

Interdependence of Nitrogen Nutrition and Photosynthesis in *Pisum sativum* L.

I. EFFECT OF COMBINED NITROGEN ON SYMBIOTIC NITROGEN FIXATION AND PHOTOSYNTHESIS¹

Received for publication November 11, 1977 and in revised form February 22, 1978

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ABSTRACT

Photosynthesis, primary productivity, N content, and N₂ fixation were determined as a function of applied NH₄⁺ in peas (*Pisum sativum* L. cv. Alaska) which were inoculated or not inoculated with *Rhizobium leguminosarum*. Carbon dioxide exchange rate (CER) increased 10-fold, total N content 7-fold, and total dry weight 3-fold in 26-day-old uninoculated plants as applied NH₄⁺ was increased from 0 to 16 millimolar. In inoculated plants of the same age CER and dry weight were maximal at 2 millimolar NH₄⁺, and total N content increased between 0 and 2 millimolar NH₄⁺ but did not change significantly with higher NH₄⁺ applications. Per cent N content of uninoculated plants was significantly lower than that of inoculated plants except at the highest NH₄⁺ concentration (16 millimolar). Symbiotic N₂ fixation by inoculated plants was maximal in peas grown with 2 millimolar NH₄⁺; and apparent relative efficiency of N₂ fixation, calculated from C₂H₂ reduction and H₂ evolution, was maximal in the 2 to 4 millimolar NH₄⁺ concentration range. The capacity to fix N₂ through the *Rhizobium*-legume symbiosis significantly enhanced the rate and efficiency of photosynthesis and plant N content when NH₄⁺ concentration in the nutrient solution was below 8 millimolar. Above 8 millimolar NH₄⁺ concentration uninoculated plants had greater CER, N content, and dry weight.

The crucial roles of photosynthesis in plant growth (10, 14) and N₂ fixation (1, 4, 6-8, 10) are well established. However, it has been pointed out that overemphasizing the importance of one aspect of plant metabolism, such as the dependence of whole plant response on photosynthetic products, may lead to an exaggerated view of that function (16). It appears more plausible that growth of the plant is subject to an interdependence among the activities of various organs. For example, photosynthate production is stimulated in proportion to N influx (14), and N deficiency reduces the rate of CO₂ assimilation (11).

Few data are available to explain the interdependence between N nutrition and photosynthesis in legumes capable of forming symbioses with N₂-fixing *Rhizobium* bacteria. It is known that symbiotic N₂ fixation is stimulated by low levels of combined N and inhibited by higher concentrations of available N (6, 8, 12). Moderate levels of combined N available to the legume from

germination do not counteract development of functional root nodules if the supply of reduced C is increased by exogenous addition of carbohydrate (17) or by raising the photosynthetic photon flux density (5).

The present study was conducted to ascertain the effect of different levels of NH₄⁺ on photosynthesis and N nutrition of peas grown with or without *Rhizobium* at a photosynthetic photon flux density which saturates photosynthesis (4). This information will be useful in understanding growth of legumes on marginal lands with low levels of available N and will provide data for understanding legume response to N₂ fixation by *Rhizobium* strains of differing efficiency (2).

MATERIALS AND METHODS

Pea (*Pisum sativum* L. cv. Alaska) plants were maintained in a growth chamber under a 16/8-hr light/dark cycle at 21/15 C, 50/70% RH and photosynthetic photon flux density of 800 μE/m²·sec measured in the photosynthetically active range. Plants were grown individually in sterile Vermiculite in 180-ml plastic pots. All seeds were surface-sterilized with 50% ethanol, and rinsed thoroughly with water before planting. One set of plants was inoculated with *Rhizobium leguminosarum* strain 128C53 (obtained originally from J. C. Burton, The Nitragin Co., Milwaukee); another set was not inoculated. The uninoculated plants grew through a hole in the plastic cover; watering was done carefully to minimize subsequent contamination. All plants were watered on alternate days with nutrient solutions containing 4 mM CaSO₄, 2 mM K₂HPO₄, 2 mM MgSO₄, 1 mM K₂CO₃, 4.2 mM CoCl₂, and micronutrients according to Johnson *et al.* (9). Nitrogen was added as 1, 2, 4, and 8 mM (NH₄)₂CO₃. The initial acidity of all solutions was adjusted to pH 7 with HCl. Each of the 10 treatments used in this study consisted of eight replicates at one of the five NH₄⁺ concentrations in the presence or absence of *Rhizobium*. Data were collected from 26-day-old plants. Three of the eight replicates were used for photosynthetic measurements, and five for the determination of N₂ fixation. Dry weight and N content were determined by using all eight replicates. Dry weights of plant parts were measured after 24 hr at 75 C. Photosynthesis, N₂ fixation, and N content were determined as described previously (5).

