isoenzymes was present as a major fraction. Also it appeared that each plant had its own isoenzyme pattern. Hartmann et al. (1973) found 7 isoenzymes to be present in the seeds of *Medicago sativa*. As the plants aged the patterns changed and from the remaining fractions, the author

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authors proposed GDH-I and GDH-II as the two most important fractions. Later Hartmann (1973) showed that GDH-II formation was reinforced by the presence of NH_4^+ while glucose had the opposite effect. The increase in GDH-II was associated with anabolic reactions (aminating) and the GDH-II decrease was associated with catabolic reactions (deaminating).

The second most important enzyme in this initial phase of NH_3 assimilation is glutamine synthetase which catalyses the formation of glutamine from glutamate and NH_3. It is in a polymeric association with a molecular weight about 650,000 and can be dissociated into 14 subunits with a molecular weight of about 47,000 each. Both GDH and glutamine synthetase are allosteric enzymes that can have reversible association and dissociation (Stadmann, 1966).

The inducible formation of GDH was demonstrated in the roots of rice seedlings when NH_4^+ was the N source but not when NO_3^--N was used (Kanamori, Konishi and Takahaski, 1972). The induced GDH was NADH-specific, and induction only took place in the soluble fraction. Oji and Izawa (1971) found that a very rapid synthesis of glutamine occurred when plants were supplied with NH_4^+. This increase in the glutamine level (20-fold) was related to a decrease in the glutamate and aspartate pools. They showed that for a plant as a whole (barley) the glutamate level remained constant, indicating rapid synthesis and an absolute reduction in the aspartate level. This indicates its entrance into the Krebs cycle as oxaloacetate. From their data, they suggest the formation of glutamine as a primary reaction in the assimilation of NH_3 in higher plants. Oji and Izawa (1972) found that the increase in the content of free amino acids and amides in the barley shoots was not due to synthesis but to translocation from the roots where NH_4^+-N was used, but when N was supplied as NO_3^-, the NO_3^- translocated to the shoots and synthetic production of amino acids and amides took place there. While glutamine was shown to be related to the primary processes of NH_3 assimilation, asparagine was not (Oji and Izawa, 1973). Ammonium-fed plants increased the turnover of amino acids and organic acids. From their studies with ^{14}C compounds, the authors propose an "aspartate-glutamate pathway" as an alternative for, or as an additional mechanism of, anaplerotic reactions.

Glutamate dehydrogenase holds a special position in the metabolism of plants since glutamate is a link between carbohydrates and N metabolism. Davis (1968), in a comment on the teleology of amino acid metabolism, said that two control points were present: a) the fixation of carbon dioxide and the entry into the Krebs cycle; and b) the entry of NH_3 into organic combination".

Sims, Folkes and Bussey (1968) suggested that the organism should be able to cope with the fluctuation in level and type of N source in the environment and also take advantage of the possibilities of sparing energy as is the case when NH_3 is made available instead of NO_3^- or when amino acids can be supplied together with NH_3. There is also the possibility of an over-supply of N and the organism must rid itself of the excessive amounts of some metabolites or inorganic compounds such as NH_3 that can be toxic when present in excessive amounts. In this sense, GDH provides a good regulation system and the regulation mechanism of GDH has been described by Stadmann (1966) as "concerted feedback inhibition" and by Sims et al. (1968) as "species independent cooperative inhibition".

Metabolism of N and its interaction with the metabolism of carbohydrates

Yemm (1949), while studying the metabolism of detached barley leaves, was able to relate the accumulation of asparagine and glutamine to the sugar content of the plant. Under lighted conditions, glutamine was more important than asparagine and most of the N was moving to growing plant parts as glutamine. Glutamine was found to be intimately associated with carbohydrate metabolism by Willis (1951) who, after working with the roots of barley, suggested glutamine to be a link in the transfer of energy from oxidative processes to protein synthesis. Steward and his associates (Steward, Thompson and Pollard, 1958; Margolis, 1960) were able to relate glutamine and asparagine to NH_4^+ nutrition and to non-growing tissues. They also found the following non-protein-N to protein-N ratio for growing and non-growing plants:

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	growing	0.138
potatoes	non-growing	1.180
	growing	0.200

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In an experiment with plant seedlings, Weismann (1959) found that NH_4^+-supplied plants accumulated the highest amount of amides in the shoots while NO_3^--supplied plants accumulated the minimum, and NH_4NO_3-supplied plants fell in between. Plants supplied with NH_4^+ had 12% more asparagine than glutamine and NO_3^--supplied plants had 30% more glutamine than asparagine. The NH_4NO_3-supplied plants still had 5% more asparagine than glutamine in the shoots and these plants had the maximum accumulation of proteins. Pavolv and Ivanov (1960) conducted an experiment where N was fed as NH_4^+ to the roots of corn and as urea in a foliar application. They found the NH_3 assimilation to be depressed by foliar applied urea due to the tie-up of sugars in the leaves. Only where sucrose was translocated to the roots did NH_3 assimilation take place. Bekmukhamedova (1961) found fewer nitrogenous compounds in the phloem exudates of corn plants which were supplied with NO_3^- and the translocated amino compounds changed quantitatively when NO_3^- was supplied to the plant roots. Oritani and Yoshida (1970) observed the presence of asparagine, alanine, glutamic acid and glutamine in the bleeding sap of rice plants. Asparagine and alanine were the most important compounds present and the level of alanine increased with N application. In developing a method for determination of the best timing for top dressing in rice cultures, Ozaki (1961) observed the presence of asparagine in the "needle-like" leaves but not in the old ones unless a heavy application of N was made.

Pate and Wallace (1964) observed an increase in the organic compounds of some species from the lower to upper portions of the shoots and suggested that this was not only due to export from the roots but also from reduction in the photosynthesizing organs. Later, he concluded (Pate, 1968) that roots are not programmed to store soluble N but they export it rapidly to the shoots.

Mineral nutrition and N metabolism

Nitrate is a better source of N under a wider set of environmental conditions than NH_4^+. Bennet, Derek and Hanway (1964), growing corn plants in sand and nutrient solution, observed that high levels of NH_4^+ had an adverse effect on the growth of plants while NO_3^- had a beneficial effect. Blackman (1938) observed that when light supply was reduced in a clover-grass association NH_4^+ was more efficient than NO_3^- in reducing the population of clover. In a similar study using isotopes, Vallis et al. (1967) observed the same ability of the grasses to compete for N. That grasses can expand faster than alfalfa under conditions of full sunlight was demonstrated by Wing-To Chan and McKenzie (1971) and when the level of shading was increased from 21% to 51% the grasses performed even better.

Possingham (1956) observed that Zn and Fe deficiencies were associated with an increase in the content of free glutamine and asparagine in tomato plants. Steward and Margolis (1962) observed an excessive accumulation of soluble N especially amides, when NH_4^+-N was supplied to tomato plants with this effect accentuated by a lack of Mn. When supplied with NO_3^-, the tomato plants had lower levels of amide accumulation. Working with a wide range of mineral deficiencies, Steward et al. (1959) concluded that in all cases the mineral deficiencies induced an accumulation of amides while plants grown in complete nutrient solutions tended to accumulate amines. Aubrey (1959) reported the accumulation of arginine in plant tissues. He suggested that this was due either to protein breakdown or to a blocking of amino acid metabolism such as when plants are lacking K, Co, Fe and S. Karmamenko (1968), however, found that K deficiency decreased the total amount of free N in barley leaves and under conditions of heavy N application plants accumulated arginine and asparagine. He observed that when the N nutrition of these plants was improved, there was an increase in the rate of synthesis of the "fast" amino acids (glutamate, threonine, gamma-aminobutyric, valine, phenylalanine, leucine, and particularly alanine). Gamborg (1970) demonstrated that the inhibition of the Krebs cycle produced by NH_3 can be reversed by the application of NO_3^-, succinate and malate.

Although much of the research work related to metabolic changes when N was supplied to plants in abnormal conditions shows increases in the pools of soluble amino acids and amides, Zsoldos (1957) found that when NH_4^+ was supplied to rice plants in high amounts there was an accumulation of a peptide. This author suggested that peptides may be the primary product of detoxification in some plants.

Comparing the effect of NO_3^- and NH_4^+ in the nutrition of rice plants, Malavota (1959) observed that plants did better when supplied with NO_3^-. Using 20 ppm and 100 ppm of N as "moderate" and "high" levels, Tanaka, Patnaik and Abichandani (1959) observed that plants did equally well on NO_3^- and NH_4^+ at 20 ppm but at the high level (100 ppm), NO_3^--supplied plants did better, while NH_4^+-N at this level was deleterious to root growth. Ammonium produced an increase in the soluble nitrogenous fraction while NO_3^- produced a higher uptake of K, Ca, Mg and P.

METHODS AND MATERIALS

Experimental design

Three treatment combinations were designed to study the effects of light and temperature on the metabolism of tropical rice with various rates of N supply while using either NH_4^+ or NO_3^- as the N source. The experimental combinations were designed in such a manner that the most probably combinations of light, temperature and N level that could be found in a tropical environment would be reproduced. The levels of light, temperature and N used are listed in Table 1. The three light and temperature treatment combinations tested are listed in Table 2. The temperatures indicated in Table 1 were kept the same during both light and dark periods. Three replications were used for each treatment.

Photoperiod

An alternating period of 12 hours of light and 12 hours of darkness was used.

Table 1. Levels of light, temperature, nitrogen supply and the rice varieties used in the investigation.

