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Cyclic Variations in Nitrogen Uptake Rate in Soybean Plants[1]

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LESLIE C. TOLLEY*[2] AND C. DAVID RAPER, JR.
Department of Soil Science, North Carolina State University, Box 7619, Raleigh, North Carolina 27695-7619

ABSTRACT

Uptake of NO_3^- by nonnodulated soybean plants (*Glycine max* L. Merr. cv Ransom) growing in flowing hydroponic culture at 22 and 14°C root temperatures was measured daily during a 31-day growth period. Ion chromatography was used to determine removal of NO_3^- from solution during each 24-hour period. At both root-zone temperatures, rate of NO_3^- uptake per plant oscillated with a periodicity of 3 to 5 days. The rate of NO_3^- uptake per plant was consistently lower at 14°C than 22°C. The lower rate of NO_3^- uptake at 14°C during the initial 5 to 10 days was caused by reduced uptake rates per gram root dry weight, but with time uptake rates per gram root became equal at 14 and 22°C. Thereafter, the continued reduction in rate of NO_3^- uptake per plant at 14°C was attributable to slower root growth.

The interrelationship between root function of supplying nitrogen and the shoot function of supplying photosynthate should be inherent in mechanistic models that describe the dynamics of plant growth. Raper *et al.* (8, 9, 11) have developed a conceptual model which describes nitrogen uptake in plants as a function of the balance between root and shoot activities. 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.

Absorption of nitrogen by roots is an active process requiring metabolic activity (4), and thus is responsive to soluble carbohydrate levels in the root (3, 5). Since roots are inherently low in soluble carbohydrate (8, 11), uninterrupted uptake of nitrogen is dependent upon concurrent translocation of soluble carbohydrate from the shoot.

One of the assumptions in the model of Raper *et al.* (8, 9, 11) is that when photosynthate is limiting, it is partitioned within the plant according to Thornley's scheme (18). 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. Subsequent translocation of carbohydrate is responsive to the concentration of carbohydrate in the shoot pool and the size and metabolic activity of sink pools (19, 20). As nitrogen absorbed by roots is translocated to the shoot, it stimulates initiation and expansion of new leaves (10, 13). The nitrogen-stimulated metabolic demand of new leaves reduces the availability of carbohydrate in the shoot pool for translocation to roots. Since nitrogen uptake is dependent on translocation of carbohydrate from the shoot to the roots, this model would predict that decreased translocation to roots would reduce nitrogen uptake and, ultimately, amount of nitrogen translocated to the shoot. A subsequent reduction in initiation and expansion of new leaf tissue in response to decreased translocation of nitrogen (10, 13) would reduce shoot demand for carbohydrate before reducing the canopy photosynthetic rate (10) and, thus, increase the availability of carbohydrate for transport to the 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.

Two inferences about nitrogen uptake can be drawn from this model for whole plant regulation of nitrogen uptake. First, when grown under near optimal conditions, a fluctuation should occur in the rate of nitrogen uptake which would be a function of the fluctuation in demand for 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. In this study, nonnodulated soybean seedlings were grown under near optimal environmental conditions or subjected to cool root-zone temperature to alter root function (7) and ultimately nitrogen accumulation within the plant. Nitrogen uptake from nutrient solution and nitrogen accumulation in plant tissue were followed over time to establish the existence of a pattern in nitrogen uptake and to examine possible effects of disturbed root function on the nature of this uptake pattern.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* L. Merr. cv Ransom) were germinated as described previously (13, 15). After 3 d, 48 seedlings with radical lengths of 8 to 12 cm were placed into each of four 200-L continuous-flow, hydroponic culture systems equipped for pH and temperature control (15). Each of these chambers consists of an upper compartment where the plant root systems are suspended in 100 L of complete nutrient solution, and a lower reservoir compartment containing 100 L of complete nutrient solution. Nutrient solution is continuously circulated between the two compartments. The culture systems were located in a single growth room of the North Carolina State University phytotron (2) programmed for day/night temperatures of 26/22 ± 0.3°C with abrupt day-to-night transitions. A PPFD[3] of 700 ± 50 μmol m^{-2} s^{-1} between wavelengths of 400 to 700 nm and a PR of 12 w m^{-2} between wavelengths of 700 to 850 nm were provided during the 9-h day (0800–1700 h) from a combination of cool-white fluorescent and incandescent lamps at an input wattage ratio of 10:3. The 15-h night period included a 3-h interruption after 6 h by the incandescent lamps to effect a LD photoperiod and repress floral development (16). During the 3-h interruption, PPFD was 70 ± 10 μmol m^{-2} s^{-1} and PR was 10 w m^{-2}. Ambient CO_2 concentration was maintained at 400 ± 25 μl L^{-1}.

