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Utilization of Ammonium as a Nitrogen Source[1]

EFFECTS OF AMBIENT ACIDITY ON GROWTH AND NITROGEN ACCUMULATION BY SOYBEAN

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

ABSTRACT

Dry matter accumulation of plants utilizing NH_4^+ as the sole nitrogen source generally is less than that of plants receiving NO_3^- unless acidity of the root-zone is controlled at a pH of about 6.0. To test the hypothesis that the reduction in growth is a consequence of nitrogen stress within the plant in response to effects of increased acidity during uptake of NH_4^+ by roots, nonnodulated soybean plants (*Glycine max* [L.] Merr. cv Ransom) were grown for 24 days in flowing nutrient culture containing 1.0 millimolar NH_4^+ as the nitrogen source. Acidities of the culture solutions were controlled at pH 6.1, 5.1, and 4.1 ± 0.1 by automatic additions of 0.01 N H_2SO_4 or $Ca(OH)_2$. Plants were sampled at intervals of 3 to 4 days for determination of dry matter and nitrogen accumulation. Rates of NH_4^+ uptake per gram root dry weight were calculated from these data. Net CO_2 exchange rates per unit leaf area were measured on attached leaves by infrared gas analysis. When acidity of the culture solution was increased from pH 6.1 to 5.1, dry matter and nitrogen accumulation were reduced by about 40% within 14 days. Net CO_2 exchange rates per unit leaf area, however, were not affected, and the decreased growth was associated with a reduction in rates of appearance and expansion of new leaves. The uptake rates of NH_4^+ per gram root were about 25% lower throughout the 24 days at pH 5.1 than at 6.1. A further increase in solution acidity from pH 5.1 to 4.1 resulted in cessation of net dry matter production and appearance of new leaves within 10 days. Net CO_2 exchange rates per unit leaf area declined rapidly until all viable leaves had abscised by 18 days. Uptake rates of NH_4^+, which were initially about 50% lower at pH 4.1 than at 6.1, continued to decline with time of exposure until net uptake ceased at 10 days. Since these responses also are characteristic of the sequence of responses that occur during onset and progression of a nitrogen stress, they corroborate our hypothesis.

Several plant species supplied with moderate concentrations of NH_4^+ as the sole nitrogen source generally do not grow as well as when supplied with similar amounts of NO_3^- (7, 8, 20). The reduction in growth with NH_4^+ as the nitrogen source has been attributed to the combined effects of acidification of the root-zone associated with the excess influx of cations relative to influx of anions during absorption of NH_4^+ (8) and toxic accumulation of free NH_4^+ or ammonia in plant tissues (18, 29).

Utilization of NH_4^+ is enhanced when acidification of the rhizosphere is alleviated during NH_4^+ uptake. When carbonates were applied with the nutrient solution or mixed in the substrate of beans (*Phaseolus vulgaris* L.) growing in sand culture, their buffering activity enhanced growth of NH_4^+-fed plants (1); and for both tomato (*Lycopersicon esculentum* L. Mill.) and soybean (*Glycine max* [L.] Merr.) plants in flowing hydroponic culture, growth of NH_4^+-fed plants was equal to that of NO_3^--fed plants when solution acidity was monitored and automatically controlled at pH 6.0 or 5.8 (17, 24). Increases in ambient acidity have several consequences on ammonium uptake and utilization. First, the rate of NH_4^+ uptake per unit root mass is decreased by increased acidity (12–14). Second, root growth is reduced as acidity is increased (23), which could further reduce uptake of nitrogen from an NH_4^+ source. Finally, most of the NH_4^+ entering plants is assimilated into soluble amide and amino compounds in the roots and transported to the shoot (5, 9, 10). Increased acidity of the root-zone apparently initiates a series of events which result in increased degradation of organic nitrogen compounds stored within the leaves with release of free NH_4^+ or ammonia (2, 5). Availability of carbohydrate regulates reincorporation of the released NH_4^+ or ammonia into organic compounds and thus governs the occurrence of toxic effects (5), including reduced photosynthetic activity (1, 18).