---

1. Light Levels
    - a. high light supply (HL) - 3,700 foot candles
    - b. low light supply (LL) - 1,600 foot candles
2. Ambient Temperature Levels
    - a. high temperature (HT) - 35°C
    - b. low temperature (LT) - 24°C
3. Nitrogen Levels in the Nutrient Solution
    - a. ammonium nitrogen (NH_4^+)
        - 1) 5 ppm N as ammonium sulfate [$(NH_4)_2SO_4$]
        - 2) 20 ppm N as ammonium sulfate [$(NH_4)_2SO_4$]
        - 3) 150 ppm N as ammonium sulfate [$(NH_4)_2SO_4$]
    - b. nitrate nitrogen (NO_3^-)
        - 1) 5 ppm N as calcium nitrate [$Ca(NO_3)_2$]
        - 2) 20 ppm N as calcium nitrate [$Ca(NO_3)_2$]
        - 3) 150 ppm N as calcium nitrate [$Ca(NO_3)_2$]
4. Rice Varieties
    - a. Come-Cru
    - b. IR-8

Table 2. Three treatment combinations using two varieties and three levels of nitrogen as nitrate or ammonium.

1.	high light supply - high temperature	(HL-HT)
2.	low light supply - high temperature	(LL-HT)
3.	low light supply - low temperature	(LL-LT)

Varieties

The rice varieties used were Come-Cru (C-C) a Brazilian rice from the northeastern state of Maranhao (5° S - 45° W) which is very popular with local farmers for its ability to grow well in the rather poor soils of the area, its resistance to lodging and to shedding, and because it does not suffer very much from the competition of taller plants (cassava, corn, cotton) when planted together in the typical "slash and burn" fashion of tropical agriculture. It has a plant type typical of the "old" tropical varieties (Tanaka, Kawano and Yamaguchi, 1964) and although there is no direct information available, it can be considered non-responsive to N according to the description given to such plants by Tanaka and Kawano (1966). The second variety used was IR-8.

Cropping technique

Plants were two weeks old when they were transplanted to treatment containers and were allowed to grow for 10 days in the growth chamber in nutrient solution. After the 10-day growth period, plants were harvested and the fresh weight, total free amino-N, NH_3-N and NO_3^--N, total N and sugar content were determined. Amino acid analysis (quantitative) was made for two experimental conditions (low light-high temperature and low light-low temperature) and two rates of N (20 ppm and 150 ppm).

Experimental methods and materials

Plants were grown in controlled environment chambers (Sherer, high light intensity programmable and Sherer, 511-38 Model). Rice seeds were germinated in distilled water and then transferred to a 2 liter polyethylene container filled with vermiculite. The seedlings were then placed in a growth chamber with a light intensity of 800 foot candles and a temperature of 28°C for alternating 12-hour periods of light and dark. Seedlings at this stage of the experiment received the basic nutrient solution (Table 3) plus NH_4NO_3 at a 20 ppm N rate. Iron was added as iron citrate; a 1,000 ppm stock solution was prepared and 5 ml of the stock solution was added to each liter of nutrient solution 2 to 3 times during each 10-day treatment period. After two weeks, plants were removed from the vermiculite, washed, and placed in experimental nutrient solutions supplying basic nutrients (Table 3) plus NH_4^+ or NO_3^- at the levels shown in Table 1. The 2 liter polyethylene containers had five 1.3 cm holes in the lid, and individual rice seedlings were held in each hole with flexible foam material. To avoid heating and light penetration the containers and lids were covered with aluminum foil. Finely ground calcium carbonate ($CaCO_3$) was added to the NH_4^+ solution to avoid a sharp drop in the pH (Karim and Vlamis, 1962). In the NO_3^- solution pH was adjusted with HCl. The pH of the NH_4^+ solution was kept at approximately 6.0; that of the NO_3^- solution around 5.5

Table 3. C

Salt
KH_2PO_4
$CaCl_2$
$MgSO_4$

Salt
H_3BO_3
$MnCl_2 \cdot 4$
$ZnSO_4 \cdot 7$
$CuSO_4 \cdot 5$
$H_2MoO_4 \cdot$

---

‡ Modified

Table 3. Composition of the basic nutrient solutions.

Macronutrients[*]

Salt	Concentration in meq/l nutrient sol.
KH_2PO_4	3
$CaCl_2$	1
$MgSO_4$	6

Micronutrients

Salt	Concentration in g/l
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
$H_2MoO_4 \cdot H_2O$	0.02

---

[*] Modified from Karim and Vlamis (1962).

After a 10-day growth period, the plants were harvested, and the harvest time was always between 5 and 6 p.m.

Roots and shoots from four of the 5 plants in each container were then weighed separately. The shoots were then fixed in 80% ethanol until ethanol extraction procedures were completed.

The alcohol extraction was made with a Vir-Tis 45 homogenizer. One gram or less of plant tissue was homogenized with 20 ml of 80% ethanol, the homogenate was passed through four layers of cheese cloth and the residual cake was reextracted. Combined extracts were then filtered through Whatman number one filter paper by gravity flow. Pigments and proteins remaining in solution were extracted by partition with chloroform and water and the extract was then adjusted to 50 ml with 80% ethanol and stored at -5°C until further analysis.

Free amino N was determined by the method of Moore and Stein (1968), with minor modifications: to 50 ml of citrate buffer (pH 5.0), 90 mg of $SnCl_2 \cdot H_2O$ was added. Two grams of ninhydrin were added to the 50 ml of methyl cellosolve and water and the two solutions were then mixed. Fresh solution was prepared daily.

In photometer tubes (15 x 100 ml, Bausch and Lomb) capped with aluminum foil, 0.1 ml of the sample and 1.0 ml of the ninhydrin solution were heated in boiling water for 20 minutes. Immediately after heating, 5 ml of a dilutant was added (n-propanol and water 1:1). The tubes were then well shaken, wiped dry and optical density read on a Bausch and Lomb Spectronic 20 colorimeter at 570 mμ. Optical densities were read against a standard curve of leucine.

Ammonia and NO_3^--N were determined in 5 ml of the extract by the methods of Bremner and Keeney (1965). Nonstructural sugars were determined by the anthrone method of Yemm and Willis (1954), and readings were made in a Bausch and Lomb Spectromic 20 colorimeter at 620 mμ.

Total N was determined in the residue after alcoholic extraction by a micro-Kjeldahl method. The residue was oven-dried for 24 hours and then a subsample of plant tissue residue was weighed and digested in a Kjeldahl flask with concentrated H_2SO_4 and a catalyst ($HgO + K_2SO_4$). After digestion, NH_3 was distilled, collected in H_3BO_4, and titrated against H_2SO_4.

The data for fresh weight, free-N, NH_3-N NH_3^--N, nonstructural sugars and total-N within light and temperature treatments were submitted to statistical analysis in accordance with a completely random factorial design with three replications. Differences between averages were evaluated by the least significant difference test (LSD).

Plants grown in NO_3^--containing nutrient solutions are referred to as NO_3^- (nitrate) plants. Plants grown in NH_4^+-containing nutrient solutions are referred to as NH_4^+ (ammonium) plants.

Quantitative amino acid analysis

The plant extracts (80% ethanol) were concentrated to dryness in a rotary evaporator under vacuum with temperatures below 40°C and the residue was resuspended in lithium-citrate buffer (pH 1.9). A sample containing 0.5 μmoles of amino-N was then applied to an automatic amino acid analyzer and the acidic and neutral amino acids were separated with lithium-citrate buffers as outlined by Bergen, Henneman, and Magee (1973). The peak areas for the amino acids were calculated by multiplying the height of peak by the width at half of the height.

RESULTS AND DISCUSSION

Responses of rice to forms and rates of nitrogen under conditions of environmental stress imposed by light and temperature

Fresh weight

The data show NO_3^- as a better source of N than NH_4^+ for rice plants under HL-HT and LL-HT conditions while at the LL-LT experimental condition no such difference exists (Tables 4a and 4b). The very stressing effect of the LL-HT condition on the fresh weight of rice plants is shown in the reduced fresh weight of NO_3^- plants at the 150 ppm rate, and in the NH_4^+ plants at all three rates under this condition. The fresh weight of the plants which received NH_4^+ under the LL-LT condition shows this combination to be less stressing (Table 4b). Under the conditions studied the data show 20 ppm to be the best rate of N for both varieties and carriers. This reinforces the observations of others (Tanaka, 1959; Osada, 1964) on this subject.

When conditions are favorable for growth, IR-8 uses up N better than C-C while both varieties behave similarly when under stress (Table 4c).

Fresh weight of plants fed NO_3^- under LL-HT conditions increased with increasing rates of N, while plants fed NH_4^+

Table 4a. Fresh weight responses of rice plants to three combinations of light and temperature and two N carriers.

Treatment	Carrier NO_3^-	Carrier NH_4^+	LSD (.05)
	g/4 plants		
HL-HT	1.4	0.9	0.13
LL-HT	1.5	0.7	0.13
LL-LT	1.5	1.4	0.13
LSD (0.05)	0.3	0.3	

Table 4b. Fresh weight responses of rice plants to three combinations of light and temperature and two N carriers applied at 3 rates.

Treatment	Carrier	Rate 5	 20	 150
		g/4 plants		
HL-HT	NO_3^-	1.5	1.5	1.2
	NH_4^+	0.7	1.5	0.5
LL-HT	NO_3^-	1.7	1.8	0.9
	NH_4^+	0.8	0.7	0.6
LL-LT	NO_3^-	1.3	1.8	1.5
	NH_4^+	1.2	1.5	1.6

LSD (0.05) = 0.38

Table 4c. Fresh weight responses of two rice varieties to two N carriers, applied at 3 rates.

