NITROGEN UPTAKE AND UTILIZATION BY INTACT PLANTS

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INTRODUCTION

The overall objective of the research effort at North Carolina State University is to continue development of a dynamic plant growth model that is capable of simulating dry matter productivity and distribution in response to environmental conditions. We have reported progress in model development in a previous paper in this volume. Inherent in mechanistic models that describe the dynamics of plant growth should be the interrelationship between root function of supplying nitrogen and the shoot function of supplying photosynthate. We have proposed a conceptual model (Figure 1) which describes nitrogen uptake in plants as a function of the balance between root and shoot activities (Raper et al., 1976, 1977, 1978). According to this model, nitrogen uptake is regulated by the balance between the demand for carbon and nitrogen products within the various plant parts, and thus the subsequent balancing of nitrogen flux into the shoot and carbohydrate flux into the root. Since absorption of nitrogen by roots is an active process that requires metabolic activity, nitrogen uptake is responsive to level of soluble carbohydrate in the root (Raper et al., 1978). Uninterrupted uptake of nitrogen by roots, which are inherently low in soluble carbohydrate (Raper et al., 1976, 1978), thus is dependent upon concurrent translocation of soluble carbohydrate from the shoot.
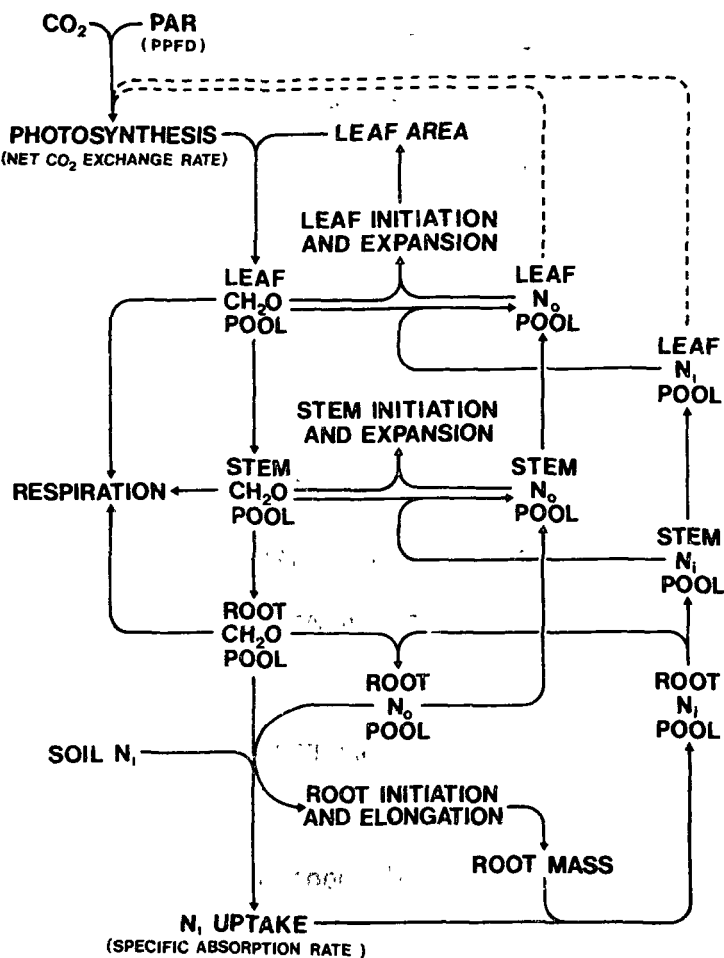
Figure 1. Scheme for balancing the nitrogen-supplying function of roots with the carbon-supplying function of shoots. N_i and N_o indicate inorganic ions (NO_3^- or NH_4^+) and organic products of nitrogen assimilation.

An assumption in the model is that when photosynthate in the shoot (leaves and stems) is limited, it is partitioned within the plant according to the scheme of Thornley (1976). The carbohydrate pool in the shoot is supplied by photosynthesis and is utilized as the source for both growth and respiration within the shoot and as the source for the root pool (Figure 1). Subsequent translocation of carbohydrate is responsive to the concentration of carbohydrate

in shoot pools and size and metabolic activity of sink pools (Wann and Raper, 1979, 1984). As nitrogen absorbed by roots is translocated to shoots, it stimulates initiation and expansion of new leaves (Raper and Peedin, 1978; Rufty et al., 1984). Nitrogen-stimulated metabolic demand of new leaves reduces availability of carbohydrate in the shoot pool for translocation to roots. Since nitrogen uptake is dependent on translocation of carbohydrate from shoot to roots, this model would predict that decreased translocation to roots would reduce nitrogen uptake and, ultimately, amount of nitrogen translocated to shoots. A subsequent reduction in initiation and expansion of new leaf tissue in response to decreased translocation of nitrogen (Raper and Peedin, 1978; Rufty et al., 1984) would reduce shoot demand for carbohydrate before reducing canopy photosynthetic rate (Raper and Peedin, 1978) and, thus, increase availability of carbohydrate for transport to roots. Thus, uptake of nitrogen and partitioning of carbon and nitrogen within the plant are regulated to maintain a functional balance between root and shoot growth.

