Plant Physiol. (1989) 91, 1345-1350
0032-0889/89/91/1345/06/$01.00/0

Received for publication April 10, 1989
and in revised form July 27, 1989

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

Ammonium as a Nitrogen Source

Leslie Tolley Henry* and C. David Raper, Jr.

Department of Forestry (L.T.H.) *and Department of Soil Science* (C.D.R.), *North Carolina State University, Raleigh, North Carolina 27695*

ABSTRACT

When NO_3^- is the sole nitrogen source in flowing solution culture, the net rate of nitrogen uptake by nonnodulated soybean (*Glycine max* L. Merr. cv Ransom) plants cycles between maxima and minima with a periodicity of oscillation that corresponds with the interval of leaf emergence. Since soybean plants accumulate similar quantities of nitrogen when either NH_4^+ or NO_3^- is the sole source in solution culture controlled at pH 6.0, an experiment was conducted to determine if the oscillations in net rate of nitrogen uptake also occur when NH_4^+ is the nitrogen source. During a 21-day period of vegetative development, net uptake of NH_4^+ was measured daily by ion chromatography as depletion of NH_4^+ from a replenished nutrient solution containing 1.0 millimolar NH_4^+. The net rate of NH_4^+ uptake oscillated with a periodicity that was similar to the interval of leaf emergence. Instances of negative net rates of uptake indicate that the transition between maxima and minima involved changes in influx and efflux components of net NH_4^+ uptake.

In studies with nonnodulated soybean plants growing in flowing solution culture, the net rate of NO_3^- uptake during vegetative growth cycles between maxima and minima with a periodicity of oscillation of 3 to 5 d (24, 28). We have proposed that this fluctuation in net rate of NO_3^- uptake occurs in response to changes in demand for carbon and nitrogen in the shoot and the availability of carbohydrate for translocation from shoot to roots associated with periodic emergence and expansion of new leaves. This hypothesis is based on the experimental observations that NO_3^- uptake and transport are dependent on respiratory energy (18) and that soluble carbohydrate status of roots generally is low (19, 20) so that continued absorption of NO_3^- depends on continued supply of carbohydrate from the shoot for respiratory energy (9). In the absence of experimental data on the dynamics of carbohydrate fluxes between organs in the whole plant, the hypothesis also takes into consideration the evidence from a simulation model (30) that translocation of carbohydrate from shoot to roots is responsive to concentration of carbohydrate in the shoot pool and to size and metabolic activity of root sinks. As nitrogen is absorbed by roots and translocated to the shoot, stimulation of shoot growth and initiation and early expansion of new leaves (25, 26) presumably reduces the availability of carbohydrate for translocation to roots as metabolic demand of the shoots is increased more rapidly than photosynthetic capacity. The reduced flow of carbohydrate to roots results in a decrease in NO_3^- uptake. The subsequent reduction in nitrogen availability to the shoot decreases initiation and expansion of new leaf tissue before reducing photosynthetic activity of the shoot (25, 26); thus, availability of carbohydrate for transport to roots, and consequently to support NO_3^- uptake, increases. From these observations, net rates of NO_3^- uptake should fluctuate with a periodicity similar to the interval between emergence of new leaves, which at about 3 to 4 d for soybean (23, 26, 28) corresponds to the observed periodicity in cycles of NO_3^- uptake (24, 28), and with the minima of net uptake coordinated with time of emergence and early expansion of leaves.

When acidity of the root zone is controlled at pH 6.0 in solution culture, nonnodulated soybean plants can utilize either NH_4^+ or NO_3^- with no differences in nitrogen accumulation or growth (21, 27). This indicates that, while fluxes of NO_3^- and NH_4^+ into roots are facilitated by separate transporters (5, 7, 8) and while NH_4^+ is assimilated almost exclusively in roots (4, 12) but NO_3^- is assimilated in both roots and leaves or stored in vacuoles (1, 6, 22), regulation of NH_4^+ uptake at the whole-plant level may involve the same mechanism as regulation of NO_3^- uptake. If so, net uptake rate of NH_4^+ would oscillate with a periodicity coordinated with the interval of leaf emergence. The objective of this experiment was to determine whether such cyclic variations occur in rates of NH_4^+ uptake under environmental conditions that do not otherwise impede NH_4^+ uptake.