Carrier	Rate	Variety IR-8	Variety C-C
	ppm	g/4 plants	
NO_3^-	5	1.7	1.3
	20	1.9	1.5
	150	1.3	1.1
NH_4^+	5	0.9	0.9
	20	1.3	1.2
	150	1.0	0.8

LSD (0.05) = 0.18

under this same experimental condition showed no differences between rates. This difference in behavior indicates that NO_3^--toxicity only develops when NO_3^- is fed in high amounts to plants under stressing conditions, while NH_4^+-toxicity develops even when the rates of NH_4^+-N applied are low (Table 4b).

Amino-N content

The free amino-N content of rice plants supplied with NO_3^- showed no significant differences among rates for the two varieties or in any of the three light and temperature combinations (Tables 5a and 5b). Rice plants supplied with NH_4^+ produced more free amino-N than when supplied with NO_3^-, and the differences are very accentuated in the HL-HT and LL-HT conditions. Here again, the less stressing effect of the LL-LT condition is observed (Table 5a).

The concentration of free amino-N in the NH_4^+-plants increases as the rates increase (Table 5b) and only at the 20 ppm rates did IR-8 accumulate higher levels than C-C.

Figures 2 through 5 show that a negative relationship exists between the levels of free amino-N and the fresh weight of the plants fed NH_4^+ while no such relationship can be established between the content of free amino-N and the fresh weight of plants fed NO_3^-.

NH_3-N content

The data for free NH_3 in the plants under the various treatments conditions are found in Table 6.

Table 5a. Free amino N in rice plants as related to three light and temperature combinations and 2 N carriers.

Treatment	Carrier NO_3^-	Carrier NH_4^+	LSD (0.05)
	uMoles/g fresh weight		
HL-HT	25.2	129.1	10.4
LL-HT	22.9	184.3	10.4
LL-LT	23.1	38.7	10.4
LSD (0.05)	22.4	22.4	

Table 5b. Free amino N in two rice varieties as related to two N carriers applied at three rates.

Carrier	Rate	Variety IR-8	Variety C-C
	ppm	uMoles/g fresh weight	
NO_3^-	5	15.8	20.2
	20	24.5	26.7
	150	26.6	28.8
NH_4^+	5	73.8	61.4
	20	88.5	62.8
	150	214.1	203.6

LSD (0.05) = 14.7

Rice plants supplied with NO_3^--N showed no significant differences in free NH_3-N as the result of different levels of N supply. The free NH_3 level was low for plants supplied with NO_3^--N at all light and temperature combinations. These results for the free NH_3 levels of plants supplied with NO_3^--N are consistent with the results for free amino N levels found in these same plants (Tables 5a, 5b).

Ammonium-fed plants accumulated higher levels of free NH_3 at the 150 ppm rate than at the other rates. The differences were very accentuated, except for the LL-LT condition where there were no significant differences in free NH_3 levels for any of the three rates. This result was in sharp contrast with the other light and temperature conditions.

Although both varieties accumulated high amounts of free-NH_3, there were differences between varieties under the various light and temperature combinations. Under LL-HT conditions and at 150 ppm NH_4-N, IR-8 accumulated 68 percent more free NH_3 than the C-C plants. The accumulation of free NH_3 occurred only under conditions where free amino-N accumulated to very high levels (Figures 2 through 5).

Influences on the plant NO_3^- content

The data for plant NO_3^- content are given in Tables 7a and 7b.

The rice plants accumulated higher levels of NO_3^- in the tissues when fed NO_3^-N at higher levels (150 ppm). Of the three light and temperature combinations, the LL-HT condition

Table 6. Free NH_3-N in two varieties of rice as related to three light and temperature combinations, two N carriers and three rates of application.

Treatment	Carrier	Rate	Variety IR-8	Variety C-C
		ppm	uMoles/g fresh weight	
HL-HT	NO_3^-	5	2.6	1.6
		20	0.1	0.1
		150	1.6	1.1
	NH_4^+	5	1.7	5.2
		20	1.3	3.1
		150	56.7	72.3
LL-HT	NO_3^-	5	2.2	0.9
		20	4.4	7.0
		150	1.4	4.5
	NH_4^+	5	5.2	10.5
		20	12.0	12.0
		150	81.5	26.2
LL-LT	NO_3^-	5	tr.	0.4
		20	3.9	5.5
		150	2.9	3.4
	NH_4^+	5	1.0	2.4
		20	3.3	0.3
		150	7.1	4.1

LSD (0.05) = 14.5

Figure 2. Modifications in the N-metabolism of IR-8 plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under HL-HT conditions.

8 plants
s under

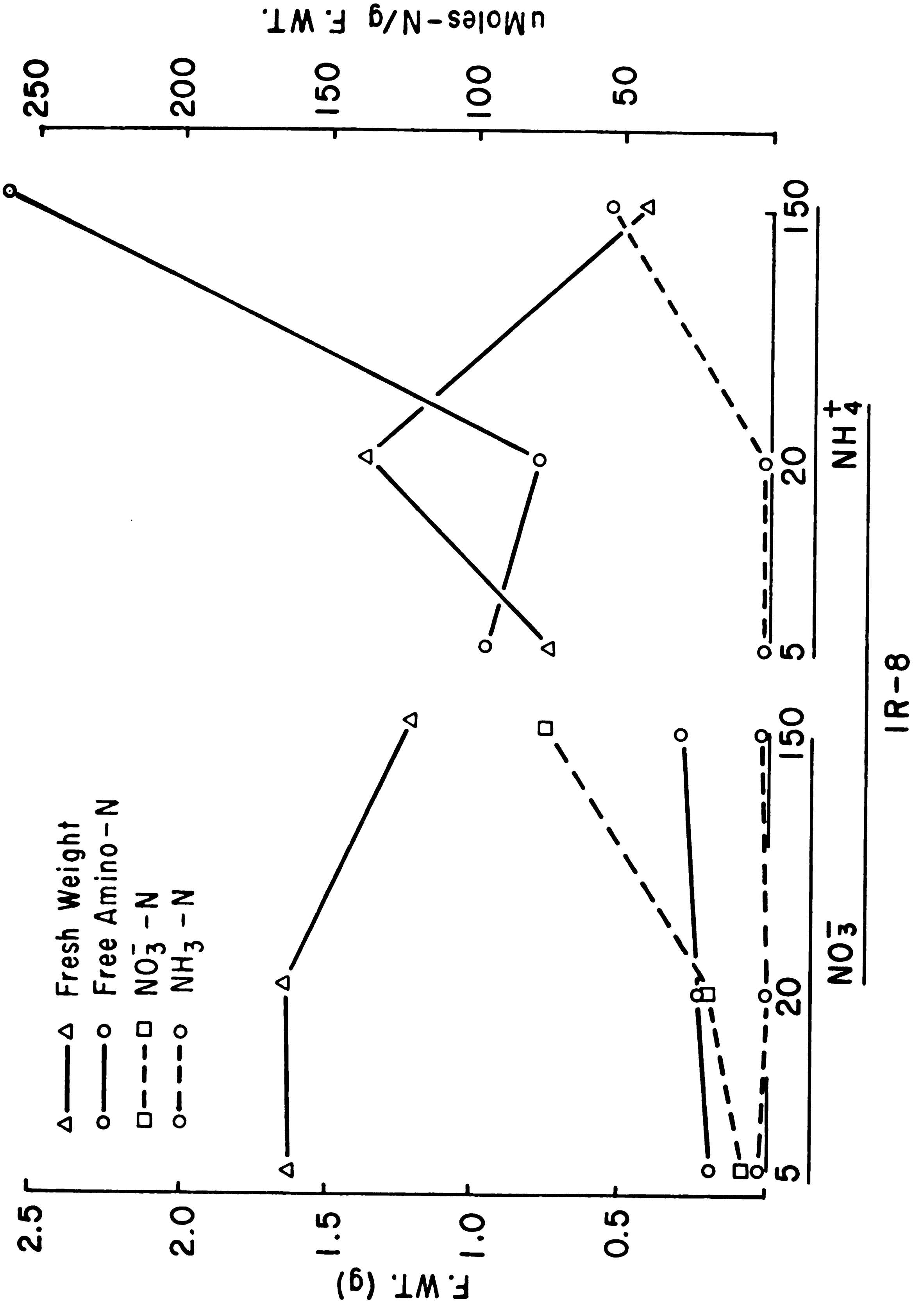


Figure 3. Modifications in the N-metabolism of C-C plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under HL-HT conditions.