Four inferences about nitrogen uptake can be drawn from this model for whole plant regulation of nitrogen uptake. First, when grown under near optimum conditions, a fluctuation should occur in the rate of nitrogen uptake which would be a function of the fluctuation in demand of carbon and nitrogen in the shoot and availability of carbohydrate within the roots to support the uptake process. Second, if root function is disturbed, an alteration should occur in rate of nitrogen uptake which might also be associated with a change in pattern of uptake as the plant establishes a new balance between root and shoot function. Third, if external nitrogen supply for the roots is discontinued, initial shifts in dry matter and nitrogen partitioning within the

plant should include a rapid decline in nitrogen content of shoots, with little immediate reduction in photosynthetic rate, and an increase in root dry weight. As the nitrogen stress continues and the nitrogen content of leaves fall below critical levels, photosynthetic rate should drop abruptly and dry weights of roots should decline relative to nonstressed plants. Fourth, if the balance between carbon and nitrogen supplies remains tightly coupled during vegetative growth, there should be little distinction in the utilization of ammonium and nitrate as sources of nitrogen if pH of the nutrient solution is controlled. We have designed experiments to test the validity of each of these inferences as a challenge to the concept of regulation of nitrogen uptake at the whole plant level by the interdependence of root and shoot functionality.

EXPERIMENTAL SYSTEM

In our experiments we use a walk-in growth room of the Phytotron at North Carolina State University (Downs and Thomas, 1983) which has a growing area of 8.92 m^2 and a height of 2.13 m between floor and light barrier. Aerial temperature is monitored and controlled within the growth room to within 0.3 C over an operational range of 7 to 40 C. A combination of cool white fluorescent and incandescent lamps, at an input wattage ratio of 10:3, provide a photosynthetic photon flux density (PPFD) of up to 750 μmol s^{-1} m^{-2} between wavelengths of 400 and 700 nm and photomorphogenic radiation (PR) of 10 w m^{-2} between wavelengths of 700 and 850 nm. Ambient CO_2 is monitored and maintained with injection of commercial grade gas.

A continuous-flow, hydroponic culture system has been constructed to operate within the growth room. The system includes four independent units with continuous monitoring and control of pH (±0.05 pH unit) and temperature (±0.2 C) of the solution. Each of the four units consists of an upper compartment where the plant root systems are suspended in 100 L of nutrient solution and a lower reservoir compartment containing 100 L of solution. Temperature and pH monitoring and control occurs in the reservoir compartment, and the nutrient solution is continuously circulated between the upper and lower compartments at 0.38 L s^{-1}. The upper compartment is divided into 12 8.3-L chambers with individual supply and return lines with the common reservoir. Each of the 12 root chambers per unit can contain one to four plants.

A Dionex Ion Chromatograph system 2110 with dual cation and anion columns is located adjacent to the growth room. From a single injection, anions plus monovalent cations in a sample of nutrient solution can be determined in less than 8 minutes. (With substitution of a new cation column being developed for Dionex, both monovalent and divalent cations can be separated in one column simultaneously.) Sampling and injection currently is done manually, but in the future the chromatograph will be connected on-line with the hydroponics system for automated sampling. Nutrient uptake rates and total nutrient accumulation by plants can be determined by depletion from the nutrient solution. Nutrient concentrations in solutions are replenished to treatment levels by additions of salts in response to depletion during a sampling interval of one day or less.