Based on this analysis of the literature, we propose that the reduction in plant growth associated with NH_4^+ nutrition begins with the reduction in both root mass and uptake rate of NH_4^+ per unit root mass in response to acidification of the root-zone during absorption of NH_4^+. The initial consequences of NH_4^+ uptake on plant growth thus would be related to a restriction in nitrogen availability, including a reduction in leaf initiation and expansion and total dry matter production, but not a reduction in the photosynthetic rate per unit leaf area (21, 27, 28). With further increases in root-zone acidity during continued absorption of NH_4^+, nitrogen stress within the plant would increase, ultimately leading to a decline in photosynthetic activity of the leaves and a limitation of carbohydrate reserves within the plant. As carbohydrate availability declined to levels below those necessary to support growth respiration and approached the levels required to meet the more inflexible demands of maintenance respiration, the carbon skeletons from continued degradation of soluble organic nitrogenous compounds would be rapidly consumed by maintenance respiration. The resulting accumulation of free NH_4^+ or ammonia within leaf tissues would then lead to the appearance of toxicity symptoms. The objective of this study, therefore, was to evaluate the hypothesis that reductions in plant growth and appearance of symptoms of NH_4^+ toxicity are the results of nitrogen stress within the plant in response to increased acidity in the root-zone.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr. cv Ransom) seeds were germinated in moist paper towels in the dark at 25°C and 98%

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RH. When radicle lengths were between 9 and 12 cm, 32 seedlings were transplanted into each of three 200-L continuous-flow, hydroponic culture systems equipped with pH and temperature control (16, 23) and located within a walk-in growth room of the phytotron at North Carolina State University (4). Each hydroponic system consists of 12 separate 8.3-L root compartments and a 100-L reservoir. Nutrient solution is pumped directly from the reservoir, which contains heating and cooling coils and pH controls, to each root compartment at a flow rate of 1.4 L min^{-1}. The solution is saturated with O_2 as it enters the compartments and is re-aerated by tumbling action as it is returned to the reservoir.

Throughout the experiment the growth room was programmed for day/night aerial temperatures of 26/22°C. During the 9-h d period, PPFD[3] of 700 ± 25 μmol m^{-2} s^{-1} between wavelengths of 400 to 700 nm and PR of 12 W m^{-2} between wavelengths of 700 to 850 nm were provided by a combination of cool-white fluorescent and incandescent lamps with an input wattage ratio of 3:1. The incandescent lamps were turned on for 3 h midway through the 15-h night period to effect a long-day photoperiod and repress floral development (25). During the interruption, PPFD was 70 ± 10 μmol m^{-2} s^{-1} and PR was 10 W m^{-2}. Radiation was measured 95 cm below the lamps with a LI-COR[4] radiometer and sensors. Ambient CO_2 concentration was monitored by an IR gas analyzer and maintained at 400 ± 25 μl L^{-1}.

The seedlings were not inoculated with symbiotic N_2-fixing bacteria and no nodules were observed during the experiment. During the pretreatment period, the plants were grown in a complete nutrient solution containing NO_3^- as the nitrogen source. Initial concentrations of nutrients in the culture solution were 1.0 mM NO_3^-, 1.65 mM K^+, 0.5 mM $H_2PO_4^-$, 0.5 mM Ca^{2+}, 1.0 mM Mg^{2+}, 1.64 mM SO_4^{2-}, 17 μM B, 3.0 μM Mn, 2.2 μM Cl, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 1.0 mg Fe L^{-1} as Fe-EDTA. Solutions were changed every 2 d to avoid depletion effects (26). A constant pH of 6.1 ± 0.1 was maintained by automatic additions of 0.01 N H_2SO_4 or $Ca(OH)_2$. Temperature of the culture solutions was 24.0 ± 0.1°C.

Treatments were initiated after 14 d when the third trifoliate had expanded and plants were in the exponential phase of vegetative growth. Nutrient solutions in all culture systems were changed to a complete solution with 1.0 mM NH_4^+ as the sole nitrogen source. Concentration of SO_4^{2-} in the treatment solution was 2.65 mM and concentrations of all other nutrients were the same as in the pretreatment solution. Solutions were changed every 2 d throughout the treatment period. Acidity in the separate culture systems was adjusted and constantly maintained during the treatment period at pH 6.1, 5.1, or 4.1. All other pretreatment conditions were continued during the treatment period.

Beginning on the d that treatments were initiated, four plants from each pH treatment were sampled at intervals of 3 to 4 d over a 24-d experimental period. For each plant sampled, mainstem and branch trifoliolates were counted, and total leaf area was measured photometrically with a Hayashi Denko AAM-5 area meter. Leaves (leaflets minus petioles), stems (including petioles), and roots were separated and immediately frozen. The plant parts were freeze-dried, weighed, and ground. Total nitrogen in samples of the dried tissues was determined by a Kjeldahl procedure modified to digest all nitrogenous compounds, including NO_3^-, to NH_4^+ (15) for colorimetric determination (3). The remaining samples of each plant part for the four plants harvested from each treatment at each date were combined, and hot water extracts of the tissues were analyzed for NO_3^- (11). Reduced nitrogen of the tissues was calculated as the residual component of mean total nitrogen minus NO_3^- nitrogen of the composite sample.

Net CO_2 exchange rates were measured between 1200 and 1300 h on the second or third youngest, fully expanded mainstem leaf of six plants per treatment. Upper and lower surfaces of a 10-cm^2 area of 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 infrared gas analyzer.