RESULTS

The effect of NH₄⁺ on N₂ fixation was most stimulatory at a concentration of 2 mM and inhibitory at 8 and 16 mM compared with the 0 mM controls (Fig. 1). Optimal N₂ fixation was reflected in most of the other parameters measured in inoculated peas: total plant dry weight (Fig. 2), CER³ (Fig. 3), CE (Table I), and the

¹ This material is based on research supported by National Science Foundation Grant AER 77-07301. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Science Foundation.

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³ Abbreviations: CE: carboxylation efficiency; CER: CO₂ exchange rate; C₂H₂ reduction: C₂H₂-dependent C₂H₄ production; RE: apparent relative efficiency of N₂ fixation [1-(H₂ evolved in air/C₂H₄ reduced)].

specific activity of N_2 fixation (Table II) were all maximal at 2 mM NH_4^+ . Plant N content increased between 0 and 2 mM NH_4^+ treatments of inoculated peas but did not change significantly thereafter with increasing NH_4^+ application (Fig. 4). Per cent N content, however, increased with increasing NH_4^+ concentration (Fig. 5) for both inoculated and uninoculated peas.

No NH_4^+ concentration-dependent maxima were evident in data from uninoculated plants. Total plant dry weight (Fig. 2), CER (Fig. 3), total N (Fig. 4), and per cent N content (Fig. 5) increased with increasing NH_4^+ concentration. A rapid increase in dry weight occurred as N content/plant increased from 8 to 40 mg, corresponding to an increase in the N concentration of the nutrient solution from 0 to 4 mM NH_4^+ (Fig. 6). A 4-fold increase in the NH_4^+ concentration (4–16 mM) resulted in nearly a 50% increase in N content/plant, but only a 7% increase in dry weight.

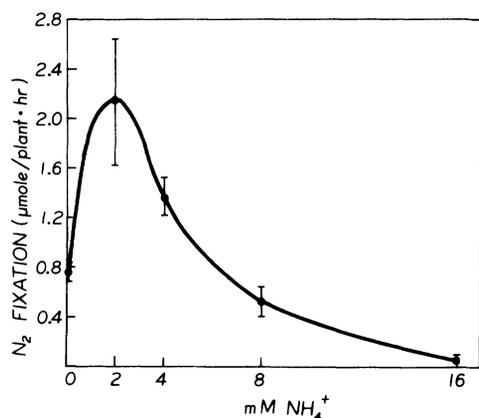


FIG. 1. Symbiotic nitrogen fixation in 26-day-old pea plants as a function of NH_4^+ concentration supplied during growth. Data points represent the mean \pm SE of five replicates and were calculated from C_2H_2 reduction and H_2 evolution data as: N_2 fixed = $(C_2H_2 \text{ reduced} - H_2 \text{ evolution})/3$.

Total plant dry weight, CER, and total N were significantly higher ($P \leq 0.01$) in uninoculated than in inoculated plants grown with 16 mM NH_4^+ , but were lower at 0 and 2 mM NH_4^+ (Figs. 2, 3, and 4). Per cent N content increased more rapidly with increasing NH_4^+ concentration in uninoculated than in inoculated plants but converged in both treatments at 16 mM NH_4^+ (Fig. 5). A factorial analysis of per cent N content data with NH_4^+ concentration and the presence or absence of *Rhizobium* as main effects showed both effects and their interaction to be highly significant ($P \leq 0.005$).