Figure 2. Effect of nitrogen stress and restoration of nitrogen availability on (A) number of mainstem and branch (inset) leaves and (B) canopy leaf area of soybean.
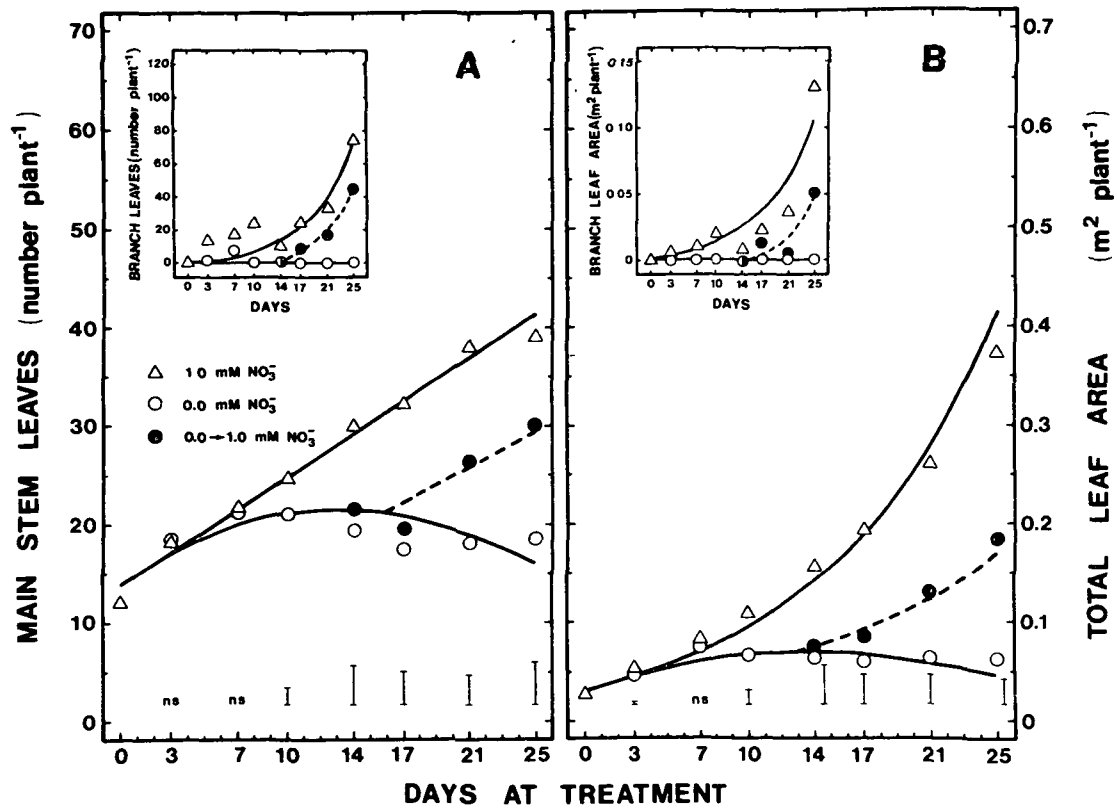
Nonnodulated soybean (<u>Glycine</u> <u>max</u> (L.) Merrill 'Ransom') plants were grown hydroponically for 14 days with 1.0 mM NO_3^- in a complete nutrient solution. Plants then were transferred to complete nutrient solution with 1.0 mM NO_3^- for 25 days, to a minus-nitrogen solution (0.0 mM NO_3^-) for 25 days, or to the 0.0 mM NO_3^- solution for 10 days followed by transfer to the 1.0 mM NO_3^- solution for 15 days. Throughout the experiment, day/night aerial temperature was 24 C. PPFD during the 9-h day was 700 µmol s^{-1} m^{-2}, and a 3-h interruption of the dark period by incandescent lamps was used to repress floral initiation. Ambient CO_2 concentration was 400 µL L^{-1}. The pH of the nutrient solution was maintained at 6.0.

When NO_3^- was removed from solution, NO_3^- storage pools in the plant were reduced rapidly (data not shown). As nitrogen became limiting for sustained growth and meristematic activity, initiation of both mainstem and branch leaves ceased within 7 days, along with expansion of canopy leaf area (Figure 2). In contrast, root growth of the nitrogen-stressed plants during this first week was increased relative to that of nonstressed plants (Figure 3), and thereafter was reduced as the nitrogen stress continued. Thus, under long-term nitrogen stress, there was an alteration in carbon and nitrogen partitioning predicted by the model (Figure 1). While nitrogen stress reduced the photosynthetic rate of leaves (Figure 4), it resulted in a greater reduction in the initiation and expansion of leaves (Figure 2). As a consequence, the activity of leaves as a sink for photosynthate was reduced more than their activity as a source. Until the total photosynthetic area