For statistical analysis of dry weights, total nitrogen contents, leaf numbers, leaf areas, and net CO_2 exchange rates per unit leaf area, the experiment was considered as a completely randomized design with the entire variation among the four individual plants sampled at each date assigned to the experimental error term in a one-way analysis of variance. LSD values are reported at the 0.05 level of probability. As the NO_3^- nitrogen and reduced nitrogen data represent values determined from a composite sample, statistical comparisons are not presented. Logarithmic, linear, quadratic, and cubic equations were fitted by regression procedures to the data for each treatment as a function of day at treatment, and the equation with the best-fit is plotted in each figure. The best-fit regression equations for nitrogen content of the whole plant and dry weight of roots were used to estimate the amount of NH_4^+ uptake during the day of each sample and the total dry weight of roots at each harvest. The specific uptake rates of NH_4^+ then were calculated from the estimated values.

RESULTS AND DISCUSSION

In whole soybean plants receiving 1.0 mM NH_4^+ as the sole nitrogen source, accumulation of dry matter (Fig. 1) and total nitrogen (Fig. 2), as well as distribution within the component parts, were nearly the same at pH 6.1 as previously observed under both NO_3^- and NH_4^+ nutrition at pH 5.8 (24) and under NO_3^- nutrition at pH 6.1 (23). When acidity of the culture solution was increased from pH 6.1 to 5.1, both dry matter (Fig. 1) and nitrogen (Fig. 2) accumulated more slowly in the whole plant and the plant parts. The reductions in rates of accumulation of dry matter and nitrogen had begun within the initial sampling interval (Figs. 1 and 2, insets) and were proportional. Thus, percentages of total nitrogen contents of plants at pH 6.1 and 5.1 were similar (data not shown). Most of the reduction in dry matter and nitrogen accumulation in shoots occurred during the first 10 to 14 d of treatment (Figs. 1 and 2, insets). With continued exposure of plants to a root-zone pH of 5.1, dry matter and nitrogen contents of shoots reached constant percentages of those at pH 6.1 as plants acclimated to the lower pH. The decline in dry matter and nitrogen accumulation in roots initially was slower than that in shoots, and dry matter and nitrogen contents of roots reached constant percentages of those at pH 6.1 later than for shoots. These responses in accumulation of dry matter and nitrogen for an increase in ambient acidity between pH 6.1 and 5.1 are similar to the responses observed during onset of a nitrogen stress within the plant (21, 27).

As acidity of the root-zone is increased, uptake rate of NH_4^+ per unit root mass has been reported (12–14) to be reduced during short-term exposure. The rates of uptake of NH_4^+ per g root dry weight calculated for this study (Fig. 3A) were lower at pH 5.1 than at pH 6.1 throughout the 24 d exposure period. The reduction in uptake rate between pH 6.1 and 5.1 was nearly constant over the 24 d, but the reduced uptake rate at pH 5.1 was still rapid enough to permit acclimation by the plant through reduced meristematic activity (19, 21, 27).

Meristematic activity, including initiation and emergence of

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

[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.