The number of nodules/plant remained stable in plants given nutrient solutions containing up to 8 mM NH_4^+ and declined at 16 mM NH_4^+ , but total nodule mass declined with nutrient solutions containing more than 2 mM NH_4^+ (Table III). Calculations from Table III revealed that mean nodule mass declined with NH_4^+

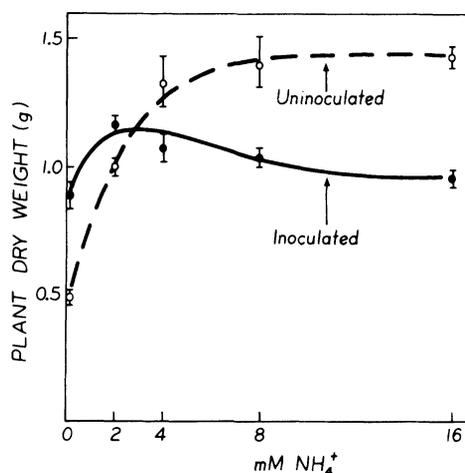


FIG. 2. Dry weight of 26-day-old pea plants which were inoculated or not inoculated with *R. leguminosarum* and grown with various amounts of supplied NH_4^+ . Dry weight data/plant represent the mean \pm SE of eight replicates.

Table I. Carboxylation efficiency (CE) in the youngest fully expanded leaf of 26-day-old pea plants. Data represent the mean \pm SE of three replicates. Mean values for inoculated and uninoculated plants at each NH_4^+ concentration were compared with a 2-tailed t test.

	$mM NH_4^+$				
	0	2	8	16	
	[$mg CO_2 dm^{-2} hr^{-1} (ppm CO_2)^{-1}$]				
Uninoculated	0.100 \pm 0.008	0.131 \pm 0.009	0.240 \pm 0.006	0.242 \pm 0.030	0.208 \pm 0.012
Inoculated	0.250 \pm 0.013	0.328 \pm 0.039	0.270 \pm 0.034	0.243 \pm 0.035	0.238 \pm 0.016
	$p \leq 0.001$	$p \leq 0.01$	n.s.	n.s.	n.s.

Table II. Symbiotic nitrogen fixation in 26-day-old Alaska peas. Nitrogen fixation was calculated as $[(C_2H_2 \text{ reduced} - H_2 \text{ evolved})/3]/g \text{ nodule dry wt} \cdot hr$. Data represent the mean \pm SE of five replicates.

	$mM NH_4^+$				
Trait	0	2	8	16	
C_2H_2 reduction ($\mu\text{mole } C_2H_4/\text{plant} \cdot \text{hr}$)	5.02 \pm 0.27	8.30 \pm 1.58	5.37 \pm 0.46	2.37 \pm 0.33	0.23 \pm 0.05
H_2 evolution ($\mu\text{mole } H_2/\text{plant} \cdot \text{hr}$)	2.64 \pm 0.30	1.90 \pm 0.24	1.11 \pm 0.14	0.80 \pm 0.09	0.11 \pm 0.04
N_2 fixation ($\mu\text{mole } N_2/g \text{ nodule dry wt} \cdot \text{hr}$)	13.8 \pm 2.1	34.4 \pm 5.4	29.2 \pm 2.3	13.7 \pm 2.0	12.0 \pm 2.4
RE [$1 - (H_2 \text{ evolved}/C_2H_2 \text{ reduced})$]	0.47 \pm 0.04	0.74 \pm 0.05	0.78 \pm 0.03	0.63 \pm 0.05	0.57 \pm 0.06

concentrations above 2 mM. This trend paralleled a similar decline in the specific activity of N₂ fixation (Table II). Hydrogen evolution declined throughout the NH₄⁺ concentration range, but C₂H₂ reduction was maximal at 2 mM. The result was that the calculated RE (15) was maximal in the 2 to 4 mM NH₄⁺ range and declined toward both lower and higher NH₄⁺ concentrations.