Figure 3. Effect of nitrogen stress and restoration of nitrogen availability

on dry matter accumulation in (A) whole plants, (B) leaves, (C) stems,

and (D) roots of soybean plants. Insets show dry weights of (A) shoot,

(B) leaves, (C) stems, and (D) roots of nitrogen-stressed plants as
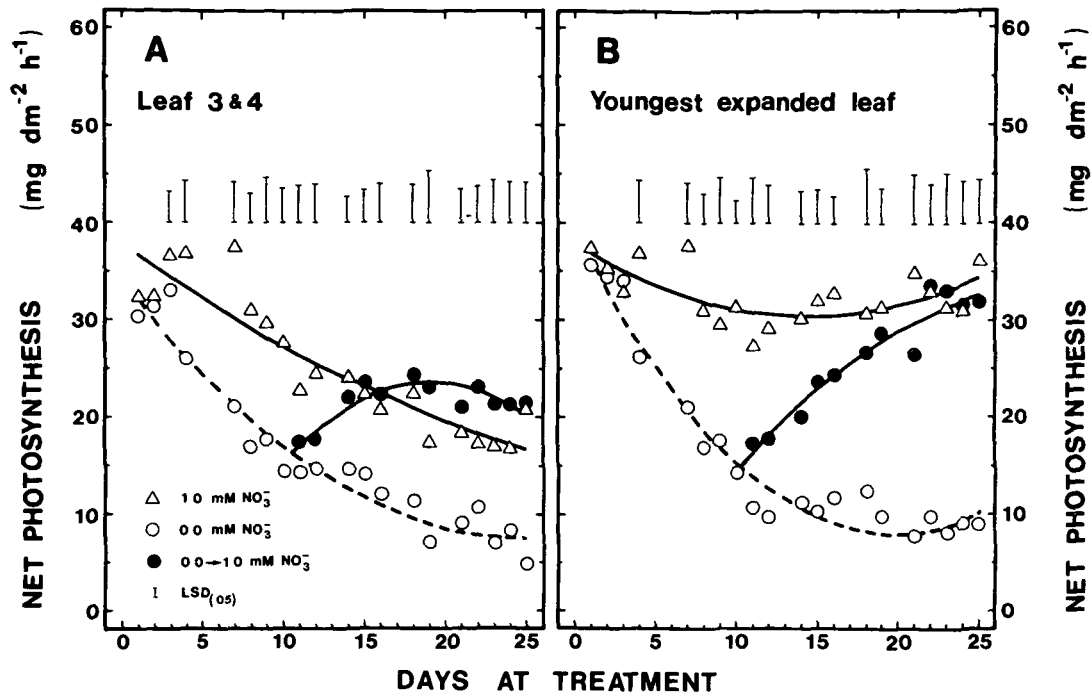
percentages of dry weights of nonstressed plants.
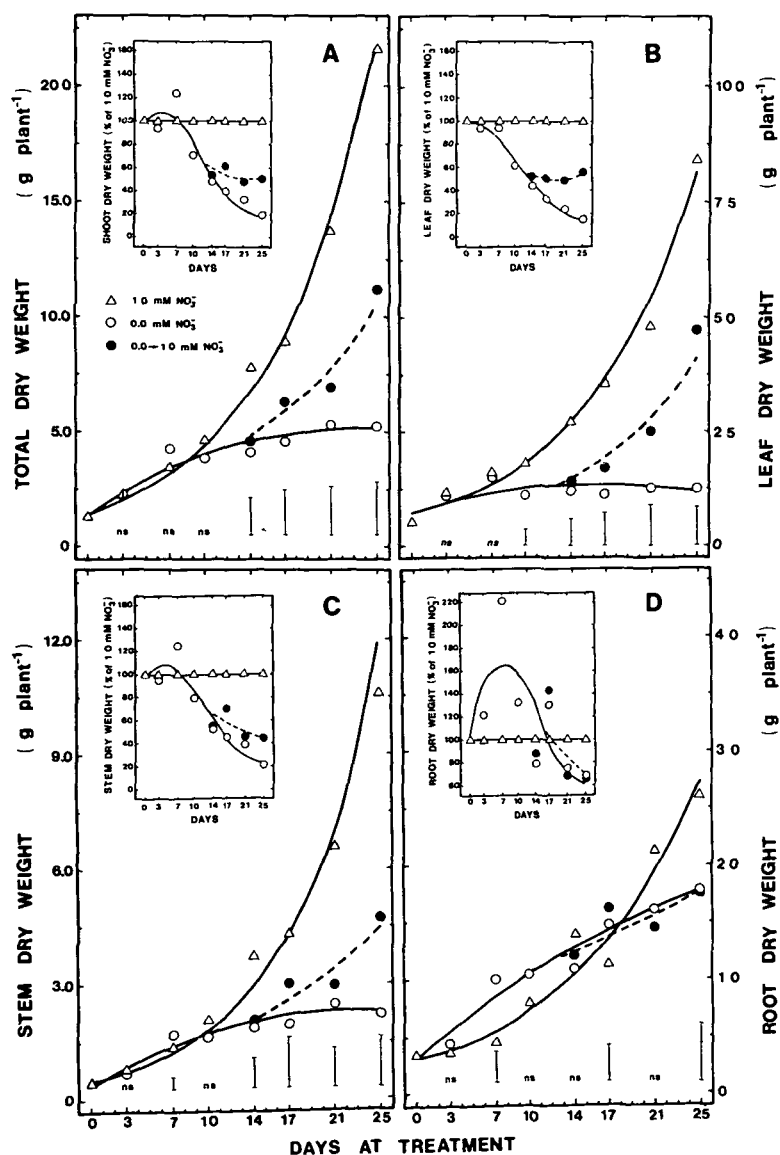
Figure 4. Effect of nitrogen stress and restoration of nitrogen availability on photosynthetic rate of (A) older, mature leaves, and (B) youngest fully-expanded leaves on the day of measurement.

of the canopy was reduced significantly, the availability of photosynthate in the leaf pool for translocation to stems and roots was increased.

FLUCTUATIONS IN NITROGEN UPTAKE RATE

Another inference from the model (Figure 1) is that uptake rates of nitrogen, rather than remaining constant during growth, should oscillate with a periodicity related to the interval of leaf emergence and the associated changes in sink activity of leaf growth. To confirm this inference of the model, soybean plants were grown in the hydroponic system for 31 days with

a complete nutrient solucion containing 1.0 mM NO_3^-. The depletion of NO_3^- from solution was monitored daily by ion chromatography, recorded, and NO_3^- was added to the solution as $Ca(NO_3)_2$ to return the concentration in solution to 1.0 mM NO_3^-. Solutions were completely changed every 2 days to avoid depletion of any nutrient below 80% of the initial concentration. Uptake rate of NO_3^- per plant during each 24-h period was calculated as mM NO_3^- removed from the solution in each hydroponic system divided by number of plants in the system during that day.