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[3] Abbreviations: PPFD, photosynthetic photon flux density; PR, photomorphogenic radiation.

During the pretreatment period, the temperature of culture solutions was maintained at 22 ± 0.2°C. Solution pH was maintained at 5.8 ± 0.1 by automated additions of 0.01 N H_2SO_4 or 0.01 N $Ca(OH)_2$. Initial concentrations of nutrients in solution were 1.0 mM NO_3^-, 0.5 mM $H_2PO_4^-$, 1.65 mM K^+, 0.5 mM Ca^{2+}, 1.0 mM Mg^{2+}, 1.65 mM SO_4^{2-}, 17.0 μM B, 3.0 μM Mn, 2.2 μM Cl, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 1 mg Fe L^{-1} as Fe-EDTA.

Treatments were started 14 d after transplanting when the third trifoliolate was unfolding. Temperature of the solution in two of the hydroponic systems was changed to 14°C, and in the other two, was maintained at 22°C. Nutrient concentrations and pH of solutions and all aerial environmental conditions were the same as during pretreatment. Samples of nutrient solutions from each system were taken daily at 1300 to 1400 h and analyzed for NO_3^- with a Dionex[4] Ion Chromatograph model 10. Depletion of NO_3^- from solution during the preceding 24 h was recorded and NO_3^- was added to the solution as $Ca(NO_3)_2$ to return the concentration to 1.0 mM NO_3^-. To avoid nutrient depletion effects, half of the solution in each hydroponic system was replaced every 2 d. At this time, the solution in each chamber was sampled to determine NO_3^- concentration, and then circulation between the two compartments of a chamber was discontinued. The lower reservoir was drained and then refilled with fresh nutrient solution, while the plant root systems remained suspended in nutrient solution in the upper compartment. This process, which takes less than 10 min, allows for replenishment of fresh nutrient solution into the hydroponic system with minimal disturbance to plant function. After remixing between the upper and lower compartments for 15 min, the solution in each system was analyzed and appropriate salts added, if necessary, to adjust nutrients to the initial concentrations. Rate of NO_3^- uptake per plant during each 24-h period was calculated as mmol of NO_3^- removed from the solution in each of the hydroponic systems divided by number of plants in the system during that day and then averaged for the two hydroponic systems at each temperature.

Beginning on the day treatments were initiated, four to eight plants were sampled from each root-temperature treatment at 2- to 3-d intervals over a 31-d growth period. Plants were sampled between 1300 and 1400 h. At each sampling, leaf area was measured photometrically with a Hayashi Denko AAM-5 area meter. Plants were separated into leaves, stems, and roots. The tissues were immediately frozen, then freeze-dried, weighed, and ground. Total nitrogen in each plant part was determined by a modified Kjeldahl procedure to digest all nitrogenous compounds, including NO_3^-, to NH_4^+ (6), and NH_4^+ was analyzed colorimetrically (1).

Net CO_2 exchange rates were obtained on every sampling date from the second or third youngest, fully expanded main-stem leaf of 8 to 10 plants per treatment. Upper and lower surfaces of a 10-cm^2 area of an attached leaf were enclosed in a clamp-on Plexiglas cuvette. Air at ambient temperature and CO_2 concentration was passed through the cuvette at a flow rate of 17 ml s^{-1}. The cuvette remained in place for 30 to 60 s while differences between CO_2 concentrations of incoming and exhaust air streams were determined with an Anarad AR-500R IR gas analyzer. Determinations of net CO_2 exchange rates were made between 1200 and 1300 h.

RESULTS AND DISCUSSION

Dry matter accumulation in whole plants (Fig. 1A) and roots (Fig. 1B) was less when soybean plants were grown at a root temperature of 14°C than at 22°C. The effect of root temperature was more pronounced on root than shoot growth. Shoot growth was affected only slightly at 14°C relative to 22°C for the initial 19 d, and shoot dry weight declined to about 70% of that at 22°C on day 31 (Fig. 1A, inset). Root growth at 14°C, however, was more severely reduced with the result that root dry weight was about 50% of that at 22°C on day 31 (Fig. 1B, inset).