DISCUSSION

Various investigators have implicated photosynthesis as a primary physiological process supporting N₂ fixation without making concurrent measurements of these two reductive events (1, 7, 10). Data from this study support the concept (6, 8, 12) that low levels of combined N promote symbiotic N₂ fixation in legumes. The mechanism of this phenomenon might now be explained as an enhancement of photosynthesis at 2 mM NH₄⁺ due to relief of N stress, but without the inhibition of N₂ fixation caused by high levels of combined N. Uninoculated pea plants in this study

showed the expected (11, 14, 16) increases in photosynthesis and primary productivity with greater availability of combined N.

Information available for soybeans shows that plants which utilize both symbiotically and nonsymbiotically derived N produce greater seed yields than plants provided with only one N source (8). The present results, taken at the end of the vegetative phase, only partially fit this concept. At lower concentrations of externally applied NH₄⁺, measured symbiotic plant responses to N availability were enhanced in comparison to those of nonsymbiotic peas. At 8 and 16 mM NH₄⁺ levels, however, it is evident that uninoculated plants had significantly more dry matter than those infected with *Rhizobium*. The slightly increased dry matter

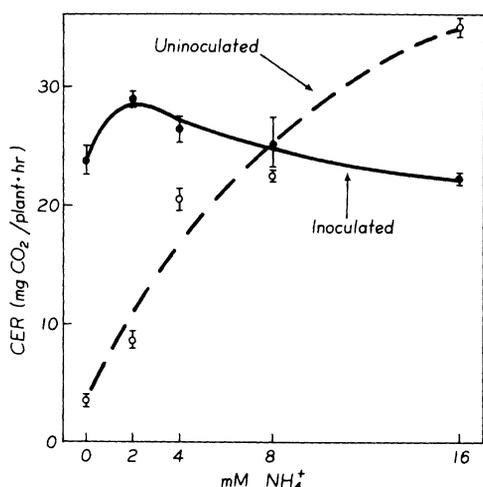


FIG. 3. CER in 26-day-old pea plants inoculated or not inoculated with *R. leguminosarum* as a function of NH₄⁺ concentration. Data represent the mean ± SE of three replicates.

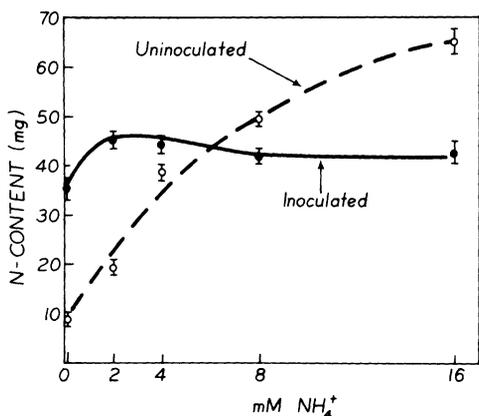


FIG. 4. Total nitrogen content of 26-day-old pea plants inoculated or not inoculated with *R. leguminosarum* as a function of NH₄⁺ concentration. Nitrogen content data/plant represent the mean ± SE of eight replicates.

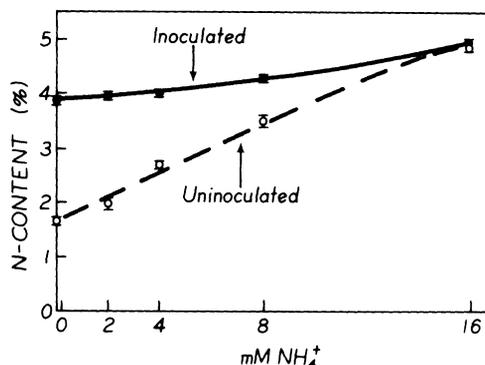


FIG. 5. Per cent N content of 26-day-old pea plants inoculated or not inoculated with *R. leguminosarum* as a function of NH₄⁺ concentration. Per cent N content calculated as (mg N/g dry wt) × 10⁻¹ is given as a mean ± SE from eight replicates. Factorial analysis of variance for the levels of NH₄⁺ and the presence or absence of *Rhizobium* showed highly significant main effects and interaction (*P* ≤ 0.005).