As predicted by the model (Figure 1), uptake rate of NO_3^- oscillated between maxima and minima with a periodicity of 3 to 5 days (Figure 5A). The interval between emergence of successive mainstem soybean leaves is
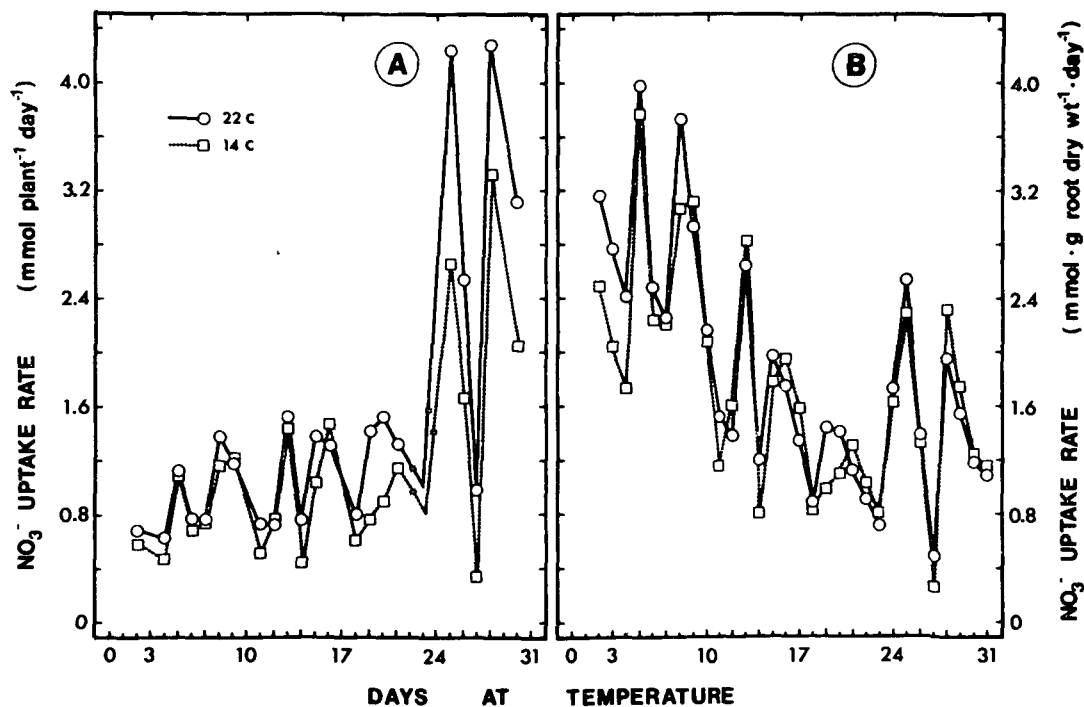

Figure 5. Uptake rate of NO_3^- per plant (A) and per g root dry weight (B) of soybean plants grown at two root-zone temperatures. Uptake rates were determined by depletion of NO_3^- from a replenished nutrient solution. (Adapted from Tolley and Raper, 1985.)

about 4 days at the 26/22 C day/night aerial temperature used in this study (Hesketh et al., 1973; Thomas and Raper, 1976). The periodicity of the oscillations in NO_3^- was not affected by the root-zone temperatures of 14 or 22 C, although uptake rates per plant were lower at 14 then 22 C. The study was repeated with concentrations of nitrogen in solution at 10.0 and 1.0 mM NO_3^-. Although the minima and maxima of the osicllations were greater at 10.0 than 1.0 mM NO_3^-, the periodicity of oscillations remained between 3 and 5 days (data not shown). These results indicate that, while the maximum rate of uptake may be regulated at the root level, the control of the actual uptake does not reside in the roots themselves, but is a function of the interdependence of roots and shoots.

DISTURBANCE OF ROOT FUNCTION

When root function is disturbed, the model indicates that an alteration should occur in rate of nitrogen uptake as the plant establishes a new balance between root and shoot function. The previous experiment serves to test this hypothesis since plants were grown at root temperatures of 14 and 22 C while aerial conditions remained the same. Total plant and root dry weights were reduced slightly at the 14 C, relative to the 22 C, root temperature (Figure 6). Nitrogen accumulation in the plants at 14 C was reduced proportionally with the dry weight (data not shown).