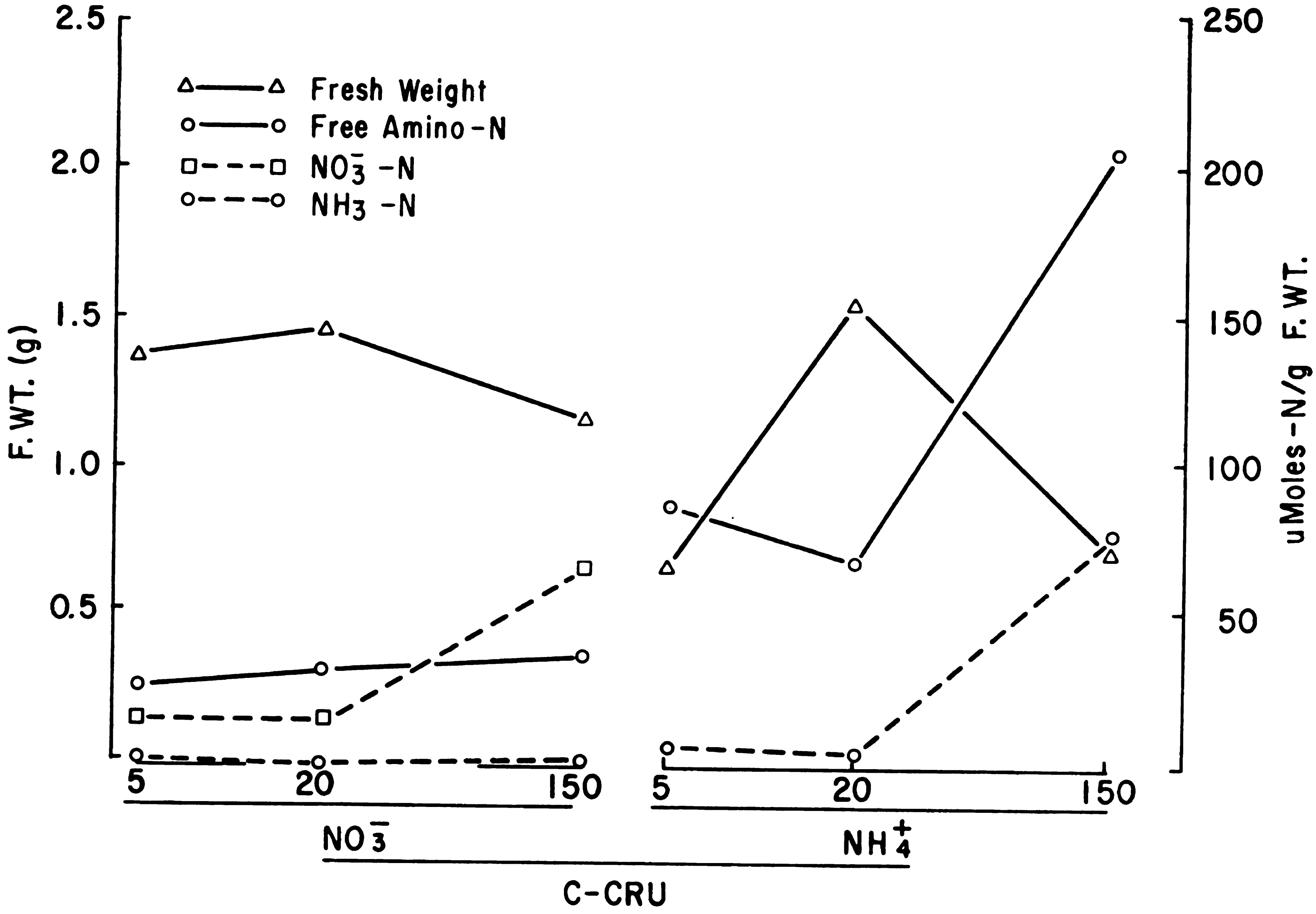

2.5
2.0
1.5
1.0
0.5
F. WT. (g)
Fresh Weight
Free Amino-N
NO_3^- -N
NH_3 -N
250
200
150
100
50
uMoles-N/g F. WT.
5
20
150
5
20
150
NO_3^-
NH_4^+
C-CRU

Figure 4. Modifications in the N-metabolism of IR-8 plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under LL-HT conditions.

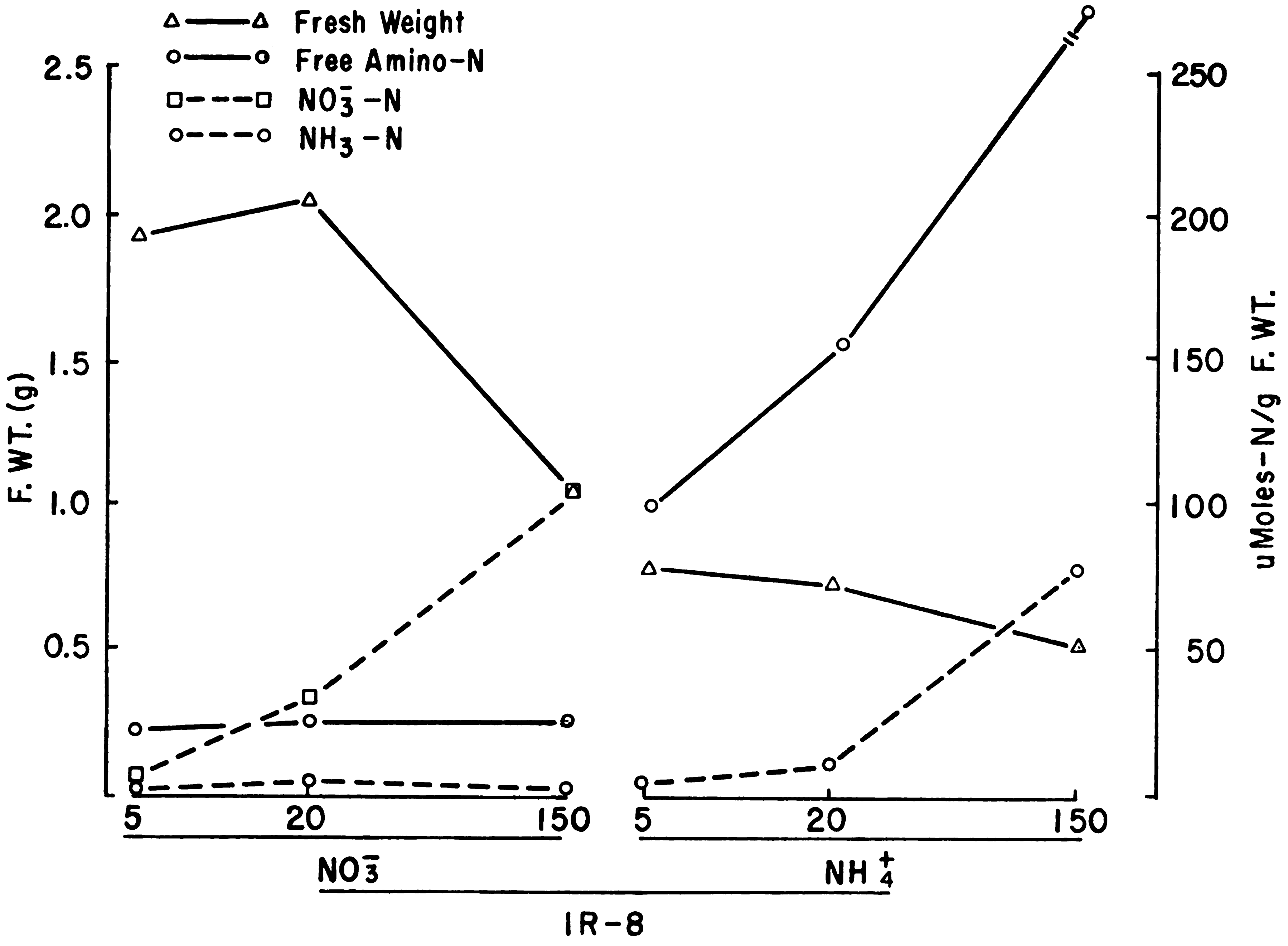

Fresh Weight
Free Amino-N
NO_3^- -N
NH_3 -N
2.5
2.0
1.5
1.0
0.5
F. WT. (g)
250
200
150
100
50
uMoles-N/g F. WT.
5
20
150
5
20
150
NO_3^-
NH_4^+
IR-8

Figure 5. Modifications in the N-metabolism of C-C plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under LL-HT conditions.