MATERIALS AND METHODS

Soybean [*Glycine max* (L.) Merr. cv Ransom] seeds were germinated as previously described (24, 28). After 3 d, 48 seedlings with radicles between 8 and 12 cm long were placed into each of two 200-L, continuous-flow, hydroponic culture systems equipped for pH and temperature monitoring and control (29). Temperature of the culture solution was main-

[1] Paper No. 12102 of the Journal series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. Supported in part by National Aeronautics and Space Administration grant NCC 2-101.

tained at 24 ± 0.5°C and acidity was maintained at pH 6.0 ± 0.1 by automated additions of 0.01 N H_2SO_4 or $Ca(OH)_2$. Initial concentrations of nutrients in the culture solution during a 14-d pretreatment period were 1.0 mM NO_3^-, 0.25 mM $H_2PO_4^-$, 1.25 mM K^+, 0.25 mM Ca^{2+}, 0.25 mM Mg^{2+}, 0.50 mM SO_4^{2-}, 19 μM B, 3.7 μM Mn, 7.2 μM Cl, 0.3 μM Zn, 0.13 μM Cu, 0.05 μM Mo, and 10.0 μmol Fe(II) as 300 Fe-Sequestrene (CIBA-GEIGY Corp).[2] Fresh solutions were supplied every 2 d to avoid nutrient depletion (24).

The culture systems were located in a controlled-environment growth room equipped for CO_2 control (29). During the pretreatment period, the growth room was programmed for the same environmental conditions as used previously (24, 27, 28). The day/night aerial temperatures were 26/22 ± 0.3°C during 9-h d (0800–1700) and 15-h night periods with abrupt day-to-night transitions. A PPFD of 700 ± 50 μmol m^{-2} s^{-1} between wavelengths of 400 to 700 nm and a PR[3] of 12 W m^{-2} between wavelengths of 700 to 850 nm were provided during the day period from a combination of cool-white fluorescent and incandescent lamps at an input wattage ratio of 10:3. To effect a long-day photoperiod and repress floral development (23), the 15-h night period included a 3-h interruption after 6 h by the incandescent lamps which provided a PPFD of 70 μmol m^{-2} s^{-1} and PR of 10 W m^{-2}. Ambient CO_2 concentration was maintained at 400 ± 25 μLL^{-1}. At the end of the pretreatment period, the population of plants was thinned to 12 plants per culture system. Details of spacing between plants to maximize light interception for individual plants and uniformity of environmental conditions have been presented elsewhere (29).

During the treatment period, which was initiated 14 d after transplanting when the third trifoliate was unfolding, day/night aerial temperatures were changed to 18°C/14°C. Other aerial conditions remained the same as during pretreatment. Nutrient solution in the culture systems was 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 1.50 mM and concentrations of all other nutrients were the same as in the pretreatment solution. Temperature of the solution was maintained at 24°C, and acidity was maintained at pH 6.0. Samples of nutrient solution from each system were taken daily between 1200 and 1300 h and analyzed for NH_4^+ with a Dionex Ion Chromatograph model 2000, with an accuracy of better than ±15 μmol in a 1.0 mM solution. Depletion (or enrichment) of NH_4^+ from solution during the preceding 24 h was recorded, and NH_4^+ was added to the solution as $(NH_4)_2SO_4$ to replace the amount depleted. Every 2 d after determination of NH_4^+, half of the solution in each system was replaced to avoid excessive depletion or accumulation of other nutrients (24, 28). After 15 min for remixing, $(NH_4)_2SO_4$ was added to return NH_4^+ to 1.0 mM. Net rate of NH_4^+ uptake per plant during each 24-h period was determined as mmol NH_4^+ removed from (or accumulated in) the solution in each culture system divided by the number of plants in the system during that day. No nodules were observed on roots, which had not been inoculated with rhizobium, and NO_3^- was not detected in the culture solutions by ion chromatography; thus, NH_4^+ was the only nitrogen source available to the plants.