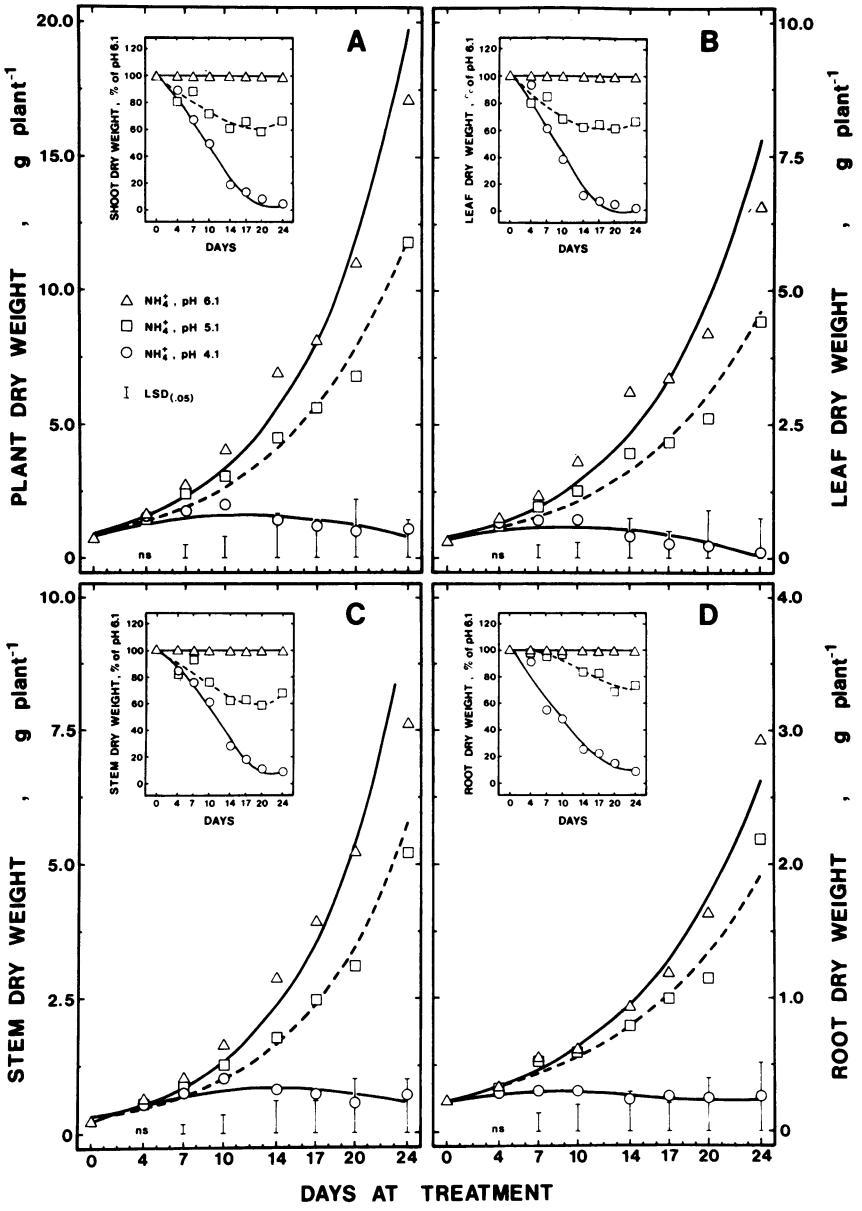

FIG. 1. Accumulation of dry matter in whole plants (A), leaves (B), stems (C), and roots (D) in response to pH of culture solutions containing NH_4^+ as the sole nitrogen source. The insets show shoot (A), leaf (B), stem (C), and root (D) dry weights of plants at pH 5.1 and 4.1 expressed as percent of dry weight of plants at pH 6.1. Symbols represent means of four plants.

new leaves and axillary branches as well as expansion of individual leaves, generally declines before net CO_2 exchange rate per unit leaf area when acquisition of nitrogen by the plant is decreased (6, 19, 21, 27, 28). The reduction between pH 6.1 and 5.1 in rate of increase in weight (Fig. 1B) and total area (Fig. 4A) of leaves was attributable primarily to a reduction in rate of initiation and emergence of lateral branches and leaves (Fig. 4B, inset). The net CO_2 exchange rate per unit area of leaves (Fig. 3B) was not significantly affected by the change in pH from 6.1 to 5.1. These responses to the increased acidity between pH 6.1 and 5.1 are thus responses characteristic of acclimation to an internal nitrogen stress.

When acidity of the culture solution was further increased from pH 5.1 to 4.1, the specific uptake rate of NH_4^+ declined rapidly (Fig. 3A). The rate of appearance of new mainstem trifoliolate leaves for plants at pH 4.1 had declined significantly relative to plants at pH 6.1 and 5.1 after 7 d of treatment, and the number of mainstem leaves for plants at pH 4.1 reached a maximum between 7 and 10 d (Fig. 4B). No branch leaves appeared (Fig. 4B, inset). Net growth had ceased by 10 d of exposure to pH 4.1, and thereafter dry matter and nitrogen contents of the plants declined (Figs. 1 and 2). The decline in dry matter and nitrogen contents (Figs. 1 and 2) of the plants after 10 d was associated primarily with the abscission of leaves. For plants at pH 4.1, the photosynthetic activity of leaves had declined significantly at the time of the first measurement on d 4 and continued to decline rapidly through d 18 (Fig. 3B). Beyond d 18, too few viable leaves remained on plants at pH 4.1 for measurement of net CO_2 exchange rate.

Functions of roots other than nitrogen uptake can be altered by rhizosphere acidity. Production and translocation of such growth hormones as cytokinins possibly are affected by acidity. Certainly, the growth rate of roots is reduced as acidity increases (23). When soybean plants that received NO_3^- as the sole nitrogen source were grown at pH 6.1, 5.1, and 4.1, an initial reduction in rate of root growth at the lower pH levels was followed by a reduction in rate of shoot growth (23). The uptake rate of NO_3^- per unit root mass, however, is enhanced by increased acidity (12, 23) and after 20 d of exposure to pH 5.1 and 4.1 rates of accumulation of nitrogen and dry matter by the plants exceeded those of plants at pH 6.1 (23). These data support the conclusion that the eventual acclimation of NO_3^--fed plants to initial effects of acidity on root growth involves avoidance of a nitrogen stress within the plant by the enhanced rate of NO_3^- uptake with