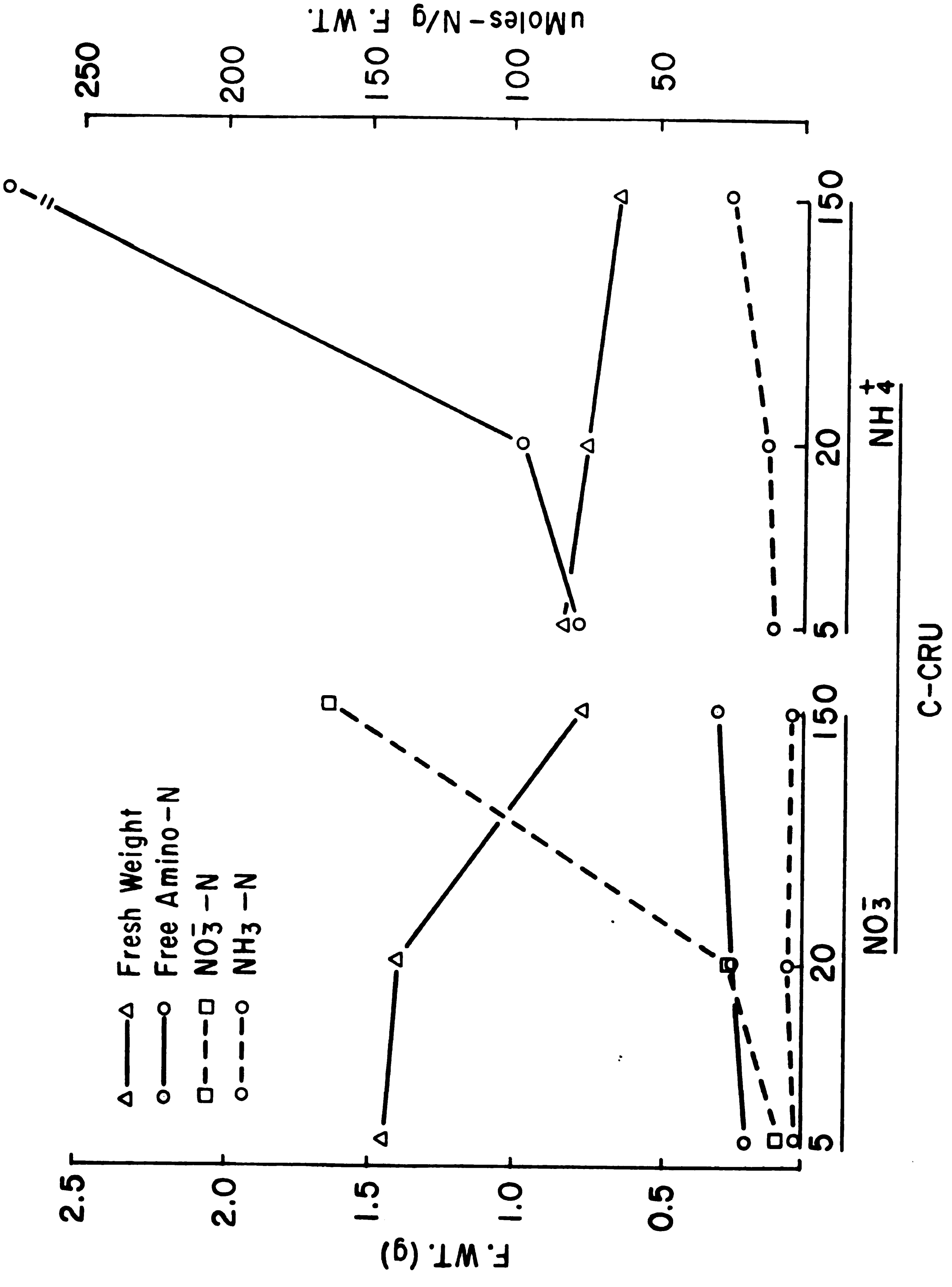

F. WT. (g)
2.5
2.0
1.5
1.0
0.5
uMoles - N/g F. WT.
250
200
150
100
50
Fresh Weight
Free Amino-N
NO3- -N
NH3 -N
5
20
150
NO3-
5
20
150
NH4+
C-CRU

Figure 6. Modifications in the N-metabolism of IR-8 plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under LL-LT conditions.

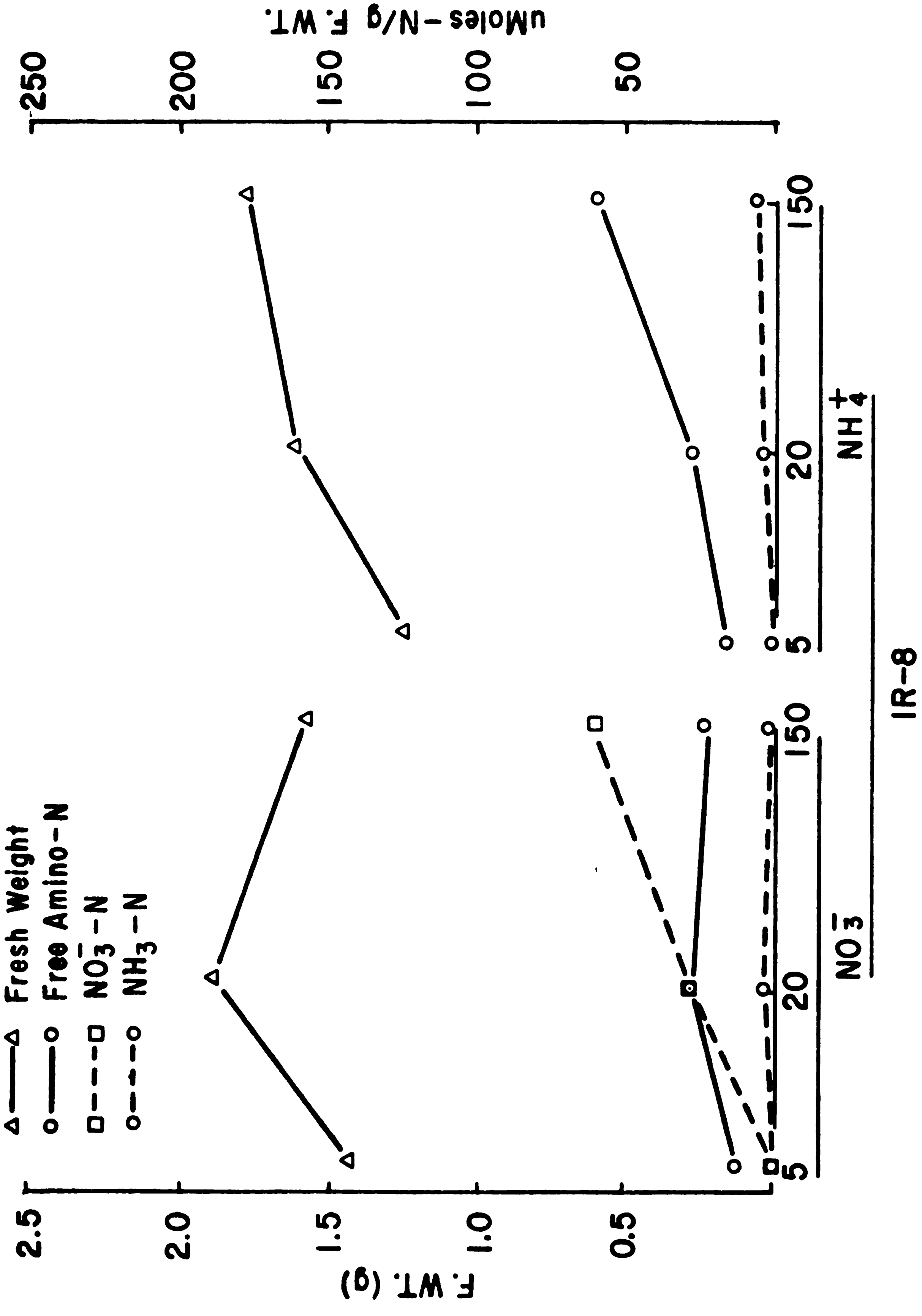
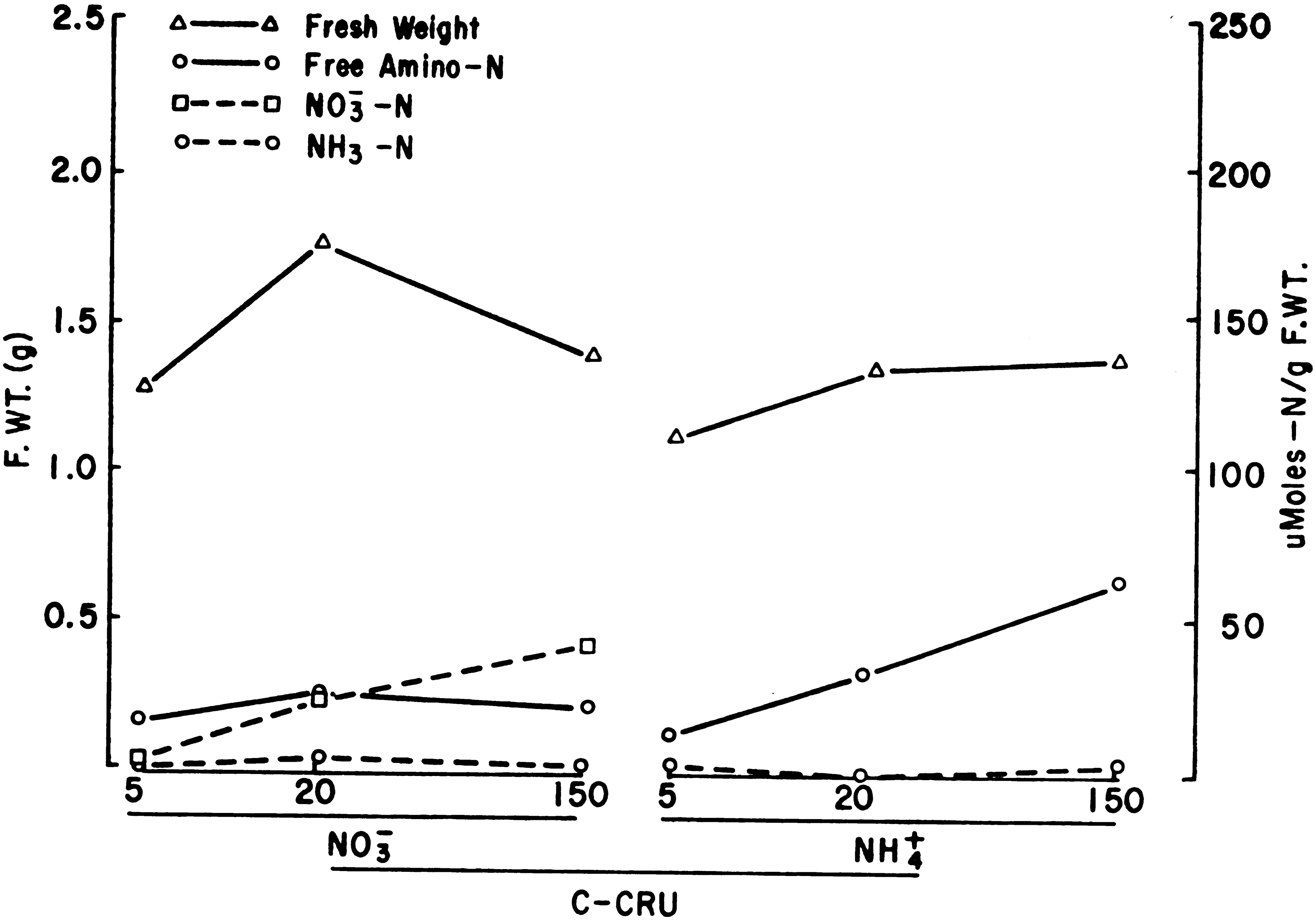

Fresh Weight
Free Amino-N
NO_3^--N
NH_3-N
F. WT. (g)
2.5
2.0
1.5
1.0
0.5
uMoles-N/g F. WT.
250
200
150
100
50
5
20
150
NO_3^-
5
20
150
NH_4^+
IR-8

Figure 7. Modifications in the N-metabolism of C-C plants fed NO_3^- or NH_4^+ at 5, 20 and 150 ppm rates under LL-LT conditions.