At 3- to 4-d intervals over a 21-d treatment period, beginning on the day that the nitrogen source was changed from NO_3^- to NH_4^+, a total of three plants was sampled between 1200 and 1300 h from the two culture systems. The number of plants harvested from each of the two culture systems alternated between one and two on each sample day. Plants were separated into leaves, stems plus petioles, and roots. Numbers of mainstem and branch leaves greater than 2 cm^2 were recorded, and the tissues were immediately frozen. After the tissues were freeze-dried, weighed, and ground, total nitrogen in each plant part was determined by a modified micro-Kjeldahl procedure to digest nitrogenous compounds to NH_4^+ (17), and NH_4^+ was analyzed colorimetrically (2).

Under the conditions of this experiment, accumulation of dry matter in shoot and roots and of nitrogen in the whole plant are positive and can be estimated as characteristics of the population by linear regression models of the natural logarithm of dry weight or nitrogen content of plants at each sample date as a function of days at treatment (19, 25, 30). For calculation of daily net uptake rates of NH_4^+ per gram root dry weight, the net uptake rate per plant determined by depletion from the replenished nutrient solution during each 24-h interval (which is a characteristic of the nonsampled portion of the experimental population) was divided by the dry weight of roots predicted by the regression analysis.

RESULTS AND DISCUSSION

Based on previous investigation (24, 28), we have proposed that net uptake of nitrogen from flowing solution culture fluctuates during vegetative growth in response to changes in demand for carbon and nitrogen in the shoot and availability of carbohydrate for translocation from shoot to roots associated with periodic emergence and expansion of new leaves. The sole nitrogen source for soybean plants in these prior experiments was NO_3^-. Since soybean plants can utilize NH_4^+ equally as effectively as NO_3^- when acidity of the solution is maintained near pH 6.0 (27), we further proposed that, although fluxes of NH_4^+ and NO_3^- into roots are facilitated by separate transporters (5, 7, 8) and their patterns of transport and assimilation within the plant are distinct (1, 4, 6, 12, 22), regulation of NH_4^+ and NO_3^- uptake at the whole-plant level should behave similarly in response to shifting demands for carbon and nitrogen during leaf emergence and expansion.

In the present experiments with nonnodulated soybean, dry matter accumulation in shoot and root tissues (Fig. 1A) and nitrogen accumulation by the whole plant (Fig. 1B) during a 21-d period of vegetative growth in which NH_4^+ was the sole nitrogen source were within the ranges that have been observed (24, 25, 28) when NO_3^- was the sole nitrogen source. Cumulative depletion of NH_4^+-N from the replenished solutions over the treatment period was in close agreement with total nitrogen accumulation in plants as determined by tissue analysis (Fig. 1B). Net rate of NH_4^+ uptake per g root dry weight (Fig. 2B), as calculated from daily depletion from solution per plant (Fig. 2A) and predicted root dry weights (Fig. 1A), cycled between maxima and minima during the treatment period. Thus, the oscillations in net rate of uptake

[2] 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.

[3] Abbreviation: PR, photomorphogenic radiation.