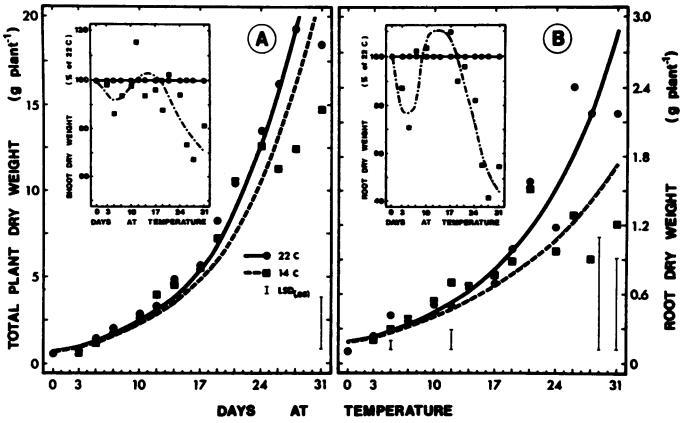

FIG. 1. Effect of root-zone temperature on dry matter accumulation in (A) whole plants and (B) roots of soybean. LSD values are shown when significant at the 0.05 level. The insets show shoot (A) and root (B) dry weights at 14°C expressed as per cent of the dry weights at 22°C. Regression equations relating dry weight of whole plants (DW_p) and roots (DW_R) to days at temperature (d) are: at 22°C, ln DW_p = −0.304 + 0.118(d), r = 0.98, and ln DW_R = −1.716 + 0.089(d), r = 0.96; at 14°C, ln DW_p = −0.273 + 0.109(d), r = 0.97, and ln DW_R = −1.579 + 0.069(d), r = 0.90.

Net CO_2 exchange rates of fully expanded upper leaves averaged over the treatment period were 18.4 ± 2.1 (SD) and 18.5 ± 1.7 (SD) μmol m^{-2} s^{-1} for plants at 22 and 14°C root temperatures. Since net CO_2 exchange rates were nearly equal throughout the treatment period, the reduction in dry matter production of plants at 14°C resulted predominately from the decline in rate of leaf production. Rates of both leaf emergence and leaf area production were significantly reduced at 14°C relative to 22°C (data not shown). These results concur with previous findings (14). The reduction in leaf production may have been related to number of branch stems and leaves since temperature has a greater effect on initiation of axillary branches than on main-stem leaves (16, 17).

From the model for balanced root and shoot functioning (8, 9, 11), we proposed that the rate of nitrogen uptake would fluctuate as a result of fluctuations in the demand for carbon and nitrogen in the shoot and availability of carbohydrate within the root to support the uptake process. Rate of NO_3^- uptake per plant as calculated from solution depletion, oscillated between maxima and minima with a periodicity of 3 to 5 d (Fig. 2A). The oscillations in rate of NO_3^- uptake occurred at both root temperatures. However, periodicity was not affected by root-zone temperature, although uptake rates per plant were lower at 14°C than 22°C. These results indicate that control of the periodicity of NO_3^- uptake may be associated more closely with shoot development, whereas fluctuations in the amplitude of the amount of NO_3^- taken up may be more closely related to root function.

The cumulative depletion of nitrogen from replenished solutions over the treatment period was in close agreement with the total nitrogen accumulation in plants determined by tissue analysis (Fig. 3). The closeness of agreement between the two methods serves as a validation of the rates of NO_3^- uptake as calculated from solution depletion.

Under the conditions of this experiment, root dry matter

[4] Trade names are given as part of the exact experimental conditions and not as an endorsement to the exclusion of other products that also might be suitable.