Fresh Weight
Free Amino-N
NO_3^--N
NH_3-N
F. WT. (g)
2.5
2.0
1.5
1.0
0.5
uMoles-N/g F.WT.
250
200
150
100
50
5
20
150
5
20
150
NO_3^-
NH_4^+
C-CRU

promoted the higher accumulation of NO_3^-. The data show that high temperature promoted the uptake of high levels of NO_3^- with both high light and low light supply.

There was a negative relationship between levels of NO_3^- accumulated in the tissues and the fresh weight of the plants. The highest levels of NO_3^- accumulation (LL-HT combination at the 150 ppm rate) coincided with the lowest levels of fresh weight (Tables 4b and 7a; Figures 4 and 5).

It is noteworthy that there was no significant accumulation of free reduced N (either as amino-N or as NH_3^--N) in the plants supplied with NO_3^--N, despite the high content of NO_3^- in the plant tissues. These data indicate a reduction in the activity of the NR system in these high NO_3^- plants and point to the existence in the rice plants of distinct NO_3^- pools. The results are in agreement with the observations made by Heimer and Filner (1971) and Ferrari et al. (1973) in plant tissue cultures. The data indicates further that the effect of light supply on NR levels and activity is due, not only to an increased influx of NO_3^- as proposed by Beevers et al. (1965), but that a more complex relationship exists involving the supply of carbon skeletons to the metabolizing cells through the photosynthetic process.

In this investigation we use the expression "NO_3^- sequestration" to designate this mechanism for differential distribution of NO_3^- between the inducing and the substrate pools.

Table 7a. NO_3^- accumulation in rice plants as related to three combinations of light and temperature and three rates of NO_3^- application.

Treatment	Rate (ppm) 5	20	150	LSD (0.05)
	uMoles/g fresh weight			
HL-HT	5.2	8.8	34.5	13.8
LL-HT	4.5	14.0	67.4	13.8
LL-LT	0.9	13.3	25.4	13.8
LSD (0.05) =	20.5	20.5	20.5	

Table 7b. NO_3^- accumulation in two rice varieties as related to rate of NO_3^- application.

Carrier	Rate	Variety IR-8	 C-C
	ppm	uMoles/g fresh weight	
NO_3^-	5	5.2	8.8
	20	26.9	21.3
	150	81.0	88.6

LSD (0.05) = 16.0

Sugar content

The data for plant sugar content are given in Table 8.

Only under the LL-LT condition were the differences between rates significant for plants fed either NO_3^- or NH_4^+. These differences are apparent for IR-8 plants, that had a greater sugar content at the 5 ppm rate of N addition and a smaller sugar content at the 150 ppm rate. Under the LL-HT condition, the IR-8 plants fed NO_3^- had a significantly higher content of sugar than the NH_4^+-plants at all N rates. At the 20 ppm rate of addition, the sugar content of both varieties was significantly less with NH_4^+ than with NO_3^-.

Total N content of plant residue after alcohol extraction

The data for total N content of the plant residue after alcohol extraction are given in Tables 9a and 9b.

Plants accumulated the highest level of TN under the HL-HT condition, with no significant differences between rates. Under LL-LT condition, the accumulation of TN was smaller, and the TN levels increased with increasing levels of applied N. This parallels the tendency for accumulation of fresh weight in this same order under this experimental condition.

Total N after alcohol extraction may be considered to represent N assimilated into proteins. The low values for total N under LL-LT in Table 9b correspond to low levels for NO_3^- (Table 7a) and for reduced N (free amino N in Table 5a and free NH_3-N in Table 6) under this combination of low radiant energy and low thermal energy. These results

Table 8. Sugar accumulation in two rice varieties as related to three combinations of light and temperature, two N carriers and three rates of application.

			Variety	
Treatment	Carrier	Rate	IR-8	C-C
		ppm	% of fresh weight	
HL-HT	NO_3^-	5	2.9	2.3
		20	2.6	2.4
		150	2.5	1.7
	NH_4^+	5	1.5	1.3
		20	1.7	1.7
		150	1.2	7.3
LL-HT	NO_3^-	5	2.9	2.1
		20	2.9	2.4
		150	2.2	2.3
	NH_4^+	5	1.3	1.1
		20	1.1	0.7
		150	0.7	1.6
LL-LT	NO_3^-	5	2.8	1.8
		20	2.4	1.4
		150	1.2	2.2
	NH_4^+	5	3.5	2.1
		20	2.5	1.9
		150	0.9	1.5

LSD (0.05) = 1.4

Table 9a. Total N content of rice varieties in relation to source of N.

Carrier	Variety IR-8	 C-C
	% of dry weight	
NO_3^-	2.9	2.9
NH_4^+	3.4	2.9

LSD (0.05) = 0.3

Table 9b. Total N content in relation to light and temperature combinations and rate of N application.

Treatment	Rate 5	Rate 20	Rate 150	LSD (0.05)
	% of dry weight			
HL-HT	3.3	3.6	3.4	0.32
LL-HT	3.0	3.1	3.1	0.32
LL-LT	2.0	2.8	2.8	0.32
LSD (0.05) =	0.50	0.50	0.50	

indicate that both uptake and assimilation of N were limited by the low supply of energy available to the LL-LT plants, regardless of the N carrier used (Figures 6 and 7).

As the residues used for total N determination had been extracted with 80% ethanol before oven drying, most of the total N can be considered to be protein N. However, the occurrence of high total N in plants having a low fresh weight, and the reverse situation in other treatments (Table 9b) may suggest the possibility of the presence of peptides in the plants supplied with high levels of NH_4^+-N (Zsoldos, 1957). These peptides may have been precipitated along with the proteins as suggested by Raake (1956).

Effects on the free amino acid pool

Based on the free amino-N and NO_3^--N analyses from all experimental conditions, samples indicating extreme changes were selected for free amino acid and amide analysis. The samples selected were IR-8 under LL-HT and LL-LT conditions at the 20 and 150 ppm N rates of both NO_3^--N and NH_4^+-N. The major amino acids present were aspartic acid, serine, glutamic acid, alanine, cystine, and the amides asparagine and glutamine (Table 10).

At the 20 ppm rate under LL-HT condition the NO_3^- fed plants had a high content of aspartic and glutamic acid (10.5 and 25.1% of the total pool) while the asparagine content was lower (3.9% of the total pool) and glutamine was 12.3% of the total. When the N rate was increased (150 ppm) the levels of aspartic and glutamic acid decreased (to 5.1

Table 10. Free amino acids in rice plants (IR-8) grown under low light-high temperature and low light-low temperature conditions, with N supplied as nitrate or ammonium.

Amino Acid	LL-HT								LL-LT							
	Nitrogen supplied (ppm)								Nitrogen supplied (ppm)							
	NO_3^-				NH_4^+				NO_3^-				NH_4^+			
	20		150		20		150		20		150		20		150	
	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%
Asp.	1.34	10.5	0.92	5.1	1.70	1.4	3.89	2.3	0.55	3.3	0.79	6.3	0.58	2.7	0.47	1.3
Thre.	0.34	2.6	0.62	3.5	1.76	1.4	2.74	1.6	0.51	3.1	0.20	1.6	0.42	2.0	0.58	1.6
Ser.	1.75	13.6	2.62	14.6	2.99	2.4	2.95	1.7	0.99	6.0	1.08	8.6	0.89	4.2	0.96	2.6
Asp.NH_2	0.51	3.9	2.00	11.2	33.31	26.7	21.65	12.5	0.92	5.6	0.41	3.3	5.23	24.4	8.17	22.2
Glut.	3.21	25.1	2.92	16.3	6.9	5.5	8.66	5.0	3.80	23.0	3.37	26.9	2.58	12.0	3.57	9.7
Glut. NH_2	1.57	12.3	3.85	21.5	68.14	54.7	122.02	70.5	6.21	37.6	3.69	29.4	9.01	42.0	19.11	51.9
Pro.	nd	nd	1.33	7.4	1.69	1.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gly.	0.20	1.7	0.22	1.2	0.85	0.7	nd	nd	0.27	1.6	0.19	1.5	0.25	1.2	0.38	1.0
Ala.	1.71	13.4	1.91	10.7	3.96	3.2	3.94	2.3	2.37	14.5	1.94	15.5	1.73	8.0	2.44	6.6
Cys.	1.75	13.8	0.66	3.7	1.73	1.4	3.64	2.1	0.46	2.8	0.40	3.2	0.39	1.8	0.57	1.5
Ile.	0.07	0.5	0.20	1.1	0.41	0.3	0.98	0.6	0.09	0.6	0.08	0.6	0.08	0.4	0.16	0.4
Leu.	0.10	0.8	0.26	1.5	0.40	0.3	1.03	0.6	0.13	0.8	0.15	1.2	0.10	0.5	0.19	0.5
Tyr.	0.13	1.0	0.15	0.8	0.23	0.2	0.33	0.2	0.10	0.6	0.12	1.0	0.08	0.4	0.14	0.4
Phen.	0.12	0.9	0.27	1.5	0.42	0.3	1.17	0.7	0.10	0.6	0.12	1.0	0.10	0.5	0.10	0.3
Total	12.80	100	17.93	100	124.50	100	173.00	100	16.50	100	12.54	100	21.45	100	36.84	100
Amino/amide	5.17		2.06		2.23		0.20		1.31		2.05		0.51		0.35	