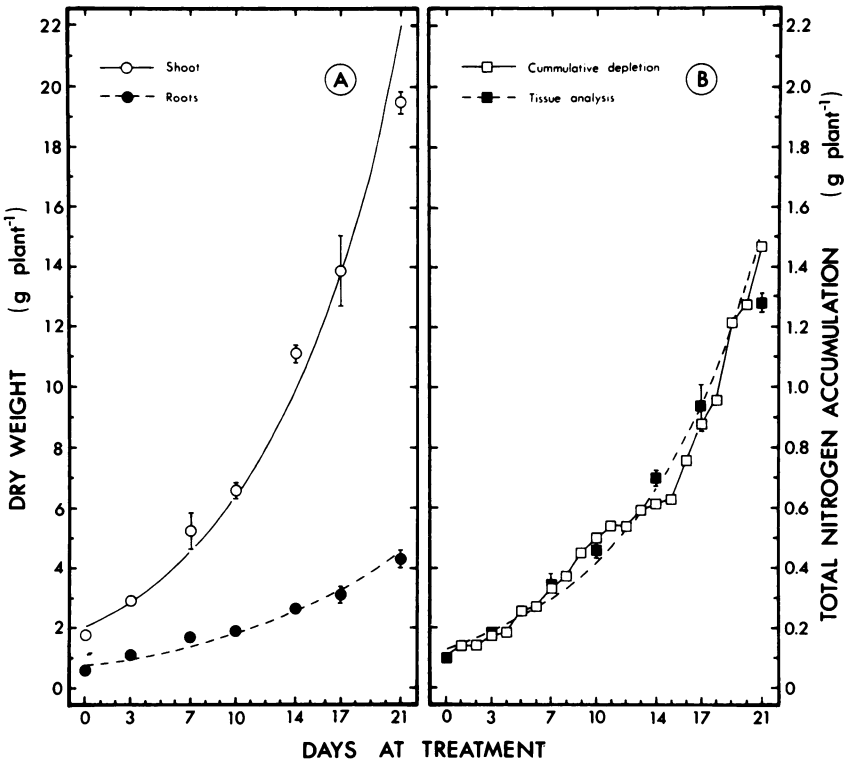

Figure 1. Dry matter accumulation in shoot and roots (A) and total nitrogen accumulation as determined by cumulative depletion of NH_4^+ from replenished solution and by analysis of tissues (B). Each value for dry weight and tissue analysis is the mean of three plants with vertical bars representing ±1 SD of the mean when greater than the size of the symbol. Regression equations relating grams of dry weight for shoot (DW_s) and roots (DW_r) and total nitrogen in plants (N) to days (d) at treatment are: ln DW_s = 0.114(d) + 0.714, r = 0.99; ln DW_r = 0.087(d) − 0.280, r = 0.98; ln N = 0.118(d) − 2.065, r = 0.99.

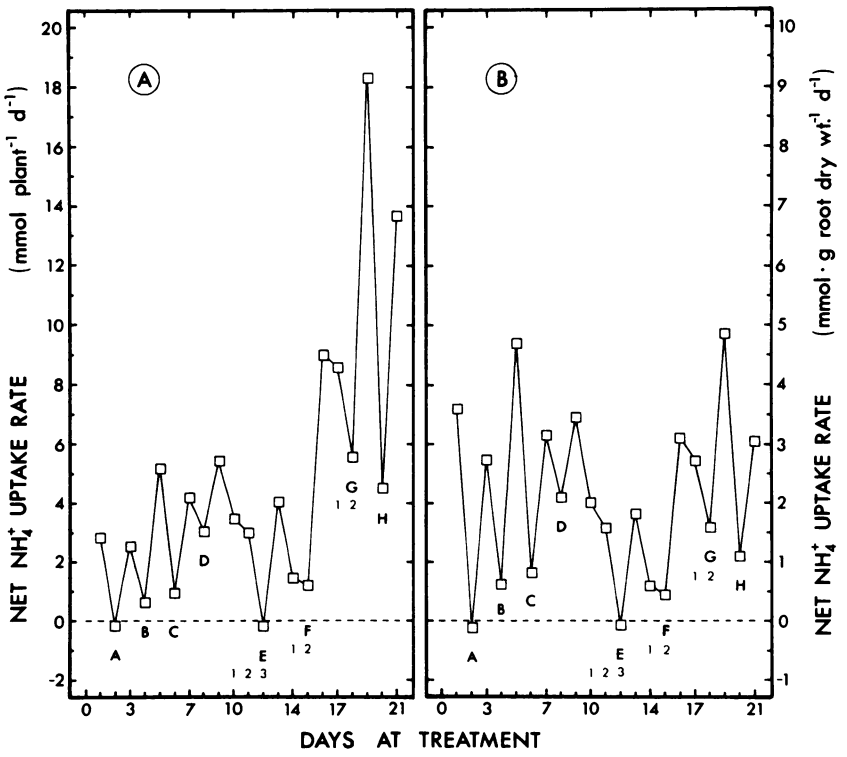

Figure 2. Net rate of NH_4^+ uptake per plant calculated from daily depletion of NH_4^+ from replenished solution (A) and net rate of NH_4^+ uptake per gram root dry weight (B) calculated from daily depletion per plant and root dry weights estimated from regression equation in Figure 1A. Minima in net uptake rate are labeled by upper case letters, and shoulders on the down slope of minima E, F, and G are labeled in numerical order for identification in Figure 4. Variations in net uptake rates between the two hydroponic systems averaged less than 8% of the daily means.