During the initial 5 to 10 days following transfer of the plants to the 14 C root temperature, the uptake rate of NO_3^- per g root dry weight was lower at 14 than 22 C (Figure 5B). After this initial period, there was little
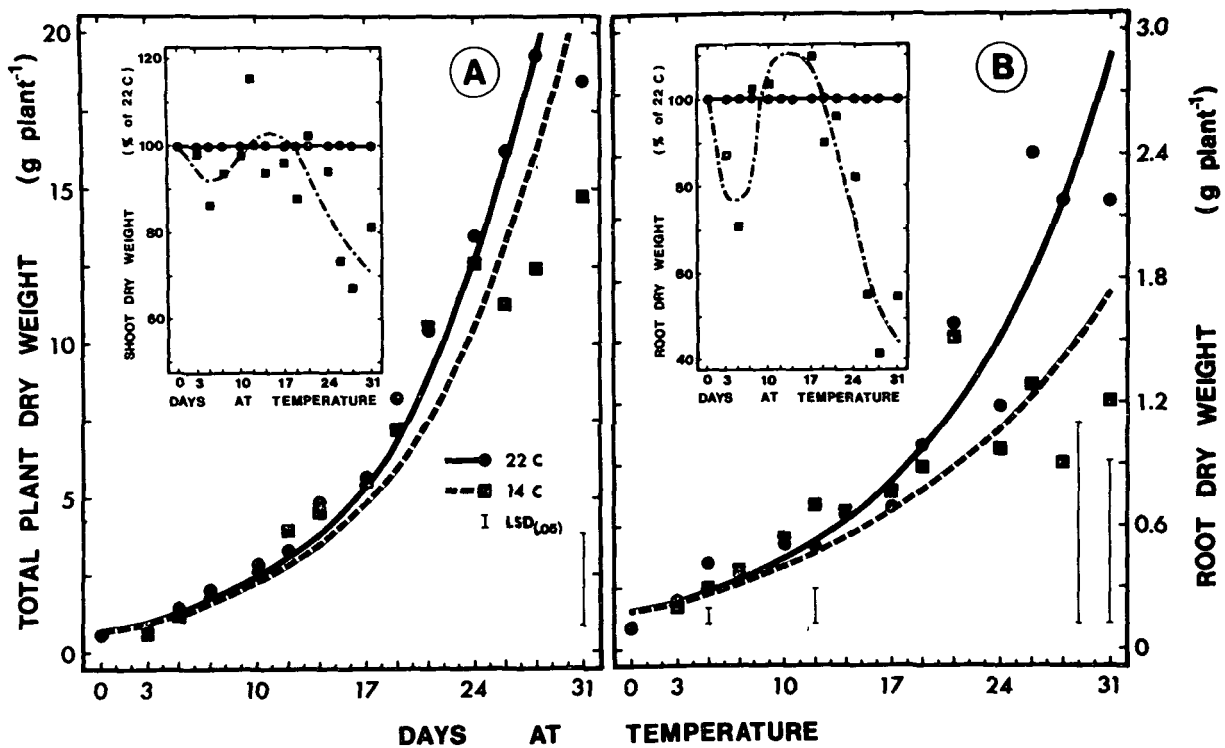
Figure 6. Effect of root-zone temperature on dry matter accumulation in (A) whole plants, and (B) roots of soybean. Shoot (A) and root (B) dry weights at 14 C are shown in insets as percentages dry weights at 22C. (Adapted from Tolley and Raper, 1985.)

difference in uptake rate per g root. The initial reduction in rate of NO_3^- uptake per g root presumably was a direct response of root metabolism or membrane permeability to the lower temperature (Osmond et al., 1982). As root growth continued, however, uptake rate per g root at 14 C became indistinguishable from that at 22 C. Thus, the initial reduction in uptake rate of NO_3^- per plant (Figure 5A), as well as the initial decrease in total nitrogen accumulation by plants (data not shown), at 14 C can be attributed to an effect of temperature on the absorption processes of NO_3^- by roots.

The continued reduction in uptake rate of NO_3^- per plant at 14 C after the initial period of exposure is a consequence of the reduction in root growth (Figure 6B).

UTILIZATION OF AMMONIUM AND NITRATE SOURCES

Plants supplied with moderate concentrations of NH_4^+ in solution generally grow poorly compared with plants supplied with NO_3^-. Experiments were conducted in the flowing hydroponics system to determine whether growth restrictions could be avoided over an extended period in the presence of NH_4^+ if root-zone pH were controlled and if plants were exposed to NH_4^+ during exponential growth when carbohydrate fluxes to the root are coordinated with the rate of nitrogen acquisition.

In an initial experiment, vegetative soybean plants at the beginning of the exponential growth stage were transferred to complete nutrient solutions containing nitrogen as either 1.0 mM NO_3^- or 1.0 mM NH_4^+. Acidity of the solutions was constantly monitored and maintained at pH 5.8. Experiments were conducted under three sets of aerial environments: (1) standard conditions with PPFD of 700 μmol $s^{-1}m^{-2}$ and CO_2 at 400 μL L^{-1}, (2) low PPFD conditions with PPFD of 350 μmol $s^{-1}m^{-2}$ and CO_2 at 400 μL L^{-1}, and (3) high CO_2 conditions with PPFD of 700 μmol $s^{-1}m^{-2}$ and CO_2 at 1000 μL L^{-1}. The source of nitrogen did not alter growth or nitrogen accumulation of plants over a 4-week growth interval (Figure 7) under any of the three environmental conditions. In a related experiment with tomato (Lycopersicon esculentum L. Mill. 'Vendor'), growth and nitrogen accumulation by plants during exponential

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Figure 7. Effect of NO_3^- and NH_4^+ sources on dry matter and nitrogen accumulation in soybean plants grown under standard (PPFD = 700 μmol $s^{-1}m^{-2}$ and CO_2 = 400 μL L^{-1}), low PPFD (PPFD = 325 μmol $s^{-1}m^{-2}$ and CO_2 = 400 μL L^{-1}), and high CO_2 (PPFD = 700 μmol $s^{-1}m^{-2}$ and CO_2 = 1000 μL L^{-1}) conditions. (Adapted from Rufty et al., 1983.)