nd = none detected

and 16.3%, respectively) while the levels of asparagine and glutamine increased (to 11.2 and 21.5%, respectively) There was also a sharp drop in the content of cystine for plants at the 20 ppm rate when compared with the 150 ppm N rate (13.8 to 3.7%). The contents of serine and alanine were high and remained about the same despite the changes in the N rate of the nutrient solution (serine, 13.6-14.6%; alanine, 13.4-19.7%). The plants at the 20 ppm rate also had a lower accumulation of total free amino acids than the plants at the 150 ppm rate (12.80-17.90 uMoles/g fresh weight). The amino to amide ratio was 5.17 for 20 ppm and 2.06 for 150 ppm in this experimental condition.

When the experimental conditions were changed to low light and low temperature there was in increase in the content of aspartic acid (3.3-6.3%), serine (6.0-8.6%), and glutamic acid (23.0-26.9%) of the total pool while the contents of asparagine (5.6-3.3%) and glutamine (37.6-29.4%) decreased as the level of NO_3^--N in the nutrient solution increased from 20 ppm to 150 ppm. The content of alanine remained about the same (14.5-15.5%). The total content of the amino acid and amide pool was higher for the 20 ppm rate (16.50 uMoles/g fresh weight) than for the 150 ppm rate (12.54 uMoles/g fresh weight). The amino/amide ratio was 1.31 for 20 ppm N and 2.05 for 150 ppm.

The inverse of the situation observed in the previous experiment occurred when the plants were under the low light and low temperature experimental condition. The levels of asparagine and glutamine decreased while the levels of glutamic acid, aspartic acid and serine increased as the NO_3^--N level increased from the 20 to 150 ppm rate (Table 10).

The data show that when the conditions are favorable for growth (LL-HT, 20 ppm) there is a more balanced distribution of amino acids in the pool (amino/amide ratio), and glutamate is the major amino acid present and asparagine is only present in small amounts. As the environmental conditions cause greater stress (high NO_3^-) there is a tendency for the accumulation of amides (asparagine and glutamine) with a decrease in the content of the respective amino acids (aspartic and glutamic).

We should note, however, that the content of serine in the LL-HT condition and of alanine in both experimental conditions are almost unchanged as the level of NO_3^- in the nutrient solution increases from 20 ppm to 150 ppm; they make up a significant amount of the total pool.

These data indicate that despite the drastic reduction in fresh weight and increase in the NO_3^- content of the plant tissues as the rates of N increase from 20 ppm to 150 ppm of NO_3^- (2.17 to 1.01 g fresh weight), and 33.3 to 106.5 uMoles NO_3^--N/g fresh weight) in the low light and high temperature condition, the changes in the amino acid pool are not so drastic. This indicates that although the conditions under which the "NO_3^- sequestration" is activated produce a reduction in the fresh weight of the plants, the mechanism is efficient enough to keep the free amino acid pool composition from becoming too unbalanced. That may be one of the reasons for the superior performance of plants fed NO_3^- when submitted to environmental stress.

It is clear that the 150 ppm rate in the LL-HT combination causes the greatest stress, and this is the reason why there is an accumulation of amides at the expense of the amino acids at this rate of NO_3^-.

Under the LL-LT condition, amides are higher at the lower level of N addition. In this case, there are no clear indications that plants receiving 20 ppm N were under more stress than those receiving 150 ppm.

We should also note that when the amino acid pool has a more favorable composition it also has a smaller content of total free amino-N. This indicates a better utilization of the amino acids by the plants under this condition.

The data on the low light and high temperature experiment show that when plants are fed high levels of NO_3^- there is an increase in the N uptake (Tables 7a and 7b) while the utilization of amino acids from the free pool decreased (Table 10). Since there is no accumulation of free amino-N in the tissues of these plants (Tables 5a and 5b), the low fresh weight of the plants fed 150 ppm NO_3^--N is due to a lack of carbon skeletons in the low light supply while the high temperature promotes an increase in the uptake of NO_3^-. The low accumulation of amino-N in these plants points out the fact that the excess NO_3^- taken up is sequestered into the substrate pool and there is no increase in the NR level or activity. The composition of the free amino acid pool as compared with the pools of the LL-LT NO_3^- plants also points in the same direction. Also, high temperature affects the uptake of NO_3^- and restricts the availability of carbon skeletons, therefore, the effect of NR is this process must be a secondary one.

When plants are fed NH_4^+ under LL-HT conditions, asparagine and glutamine are the two major amino compounds present making up 81.4% of the total pool in the 20 ppm rate plants and 83.0% of the total in the 150 ppm rate plants (Table 10). After the amides, glutamate is the amino acid present in the highest amount: 5.5% of the total in the 20 ppm plants and 5.0% in the plants fed NH_4^+ at the 150 ppm rate. Alanine comes next with 3.2% and 2.3% of the total for the two rates. Serine accumulated more in the 20 ppm plants (2.4%) and a higher amount of aspartic acid was present at the 150 ppm rate. Cystine was only present in high amounts in the 150 ppm plants (2.1%). The amino/amide ratio was 2.23 at the 20 ppm and 0.20 at the 150 ppm NH_4^+ for this experimental condition.

Under the LL-LT experimental condition there was a more balanced distribution of amino acids and amides than under the previous condition. At the 20 ppm rate, asparagine and glutamine are the major fraction and make up 66.4% of the total pool, glutamic acid is present in high amounts (12.0%

of the total) and the contents of alanine, serine and aspartic acid are also high (8.0, 4.2, and 2.7% of the total pool, respectively). At the 150 ppm rate asparagine and glutamine make up 74.1% of the total pool while the level of glutamate falls to 9.7%. The content of alanine is still high (6.6%) and the content of serine is lower than at the 20 ppm rate (2.6%). The amino/amide ratio was 0.51 for the 20 ppm plants and 0.35 for the 150 ppm NH_4^+ plants. The NH_4^+ fed plants accumulated higher levels of amino acids and amides than the NO_3^- fed plants and glutamine was always the major amino compound present in the NH_4^+ plants (Table 10). These data are in agreement with the suggestion by Oji and Izawa (1972) that glutamine synthesis is a primary reaction of NH_3 assimilation in plants. Also, there is a high content of alanine in the NH_4^+ plants (in absolute amounts) at both the 20 ppm and the 150 ppm rate in the LL-HT experimental conditions (3.96 and 3.94 uMoles/g fresh weight). Serine is also present in relatively high amounts in both experimental conditions and for both N carriers and rates (Table 10). Under conditions of high NH_4^+ supply, however, the high accumulation of amides makes the percent contribution of serine and alanine to the total pool look rather small.

The data show a better balance in the distribution of amino acids in the free pool (higher amino/amide ratio) to be related to the more favorable growing conditions, while the plants subjected to stress conditions show an accumulation of amides that become the dominant fraction of the free pool. The presence of high amounts of glutamate as a % of the total pool is also related to better growing conditions. It is of interest to note the rather high content of alanine (in absolute amounts) in the plants fed NH_4^+-N even under the most stressing conditions (LL-HT at 150 ppm rate). The data lead us to the conclusion that there is a virtual stop of the overall metabolism of the plants fed high NH_4^+ levels on the low light and high temperature condition, and glutamine accumulates to keep down the levels of free-NH_3 in the tissues.

Glutamine is the major amide present under conditions unfavorable for growth in either NO_3^- or NH_4^+ fed plants. It appears important to note the decrease in the content of asparagine and the increase in the content of glutamine as the rates of N applied increased from 20 ppm to 150 ppm in the LL-HT condition (the greatest stress) when plants are fed NH_4^+. The absolute amounts of amino acids present in the free pool of plants fed NH_4^+ under stress conditions are high if compared with the amounts found in the NO_3^- plants. The plants fed NH_4^+ are in fact under unfavorable conditions for growth (Table 4). This indicates that the imbalance in the amino-N/amide-N promoted by the accumulation of amides disrupts the metabolism of the plants under stress.

The amino-N/amide-N ratio or the glutamate/glutamine/asparagine ratios seem to be very sensitive indicators of the metabolic changes taking place in the N metabolism of the rice plants when the environmental conditions change from more favorable for growth to more stress on the plants. The stress factor can be N rate, N carrier, light or temperature.

Differences in N assimilation between varieties

The data on the fresh weight in the plants studied suggest a more rapid utilization of the NO_3^- taken up by IR-8 plants than by the C-C variety (Table 4c). This ability is best reflected in the fresh weight of plants under more favorable conditions of growth.