that have been observed for NO_3^- (24, 28) also occur for NH_4^+.

If, as proposed (24, 28), the fluctuations in uptake rate of nitrogen are a response to changes in flux of carbohydrate from shoot to root, and the availability of carbohydrate for transport to roots declines during initiation and early expansion of new leaves (19, 20, 30), the intervals between occurrence of uptake minima and emergence and early expansion of new leaves should be coincident. Since leaves are initiated on both the mainstem and axillary branches of soybean plants, synchronous emergence of groups of leaves among the multiple stems must occur for the proposed relationship to be

tenable. In the present experiments, trifoliolate leaves on the mainstem and on individual axillary branches were counted at each harvest (Fig. 3). Since plants were harvested at 3- to 4-d intervals, the most recently emerged leaves were at variable stages of development and ranged from 2 to 46 cm^2 in area when first counted. The rates of emergence of leaves (leaves d^{-1}) on the mainstem (Fig. 3A) and on each branch (Fig. 3B) thus were estimated by linear regression ($r = 0.96$ or greater), and the intervals of emergence for leaves (d $leaf^{-1}$) on each stem were calculated as the reciprocals of these rates. The calculated interval of leaf emergence on the mainstem was 2.16 d $leaf^{-1}$ during the initial 5.6 d of treatment and 3.61 d $leaf^{-1}$ during the remainder of the study. The change in rate of emergence was accepted as a consequence (23) of the decrease in aerial temperature at the initiation of the treatment period. For branch stems, the interval of leaf emergence ranged from 3.81 to 7.17 d $leaf^{-1}$.

The calculated values for interval of leaf emergence were used to reconstruct the time of emergence of each leaf on the mainstem and on each branch (Fig. 4). Based on the regression equations used in Figure 3A to relate number of leaves to day of treatment, the emergence of the first new mainstem leaf following initiation of treatments was projected at mainstem node V5 on d 1.68. At intervals of emergence 2.16 d $leaf^{-1}$ until d 5.60 and 3.61 d $leaf^{-1}$ thereafter, emergence of successive leaves on the mainstem was projected at d 3.84, 6.27, 9.88, 13.49, 17.10, and 20.71 (see horizontal line representing mainstem, MS, in Fig. 4). From Figure 3B, emergence of the first leaf of cotyledonary branch C1 was projected on d 1.93, and with an interval of emergence of 4.74 d $leaf^{-1}$, emergence of successive leaves on C1 was projected at d 6.67, 11.41, 16.15, and 20.89 (see horizontal line C1 in Fig. 4). Time of appearance of leaves on branch stems C2, P1, P2, and V1 through V5 were calculated similarly. The leaves on the various stems were sorted by projected time of emergence into eight groups (identified by vertical dashed lines A′–H′ in Fig. 4) with mean days of emergence (±SD of the mean) of 1.9 ± 0.2 (A′) 4.0 ± 0.4 (B′), 6.4 ± 0.5 (C′), 8.2 ± 0.4 (D′), 10.6 ± 1.1 (E′), 13.9 ± 0.7 (F′), 16.8 ± 0.8 (G′), and 20.4 ± 0.6 (H′). When compared on a 1:1 relationship (square symbols in inset of Fig. 4), the mean days of emergence for these eight groups correspond closely with the occurrence of net uptake minima (identified by arrows labeled A–H along the base of Fig. 4) at d 2(A), 4(B), 6(C), 8(D), 12(E), 15(F), 18(G), and 20(H). The largest standard deviations of the mean day of emergence, as well as the greatest differentials between mean day of emergence and time of observed minima of net rate of NH_4^+ uptake, occurred for leaf groups E′, F′, and G′. The contours of the slopes in uptake rate between the minima for these leaf groups and the preceeding maxima had one or two shoulders (Fig. 2). If the leaves within these groups are further sorted into subgroups labeled in Figure 4 as e1, e2, and e3 for group E′, f1 and f2 for group F′, and g1 and g2 for group G′, the 1:1 correspondence between mean days of emergence for the subgroups and the occurrence of each shoulder and the nadir of the declines in net uptake rate (circled in inset of Fig. 4) was closer than the correspondence between days of emergence for leaf groups E′, F′, and G′ and observed minima E, F, and G. These results do not establish that the interval of leaf emergence is necessarily associated with periodicity of oscillations in net uptake rate of NH_4^+. The correspondence between these two phenomena, however, is consistent with the hypothesis that the periodicity of oscillations in net uptake rate of NH_4^+, as apparently also was the