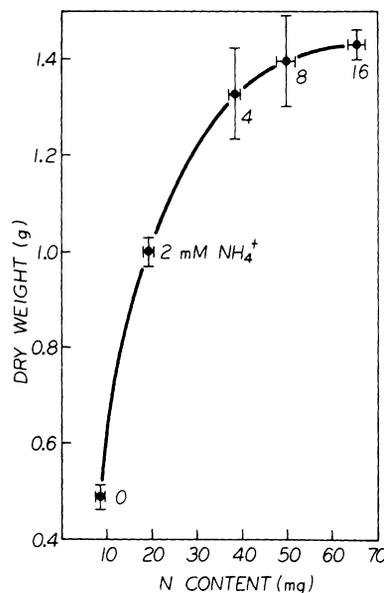


FIG. 6. Dry weight of 26-day-old pea plants grown in the absence of *Rhizobium* as a function of N content. Ammonium content (mM) of the nutrient solution is indicated at each datum point. Means ± SE were calculated from eight replicates.

Table III. Nodule dry weight and number in 26-day-old Alaska peas. Data represent the mean ± SE of five replicates.

Nodule data	0	2	mM NH ₄ ⁺	8	16
Dry weight (mg/plant)	55.8 ± 2.7	57.6 ± 3.8	38.2 ± 3.6	30.8 ± 3.6	3.4 ± 0.9
Number (no./plant)	256 ± 12	267 ± 12	227 ± 23	249 ± 30	164 ± 12

production of uninoculated peas grown with 4 mM NH_4^+ compared with inoculated plants may reflect a lower energy requirement for deriving organic N from NH_4^+ than from N_2 because inoculated peas had significant levels of N_2 fixation in the presence of 4 mM NH_4^+ . In the presence of 16 mM NH_4^+ , however, inoculated peas exhibited insignificant N_2 fixation and yet accumulated nearly 40% less N than uninoculated plants. One explanation of this phenomenon may have involved a repression of NH_4^+ utilization by the numerous small root nodules present on the inoculated plants.

A reasonable case can be made for the concept that photosynthesis was limited by available N in uninoculated plants grown at 0 and 2 mM NH_4^+ . A 5-fold increase in CER between 0 and 4 mM NH_4^+ was largely reflected in dry matter production of these plants, when initial seed dry matter (200 mg) is considered. CE values reveal that uninoculated plants grown with 0 or 2 mM NH_4^+ had significantly lower ($P \leq 0.01$) photosynthetic efficiencies on a leaf area basis than inoculated plants. Values of CE for plants grown with 4, 8, or 16 mM NH_4^+ were not affected significantly by the presence of *Rhizobium*. The fact that dry weight increments/unit of N input decline between 4 and 16 mM NH_4^+ suggests that an upper limit to photosynthetic assimilation of CO_2 had been reached. The convergence of per cent N content of inoculated and uninoculated plants supports this possibility. Although one might expect to observe ammonium toxicity effects at 16 mM NH_4^+ (5, 13), neither CE data nor CER measurements support this concern.

The RE value of 0.47 for plants grown in the absence of combined N is representative of previously reported data from nodules formed by *R. leguminosarum* strain 128C53 on Alaska peas at this stage of growth (3). The increases in RE values in the presence of 2 and 4 mM NH_4^+ followed by decreases at 8 and 16 mM NH_4^+ were unexpected. Differences in morphological stage which were associated previously with changes in RE (3) were not involved in this study because all plants had approximately the same number of leaves. Nor can different photosynthetic photon

flux densities, previously related to varying RE values (4), be used to explain the present differences. Changes in RE may involve alterations in the capacity of the nitrogenase complex to evolve H_2 or in the activity of an uptake hydrogenase system. Attempts to select between these possible explanations are being made.

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