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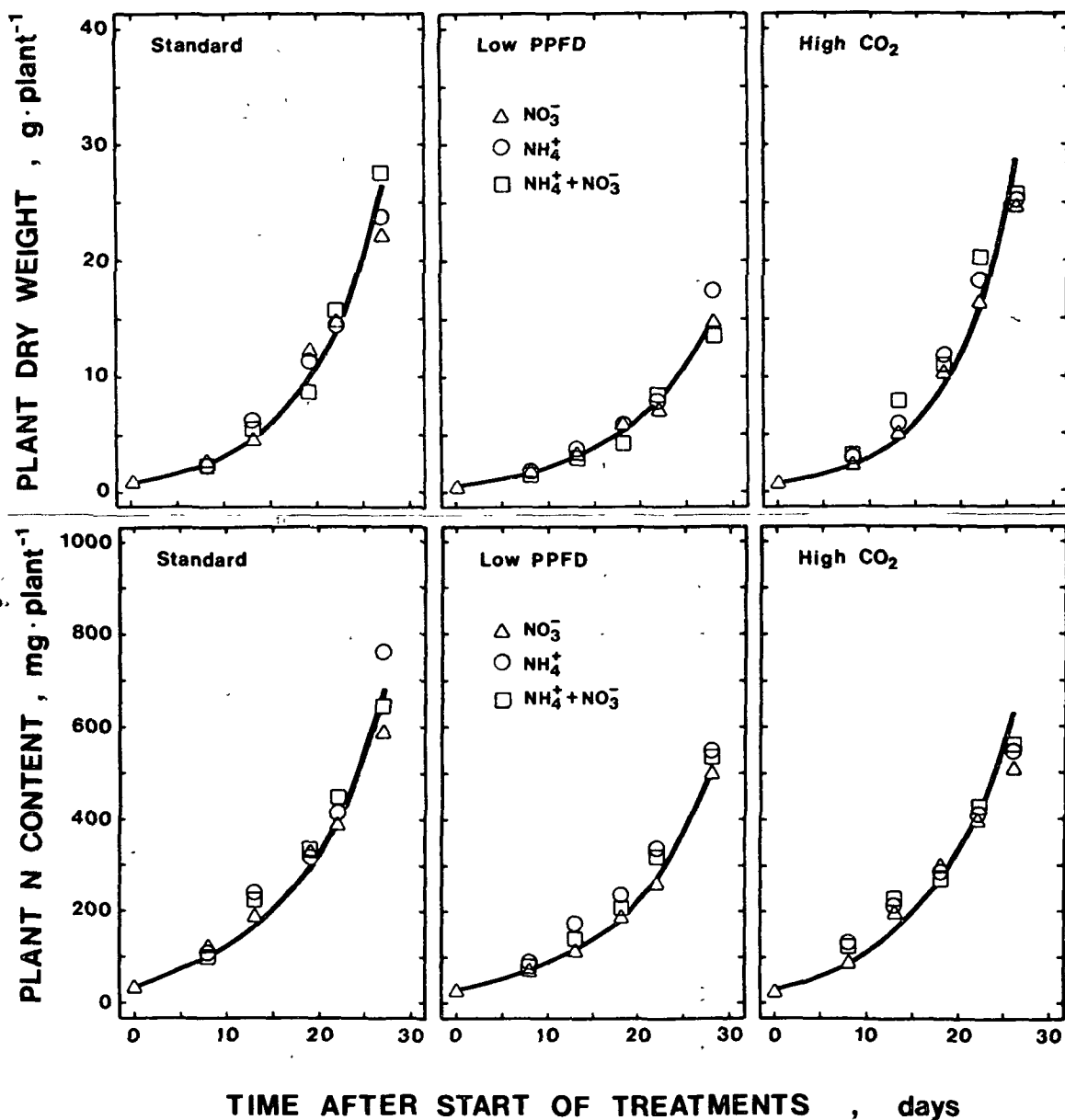
growth were not altered by source of nitrogen when acidity of the solution was controlled at pH 6.0 (data not shown).