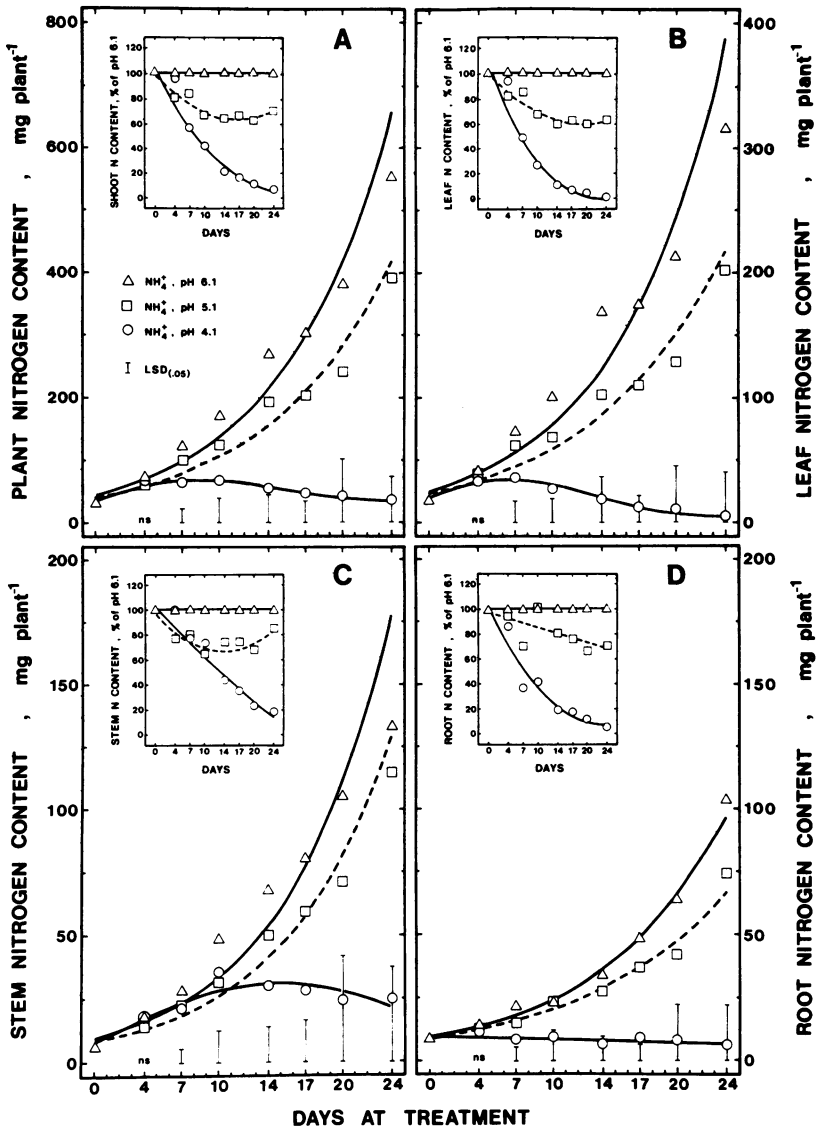

FIG. 2. Accumulation of total nitrogen in whole plants (A), leaves (B), stems (C), and roots (D) in response to pH of culture solutions containing NH_4^+ as the sole nitrogen source. The insets show shoot (A), leaf (B), stem (C), and root (D) dry weights of plants at pH 5.1 and 4.1 expressed as percent of nitrogen content of plants at pH 6.1. Symbols represent means of four plants.

increased acidity. Conversely, for NH_4^+-fed plants the combined effects of increased acidity on reductions in growth rate of roots (Fig. 1) and rate of NH_4^+ uptake per unit root mass (Fig. 3) would exacerbate development of a nitrogen stress.

During the pretreatment period, all plants received NO_3^- as the sole nitrogen source. At the time of transfer to the treatment solutions containing NH_4^+ as the sole nitrogen source, 2.5 mg of the 33.7 mg total nitrogen per plant (or about 7%) was present as NO_3^- (Figs. 5 and 2A). The extent to which this pool of NO_3^- was reduced within the plants after transfer to NH_4^+ as the nitrogen source was affected by acidity of the culture solution (Fig. 5). For plants at pH 6.1, less than half of the NO_3^- was reduced within the first 4 to 7 d of treatment, and very little of the remaining NO_3^- was reduced during the treatment period. As acidity of the culture solution was increased, more of the residual NO_3^- was reduced. The residual NO_3^- pools in the plant parts followed the general pattern of depletion after transfer to NH_4^+-containing solutions (data not shown). The extent of depletion of NO_3^- at all pH levels was greatest in roots and least in stems, and with all organs the depletion was increased in response to increased acidity of the culture solution. We assume that the amount of residual NO_3^- depleted after transfer to the NH_4^+ solution at pH 6.1 represents the portion in cytoplasmic and vascular pools which is readily accessible to reduction and assimilation. The remaining portion of the residual NO_3^- thus would represent NO_3^- sequestered in less accessible pools such as vacuoles and released into more accessible pools as current uptake of nitrogen fails to satisfy demand. Since the pool of NO_3^- in tissues is depleted very rapidly during onset of a nitrogen stress (21, 27), these results are consistent with the hypothesis that the reduction in uptake rate of NH_4^+ per unit root mass as acidity of the root-zone is increased leads to development of a nitrogen stress within the plant.