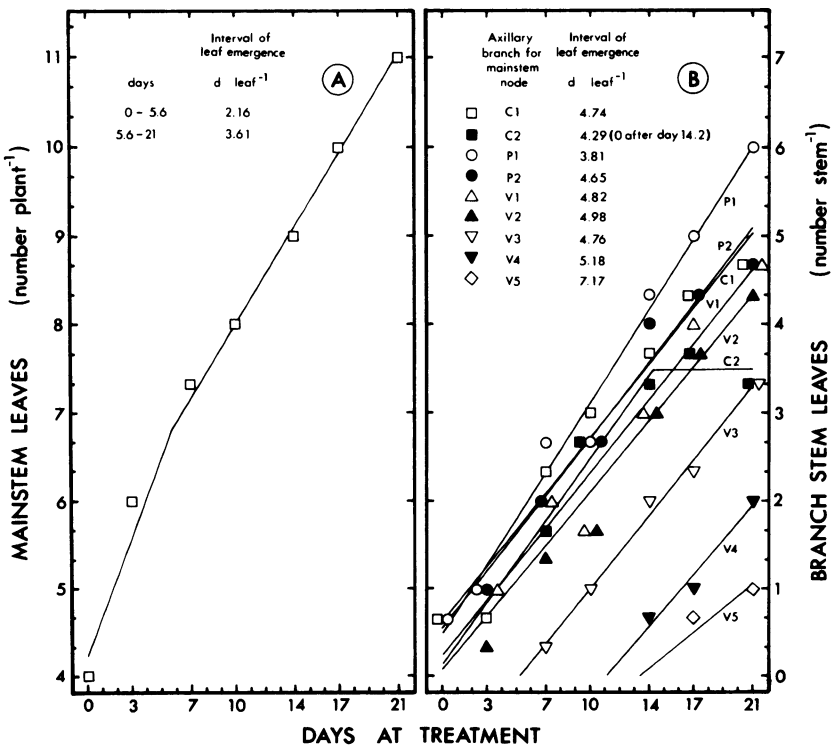

Figure 3. Number of trifoliolate leaves on the mainstem (A) and on each axillary branch (B) of plants at each harvest. Axillary branches are designated as C1 and C2 at the cotyledonary node, P1 and P2 at the primary node, and V1 to V5 at successive acropetally numbered trifoliolate nodes on the mainstem. For clarity, overlapping values for a sample day are depicted side-by-side. Each value is the mean of three plants. Intervals of leaf emergence for each stem are calculated as reciprocals of rates of leaf appearance derived as the slope of linear regression equations relating number of leaves to days of treatment. Apparent intercepts (days) for leaf emergence for each stem are: MS, −9.11; C1, −2.81; C2, −0.63; P1, −1.86; P2, −2.46; V1, −1.11; V2, −0.39; V3, 5.24; V4, 11.01; and V5, 13.35.