When plants were fed NH_4^+, IR-8 showed a tendency for higher accumulation of free amino-N under all the conditions studied, and NH_3-N under the LL-HT condition. There was also a higher accumulation of TN by IR-8 plants when fed NH_4^+ (Tables 9a and 9b).

The data indicates the physiological superiority of IR-8 plants over the Brazilian variety in the utilization of N from the nutrient solution. The results suggest this superiority to be related to higher photosynthetic ability.

Use of NO_3^- versus NH_4^+ in the nutrition of the rice plant

Both light and temperature had a positive effect on the uptake of NO_3^- (Table 7a). However, the accumulation of NO_3^- as such was higher for the LL-HT condition. This shows the effect of temperature on the NO_3^- uptake and supports the findings of George et al. (1971) and of Beevers et al. (1965).

In all the experimental situations, the accumulation of NO_3^- was associated with a reduction in fresh weight

(Figures 2 through 7).

Positive interaction between high light supply and high temperature only holds true at moderate levels of NO_3^- supply. At high rates of N supply (150 ppm) accumulation of NO_3^- is related to the reduction in fresh weight of the plants despite the high levels of light supply (Tables 4b, 5a; Figures 2 and 3).

Plants fed NH_4^+ had marked differences in fresh weight between N rates under the HL-HT condition while the differences in fresh weight were minimal among N rates for plants under the LL-HT and LL-LT conditions that were the most stressing and the least stressing, respectively (Table 4b). This indicates that, under conditions of increased respiration (high temperature) and reduced photosynthesis (low light supply), NH_4^+-N can be harmful to plants even when applied at the optimum N rate (20 ppm).

The data show that NO_3^- can be used as a source of N for rice plants over a wider range of environmental conditions than NH_4^+.

Regulation mechanisms in rice plants fed NO_3^- or NH_4^+

The most remarkable difference between plants fed NO_3^--N and plants fed NH_4^+-N in these experiments is in the levels of accumulation of reduced N (total free amino-N and free NH_3). In plants fed NO_3^--N, the level of free reduced N is kept low despite the changes taking place in the environment (temperature, light, N-rate) and also despite the level of accumulation of NO_3^- in the plant tissues.

These data indicate the regulatory efficiency of the NO_3^- sequestration mechanism in keeping excess NO_3^- out of the inducing pool and consequently controlling the activity of the NR system that must be secondary to it. In this way the rate of N assimilation and the rate of photosynthetic activity are geared to each other. This indicates that to improve the utilization of NO_3^- by plants it is the photosynthetic efficiency and not the levels of NR activity per se that should be looked upon in the first place.

When plants are fed NH_4^+-N, however, the mechanisms of regulation and detoxification acy by "locking up" the excess reduced N in amino and amide groups (Prianishnikov, 1952; Kretovich, 1958). These facts are clearly shown in the levels of accumulation of free amino-N in plants fed NH_4^+ and are very accentuated at the 150 ppm rate (Tables 5a and 5b).

The LL-HT experiment indicates a drastic mobilization of plant organic N into reduced and mineral forms, giving rise to low fresh weights (Table 4b) and high levels of amino N (Table 5a) and NH_3-N (Table 6). The contrasting situation in the LL-LT combination points to the fact that high respiratory rates promoted by high temperature produce a deficiency of carbon skeletons to react with the NH_3 present in plant tissues and leads to accumulation of amides and free NH_3.

Although the indications are that the accumulation of free NH_3 is due to exhaustion of carbon skeletons in the plants under conditions of high respiration and low photosynthesis (Table 8), a situation of metabolic disruption in these plants (LL-HT), probably due to high NH_3 content is also indicated by the very marked modifications in the free amino acid pools between the LL-HT and LL-LT experimental conditions, with a sharp increase in the content of glutamine in the LL-HT plants, especially at the 150 ppm rate (Table 10).

The N-status of the rice plants as a diagnostic tool

The N-status of plants is a good indicator of the interactions between N-applied and the conditions of the environment where the plant lives.

Several methods have been proposed to assay the N status of rice plants. Ozaki (1955) proposed the asparagine test and this method was used by Mitsui et al. (1958) and Singh et al. (1960) for the estimation of the need of topdressing in rice cultures.

Thenabadu (1972) criticized the asparagine test on the grounds that the asparagine content of plants can be increased with darkness and with heavy N application. He then proposed the use of the total N content of the first and second most recently matured leaves of 66-67 day old plants as a guide for N status of the rice plants. Youngberg (1972) working with alfalfa, observed that the daily variation in the N content was an artifact due to dilution by non-structural carbohydrates.

Our experimental results point to the fact that no method for the estimation of the N-status of the rice plants is really safe if the energy levels in the environment where the plant grows are not taken into consideration. We can see from plants fed high rates of NH_4^+ (150 ppm) that there is an inverse relationship between high free amino-N content and fresh weight (Tables 4c and 5b). The same inverse relationship can be seen between the TN content and the fresh weight of plants under HL-HT conditions (Tables 4a and 9b).

The metabolic behavior of a plant or plant part at a given stage of the life cycle can change dramatically when exposed to environmental stress. The data show that changes do take place in the N content of the rice plants when under environmental stress, no only quantitatively, but also qualitatively (Tables 5a, 5b, 6, 7a, 7b and 10).

We can thus conclude that the metabolic response of rice plants to environmental stress caused by light, temperature and N can be used as a guide for understanding the N-status of these plants.

Influences of the environment on N-utilization

The culture of upland rice follows the general patterns of tropical agriculture. The growing season is the rainy season. This is a season of low light supply and high rainfall (Figure 1). The supply of NO_3^- is quite high at the beginning of the rainy season, and then goes down quite sharply (Birch, 1958). Ammonium-N may become available in somewhat higher amounts later on, as reducing conditions develop in the soils.

Under this set of environmental conditions it is important that a plant be able to take up as much N as possible from the short-lived "flush" of NO_3^-.

The experimental results show that rice plants do indeed take up very high amounts of N, when there is a high N-supply, and favorable conditions of light and temperature (Figures 2-5).

If N is available as NH_4^+, its high uptake and accumulation in the plants is harmful, even when light and temperature are favorable (Tables 4b and 5b).

On the other hand, if N is available as NO_3^-, any excess taken up is "sequestrated" into the substrate pool (Tables 5a and 7b), from where it cannot be taken back to the inducing pool. However, the NO_3^- in the substrate pool can be withdrawn and used for plant growth (Ferrari, 1973).

Using the mechanism of "NO_3^- sequestration", plants can take up an appreciable amount of N that may compensate for the low N supply later in the growing season.

If chemical fertilizers are used, the data show that NO_3^- can be used in higher amounts, over most of the conditions prevailing during the growing season in the tropics. Although high levels of radiation and/or temperature are likely to occur during the rainy season, the increase in N-uptake due to this extra energy supply will not trigger an outburst of NR activity. The NO_3^- taken up is stored and can be used later.

However, if NH_4^+ is used, any increase in the N-uptake caused by environmental factors will result in dramatic changes in the metabolism of the plants (Tables 5a, 5b, 6 and 10).

As indicated by the meterological data (Ministerio da Agricultura, 1970 and Bastos and Sa, 1972) the periods of high light supply are short-lived. Even if temperatures are favorable, plants will end up with a high level of reduced N to tie up into organic forms. This tying up process will most probably be done in a subsequent period of low photosynthetic activity.

In a situation like this, the leafy, tall indica plants do behave as non-N responsive. Perhaps the situation could be better described as "non-NH_4^+-N responsive varieties".

If, however, the light and temperature are kept down, as in the LL-LT experimental condition, NH_4^+-N can still produce an increase in plant growth, even at high rates of application (Figures 6 and 7).

The N carrier that can be used as a fertilizer in tropical rice fields, the amount to be used, and the time of application, can be best estimated if there is a good understanding of the environment and environmental changes throughout the growing season.

SUMMARY AND CONCLUSION

The responses of N-metabolism of tropical rice plants to environmental stress was studied by subjecting plants to three combinations of light and temperature, while applying N either as NO_3^- or NH_4^+ at three rates.

A negative relationship was observed between levels of reduced-N in the tissues and the fresh weight of the plants.

The results showed the existence of different regulation mechanisms when plants are fed NO_3^- or NH_4^+ under stress. Plants fed high rates of NO_3^- under conditions of environmental stress took the excess NO_3^- off the inducing pool and into the substrate pool. The expression "NO_3^- sequestration" was used to describe this phenomenon. This mechanism prevented the accumulation of reduced-N in the tissues of the plants studied.

The results point to photosynthetic efficiency as the prime factor related to NO_3^- utilization by rice plants and indicates the activation of the NR system to be a secondary factor.

This mechanism of NO_3^- regulation may be of fundamental importance for the N-economy of plants growing in tropical environments subjected to seasonal flushes of NO_3^-.

Ammonium-fed plants rid themselves of excess NH_3 by locking it up in amino and amide groups.

It was concluded that NO_3^- is a better source of N for rice plants than NH_4^+ over a wider range of environmental conditions.

The data indicate a physiological superiority of IR-8 over C-C for the utilization of N.

Studies of the free amino acid pools of rice plants showed accumulation of amides to be related to stressing conditions while a higher amino/amide ratio was related to conditions favorable to growth. Glutamine was the major amide present in the amino acid pool of stressed plants.

The composition of the amino acid pool of rice plants was shown to be a very sensitive indicator of stressing conditions in the environment.

The study indicates that a good understanding of the environment and environmental changes throughout the growing season possibilitates a better estimation of the N-carriers, N-rates, and the best time for N-application in the tropical rice fields.

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