The concentration of the reduced nitrogen fraction (total nitrogen minus NO_3^- nitrogen) in tissues generally declined as the plants aged (Table I). Although influenced by the utilization of NO_3^- pools (Fig. 5), the total nitrogen concentrations in organs declined as expected (17, 19, 21, 22, 27) during this period of vegetative growth (*cf.* Figs. 1 and 2). In leaves and roots, the rate of decline in concentration of reduced nitrogen became more rapid as ambient acidity was increased, while in stems the rate of decline was slower as acidity was increased (Table I). The increased concentration of reduced nitrogen in stems at pH 4.1 probably reflects remobilization within the plant. The decrease in concentration of reduced nitrogen in roots grown at pH 4.1 indicates that organic nitrogen compounds were not accumulating within these tissues. Thus, end-product inhibition most likely is not associated with the effects of external acidity on the rate of NH_4^+ uptake per g root (Fig. 3), providing that NH_4^+ is primarily assimilated within the root tissues (5, 9, 10). Rather,

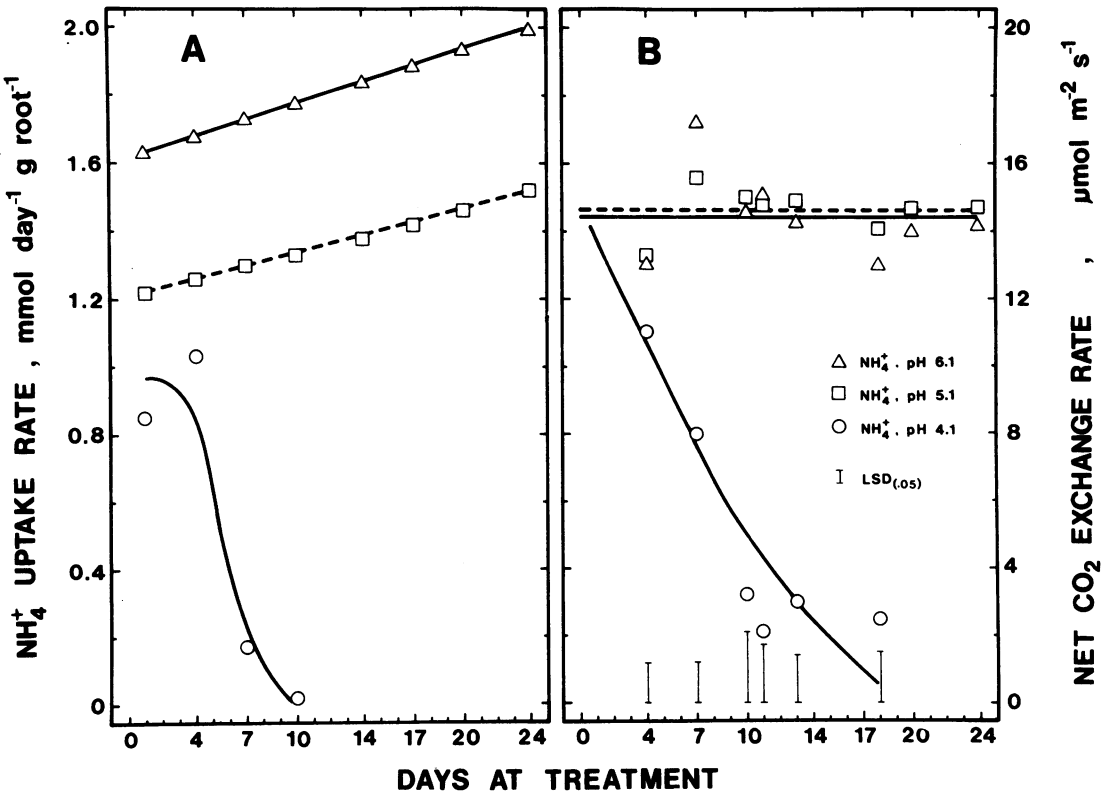

FIG. 3. Specific uptake rates of NH_4^+ by roots (A) and net CO_2 exchange rates of leaves (B) in response to pH of culture solutions containing NH_4^+ as the sole nitrogen source. Symbols for specific uptake rates represent values calculated from regression equations plotted in Figures 1 and 2. Symbols for net CO_2 exchange rates represent means of six plants.