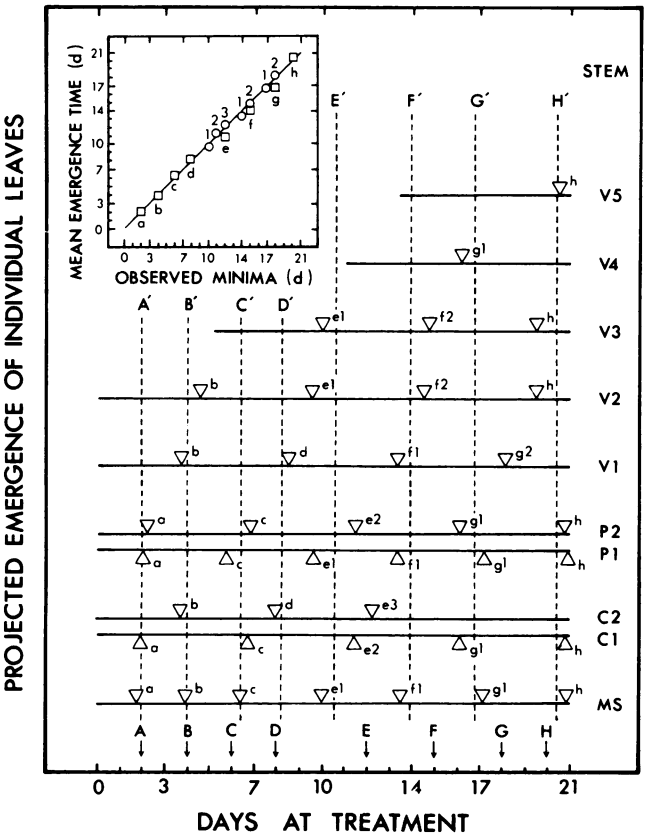

Figure 4. Estimated time of emergence of leaves on the mainstem and axillary stems for cotyledonary (C1 and C2), primary (P1 and P2), and mainstem trifoliolate (V1–V5) nodes based on calculated intervals of leaf emergence from Figure 3. Stems are represented as solid horizontal lines and time of appearance of a leaf as a triangle. Dashed vertical lines are the mean day of emergence for leaf groups A′–H′, with each of these leaf groups identified with times (A–H) of observed minima in net uptake rate of NH_4^+ from Figure 2. Lower case letters denote individual leaves assigned to each leaf group. Leaves denoted as e1, e2, e3, f1, f2, g1, and g2 represent subgroups within leaf groups E′, F′, and G′ that are associated with the shoulders and nadir of declining uptake rates as denoted in Figure 2. Inset shows the relationship between mean time for emergence of leaves within each group (squares) and subgroup (circles) and time of observed minima and shoulder in uptake rate (see text for further discussion).

case for oscillations in net uptake rate of NO_3^-, is coincident with times of emergence of discrete groups of leaves, and thus is coordinated with presumed flux of carbohydrate from shoot to root.

While the periodicity in net uptake rate of nitrogen may reflect changes in translocation rates of carbohydrates from shoot to roots, shifts in the influx and/or efflux components of NO_3^- uptake (3, 10, 11, 13) have been implicated (28) in determining the amplitudes of the maxima and minima of oscillations in net rates of NO_3^- uptake. There is evidence of independently regulated influx and efflux components of net NH_4^+ uptake (14–16). The influx and efflux components of NH_4^+ uptake were not directly estimated in the present study. The two instances of negative net rates of NH_4^+ uptake at d 2 and 12 (minima A and E, Fig. 2), however, are evidence that efflux was occurring as a component in a balance between influx and efflux which determined the amplitude of oscillations in net NH_4^+ uptake. The possible involvement of influx and efflux components in determining the amplitude of oscillations in net NH_4^+ uptake rates in response to changes in flux of carbohydrates for shoot to roots thus is consistent with the supposition that regulation of NH_4^+ uptake involves mechanisms at the whole-plant level which are similar to those regulating NO_3^- uptake.

ACKNOWLEDGMENTS

We thank Emory York and Ladda Tangbanleukal for their excellent technical assistance and the staff of the Southeastern Plant Environmental Laboratories at North Carolina State University for their assistance in the use of the phytotron facilities.

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