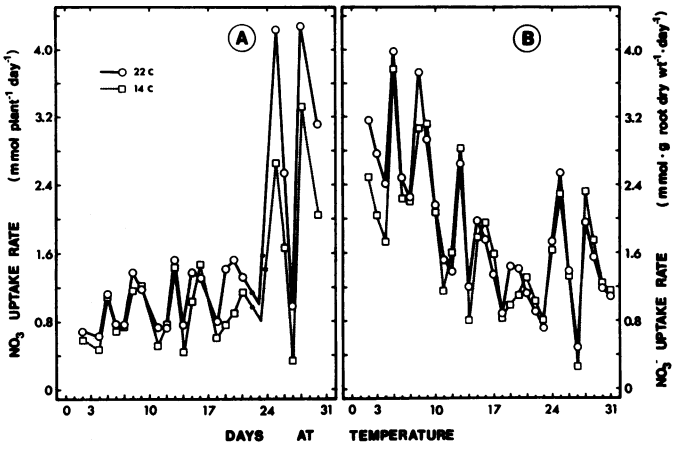

FIG. 2. Effect of root-zone temperature on (A) rate of NO_3^- uptake per plant as determined by solution depletion and (B) rate of NO_3^- uptake per g root dry weight as calculated from the measured and interpolated rates per plant shown in (A) and root dry weights estimated from regression equations for Figure 1B. Note that nitrate uptake rates on day 23 were estimated as data were incomplete for this sampling period.

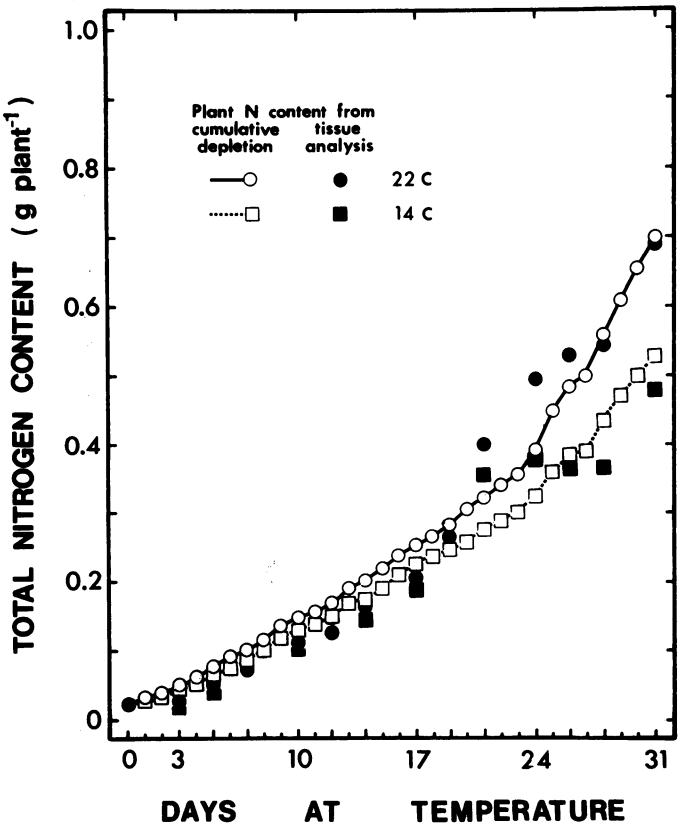

FIG. 3. Effect of root-zone temperature on total nitrogen accumulation in soybean plants. (○, □), Cumulative depletion from replenished solutions; (●, ■), analysis of tissue samples.

accumulation is positive (19, 20) and can be described by regression equations (Fig. 1B) to account for the plant-to-plant variability that is inherent in destructive sampling. The solution measurements, however, represent the functioning of a larger population. Therefore, the rate of NO_3^- uptake per g root dry weight (Fig. 2B) was estimated from the measured and interpolated rates of NO_3^- uptake per plant (Fig. 2A) and the regression equations for root growth (Fig. 1B). During the initial 5 to 10 d following transfer of plants to the 14°C root temperature, the rate of NO_3^- uptake per g root dry weight was lower at 14 than 22°C (Fig. 2B). The initial reduction in rate of NO_3^- uptake per g root perhaps was a direct response of root metabolism or membrane permeability to the lower temperature (7, 12). As root growth continued, uptake rate per g root at 14°C became indistinguishable from that at 22°C. Thus, the initial reduction in rate of NO_3^- uptake per plant (Fig. 2A), as well as the initial decrease in total nitrogen accumulation by plants (Fig. 3), at 14°C was attributable to an effect of temperature on the absorption processes of NO_3^- by roots. The continued reduction in rate of NO_3^- uptake per plant at 14°C and decrease in total nitrogen accumulation after the initial period of exposure were a consequence of the reduction in root growth (Fig. 1B).

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