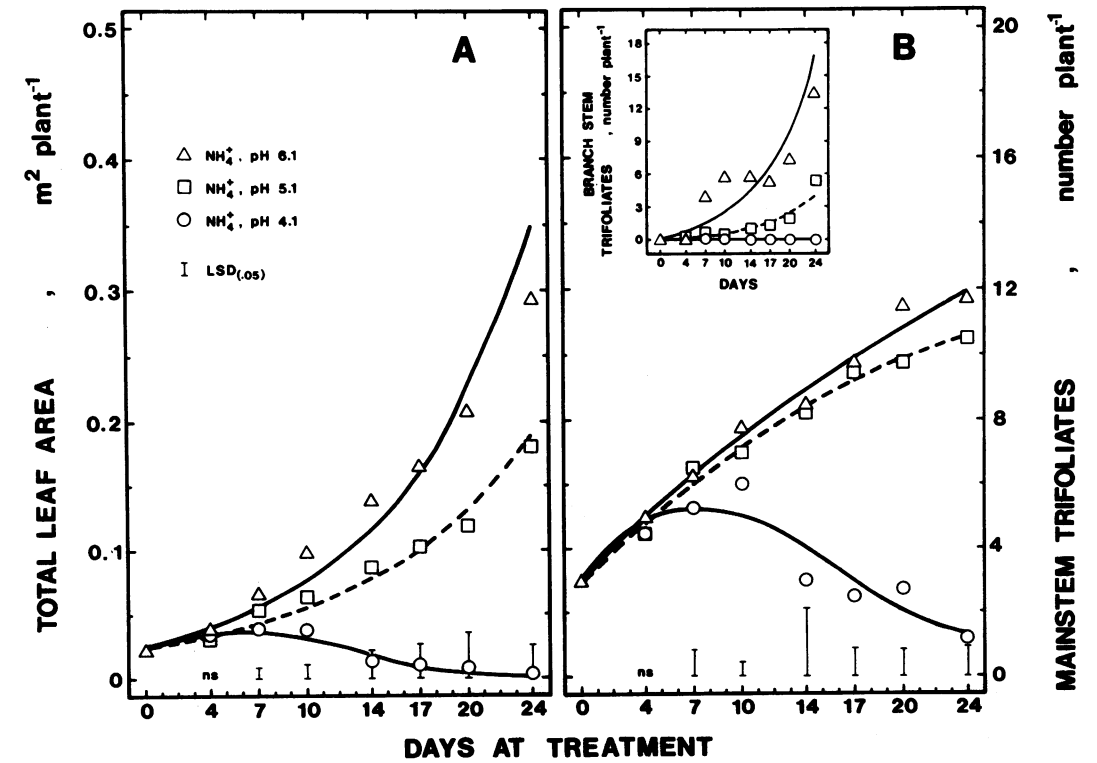

FIG. 4. Total leaf area (A), number of mainstem trifoliates (B), and number of trifoliates on branch stems (inset, B) of plants in response to pH of culture solutions containing NH_4^+ as the sole nitrogen source. Symbols represent means of four plants.

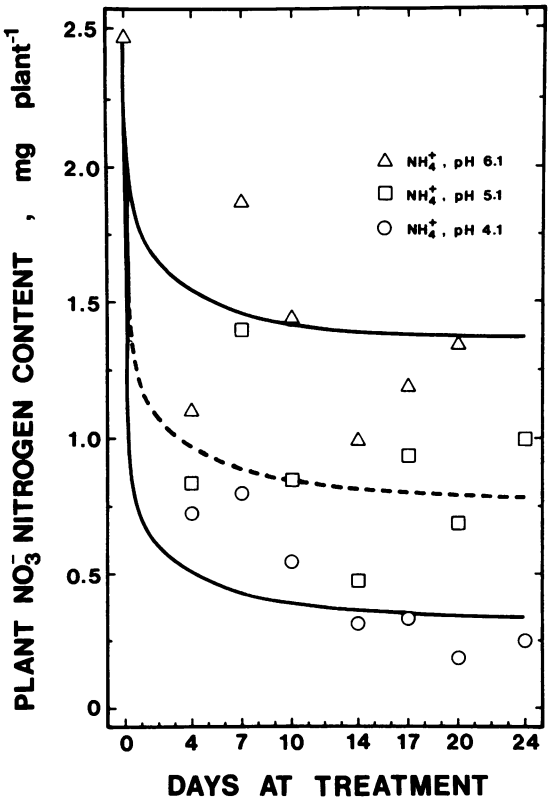

FIG. 5. Decline of NO_3^- nitrogen within plants in response to pH of culture solutions containing NH_4^+ as the sole nitrogen source after transfer from pretreatment solutions containing NO_3^- as the nitrogen source. Symbols represent analysis of a single sample composited from four plants.