In a subsequent experiment, soybean plants were grown with 1.0 mM NH_4^+ as the sole nitrogen source with acidity of the solution maintained at pH 6.1, 5.1, and 4.1. While plants exposed to NH_4^+ at pH 6.1 accumulated dry matter (Figure 8) and nitrogen (data not shown) at rates comparable to plants exposed to NO_3^- as the sole nitrogen source (cf. Figure 7), growth was reduced at pH 5.1 and ceased within days of initial exposure to NH_4^+ at pH 4.1. The decreased growth at low pH under NH_4^+ nutrition was not a singular response to acidity of the nutrient solution. In another experiment (Rufty et al., 1982) soybean plants receiving 1.0 mM NO_3^- as the nitrogen source initially responded to decreased solution acidity from pH 6.1 to pH 4.1 with a reduction in growth rate, but the plants receiving NO_3^- acclimated to the low pH and after 3 weeks had attained growth rates comparable to plants growing at pH 6.1. Apparently, there is an interaction between external pH of the nutrient solution and the ability of the plants to assimilate NH_4^+ as it enters the roots. Experiments are planned to further explore the relationship between external acidity and NH_4^+ toxicity.

CONCLUSION

The results of our experiments support the proposed conceptual model (Figure 1) that relates nitrogen uptake activity by plants as a balanced interdependence between the carbon-supplying function of the shoot and the nitrogen-supplying function of the roots. The data are being used to modify
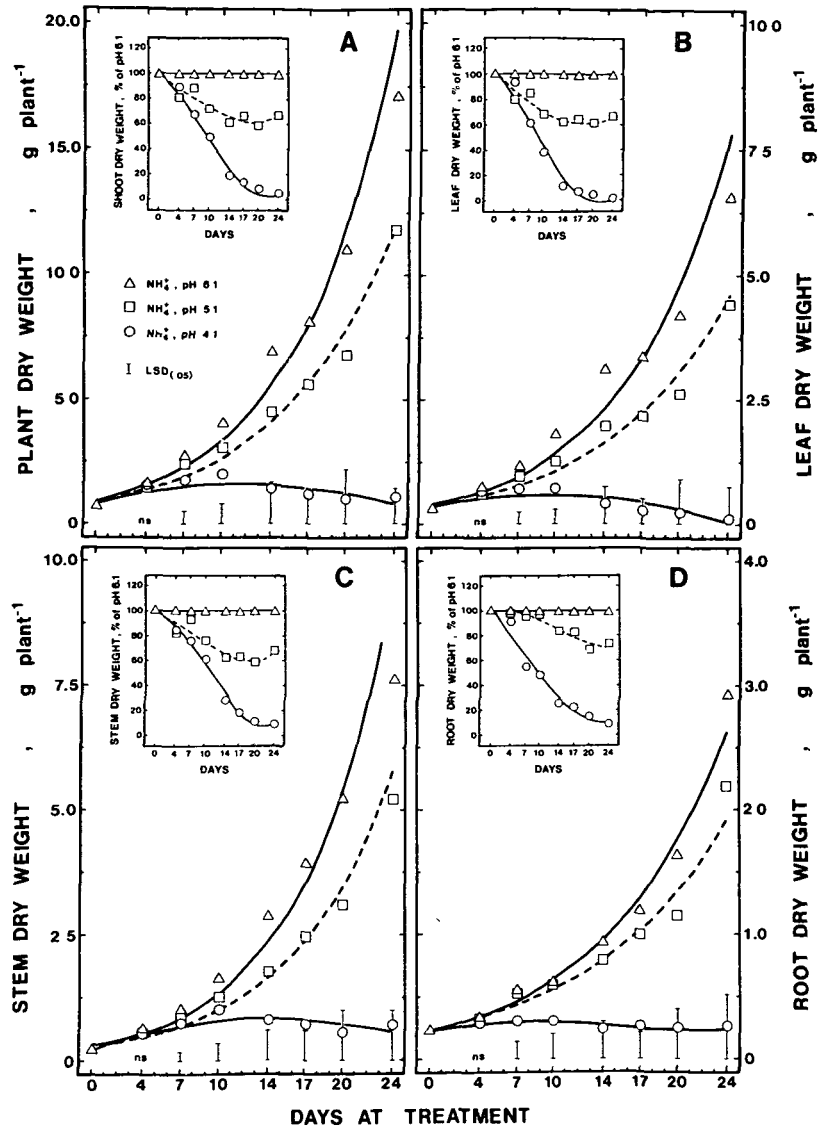
Figure 8. Effect of pH of the nutrient solution on dry matter accumulation by soybean plants receiving NH_4^+ as the sole nitrogen source.

a dynamic simulation model of plant growth, which presently describes carbon

flows through the plant (Wann and Raper, 1979, 1984), to describe nitrogen

uptake and assimilation within the plant system. Although several models have

been proposed to predict nitrogen uptake and partitioning, they emphasize

root characteristics affecting nutrient uptake and rely on empirical methods

to describe the relationship between nitrogen and carbon flows within the plant. We propose, on the other hand, to continue to attempt a mechanistic solution in which the effects of environment on nitrogen (as well as carbon) assimilation are incorporated through their direct effects on photosynthesis, respiration, and aging processes.

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