Table I. *Effect of Ambient pH on Concentration of Reduced Nitrogen (mean total nitrogen minus NO_3^- nitrogen of combined sample) in Leaves, Stems, and Roots of Soybean Plants Grown for 24 Days with NH_4^+ as the Sole Nitrogen Source*

Days	Leaves pH			Stems pH			Roots pH		
	6.1	5.1	4.1	6.1	5.1	4.1	6.1	5.1	4.1
	reduced nitrogen, % of dry weight								
4	5.7	5.7	5.7	2.9	2.8	3.6	4.1	4.0	3.9
7	6.3	6.4	5.0	2.7	2.3	2.8	3.9	3.8	2.7
10	5.5	5.4	4.7	2.9	2.5	3.6	3.7	3.9	2.6
14	5.4	5.3	4.9	2.3	2.8	3.7	3.6	3.5	2.8
17	5.2	5.0	4.7	2.2	2.4	3.9	4.0	3.7	3.2
20	5.1	5.0	4.6	2.0	2.3	4.3	3.9	3.7	3.1
24	4.8	4.6	4.3	1.7	2.2	3.6	3.5	3.4	2.3

the effect of ambient acidity on rate of NH_4^+ uptake more likely involves the development of an unfavorable concentration gradient for the countertransport of hydrogen ions associated with NH_4^+ absorption. However, the methodology used in determining reduced nitrogen does not give a good indication of the concentration of free NH_4^+ or amides within the cytoplasm of root cells. Thus, the possibility that toxic accumulation of free NH_4^+ within the root tissues contributes to the decline in the specific rate of NH_4^+ uptake cannot be excluded on the basis of the data presented here.

CONCLUSIONS

Most of the NH_4^+ entering plants apparently is assimilated into soluble amide and amino compounds in the roots before being transported to the shoot (5, 9, 10). Since the percentage of the total nitrogen content in the shoot does not differ between NH_4^+-fed and NO_3^--fed plants when ambient pH of the culture solution is controlled at a pH between 5.8 and 6.1 (17, 24) and since nonassimilated NO_3^- accounts for 5 to 10% of the total nitrogen content of NO_3^--fed plants (21–24, 27), a greater pool of organic nitrogen would be expected to occur within the shoot of NH_4^+-fed plants. It does not seem likely that the increased pool of organic nitrogen is the primary cause of reduced growth and toxicity for plants grown under NH_4^+ nutrition. Rather, the effect of acidification of the rhizosphere during absorption of NH_4^+ on rate of uptake of NH_4^+ appears to be responsible for the initial decline in growth and eventually to promote toxicity symptoms.

When acidity of the culture solution was increased from pH 6.1 to 5.1, uptake rate of NH_4^+ per unit root mass (Fig. 3A) was reduced and a reduction in growth (Fig. 1) did occur. The net photosynthetic rate per unit area of leaves (Fig. 3B) was unaffected, but meristematic activity, including appearance of new leaves and axillary branches and expansion of leaf area (Fig. 4), was reduced. The preceding responses are consistent with the effects of an internal nitrogen stress under NO_3^- nutrition (21, 27, 28) and thus can be attributed to the effect of the increased acidity on reducing the rate of NH_4^+ uptake per unit root mass (Fig. 3A).

When acidity of the culture solution was reduced further to pH 4.1, net dry matter accumulation of the plants (Fig. 1), as well as meristematic activity (Fig. 4), ceased within about 10 d. The little photosynthetic activity measured after 10 d (Fig. 3B) was insufficient to overcome the rapid abscission of leaves (Fig. 4B). That a severe nitrogen stress rapidly developed in these plants following transfer to the NH_4^+ culture solution of pH 4.1 is indicated by the rapid decline in uptake rates of NH_4^+ (Fig. 3A) and the rapid assimilation of the NO_3^- pool present at the time of transfer (Fig. 5). Maintenance respiration would be expected to continue even after nitrogen availability within the plant had declined to levels that preclude continued growth respiration (30). We postulate that, as the decline in photosynthetic activity results in a depletion of carbohydrate reserves below the critical levels to meet the demands of maintenance respiration, degradation of organic nitrogen compounds within the leaves is required as an energy source. Continued degradation of organic nitrogen compounds results in an accumulation of free NH_4^+ or ammonia within the leaf tissues that ultimately leads to the development of toxicity symptoms.

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