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Increased Arginine Biosynthesis during Phosphorus Deficiency[1]

A RESPONSE TO THE INCREASED AMMONIA CONTENT OF LEAVES

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ABSTRACT

The accumulation of arginine in leaves of four citrus rootstock cultivars during P deficiency has been demonstrated to be due to increased *de novo* synthesis rather than decreased catabolism or increased protein degradation (E Rabe, CJ Lovatt, 1984, Plant Physiol 76: 747–752). In this report, we provide evidence (a) that the increased activity of the arginine biosynthetic pathway observed for citrus rootstocks grown under P-deficient conditions for 7 months is due to an increase in the concentration of ammonia in leaves of P-deficient plants and (b) that ammonia accumulation and removal through arginine systhesis are early responses to phosphorus deficiency for both a woody perennial, rough lemon (*Citrus limon*), and an herbaceous annual, summer squash (*Cucurbita pepo*). Transferring 5-day-old squash plants to a phosphorus-deficient nutrient solution for only 10 days resulted in a 2-fold increase in the concentration of nitrate in the youngest fully expanded leaves (YFE). Concomitantly, the specific activity of nitrate reductase doubled and the ammonia content of P-deficient YFE leaves increased to a concentration significantly greater that of leaves from healthy control plants ($P < 0.05$). Consistent with increased availability of ammonia, the incorporation of $NaH^{14}CO_3$ into arginine plus urea doubled during phosphorus deficiency and arginine accumulated. Despite the accumulation of nitrate and ammonia in YFE leaves during phosphorus deficiency, the total nitrogen content of these leaves was less than that of the healthy control plants. Similar results were obtained for rough lemon. Nitrate content of the YFE leaves increased 1.5- and 3.0-fold in plants deprived of phosphorus for 6 and 12 weeks, respectively. Ammonia content of the leaves increased as P deficiency progressed to 1.4 ± 0.08 mg (± SE, $n = 4$) per gram dry weight, a level 1.8-fold greater than that of the P-sufficient control plants. During P deficiency *de novo* arginine biosynthesis in rough lemon increased 10-fold. Immersing the petiole of YFE leaves from P-sufficient squash and rough lemon plants in 50 millimolar NH_4^+ for 3 hours resulted in the accumulation of ammonia in the leaves, and a 4-fold increase in the incorporation of $NaH^{14}CO_3$ into arginine plus urea. Taken together, these results provide strong evidence that the accumulation of nitrate and ammonia in leaves is an early response of both woody and herbaceous plants to P deprivation. The data are consistent with the hypothesis that increased *de novo* arginine biosynthesis in leaves during P deficiency is in response to ammonia content of the leaves.

We have recently demonstrated that the accumulation of arginine in the leaves of four citrus rootstock cultivars during P deficiency is due to increased *de novo* synthesis of arginine, rather than decreased catabolism or increased protein degradation (25). Evidence to support a cause and effect relationship between P nutrition and arginine biosynthesis was provided by experiments that demonstrated: (a) that both arginine content and activity of the arginine pathway increased in parallel with increasing severity of P deprivation; and (b) when −P[4] seedlings were resupplied with P, activity of the *de novo* arginine pathway was reduced and accumulation of arginine ceased. The increased activity of this pathway during P deficiency, along with reports of increased nitrogen levels in citrus cultivars grown under low P fertilization (1, 23), suggested to us the possibility that P deficiency results in increased concentration of ammonia in P-deficient leaves.

In this communication, the accumulation of ammonia is demonstrated to be an early result of P deficiency in plants representing two families: Rutaceae, *Citrus limon* (L) Burm f. cv rough lemon, and Cucurbitaceae, *Cucurbita pepo* L. cv Early Prolific Straightneck.

MATERIALS AND METHODS

Chemicals. All radiolabeled chemicals were purchased from ICN Pharmaceuticals, Inc. Liquiscint (liquid scintillation cocktail) was purchased from National Diagnostics. Mineral salts for Hoagland and Shive nutrient solutions were of analytical reagent quality from Fisher Scientific. All other chemicals were purchased from Sigma Chemical Company.

Plant Materials. Seeds of *Citrus limon* (L.) Burm f cv rough lemon were grown from seed in sterile sand containing 100 μg P/g sand (P-sufficient; +P) or 5 μg P/g sand (P-deficient; −P) provided as $Ca(H_2PO_4)_2$ (25). Plants were watered with half-strength Hoagland solution without P. When the seedlings were 6 and 12 weeks old, the youngest fully expanded leaves (approximately 21-d-old) were collected for analyses and root and shoot dry weights were determined. Seeds of summer squash (*Cucurbita pepo* L. cv Early Prolific Straightneck), courtesy of the Joseph Harris Co., Inc., were imbibed in distilled H_2O at room temperature. After 24 h, the seeds were rinsed three times with distilled H_2O, spread evenly between two sheets of paper toweling in a plastic box (33 × 23 × 9.5 cm), and moistened with 30 ml of distilled H_2O. The covered box was placed in a growth chamber where the seeds germinated in the dark for 48 h at 30°C. At the end of the germination period, the seedlings were inserted into holes in the lid of a polyurethane breadbox (38 × 14 × 14 cm, 5.5 L) covered with aluminum foil and containing Shive's nu-

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[4] Abbreviations: +P, 100 μg P/g sand, phosphorus sufficient; −P, 5 μg P/g sand, phosphorus deficient; YFE, youngest fully expanded leaves, YFE leaves are 5-d-old for *Cucurbita pepo* cv. Early Prolific Straightneck and approximately 21-d-old for *Citrus limon* cv. rough lemon.

trient solution. The box was transferred to a growth chamber where the plants were hydroponically cultured in aerated solution at 30°C under continuous illumination (310 $\mu E/m^2 \cdot s$) (17). After 3 d, plants were transferred to complete Shive's nutrient solution containing 1 mM KH_2PO_4 and 2 mM K_2SO_4 (+P) or a solution from which the 1 mM KH_2PO_4 was omitted and to which 2.5 mM K_2SO_4 was added (−P). The plants were transferred to a growth chamber, aerated and allowed to grow at 30°C under continuous illumination for 10 d. Nutrient solutions were decanted and replaced by fresh solutions on d 4 and 7. Analyses employed the youngest fully expanded leaves (5-d-old) or mature leaves (approximately 10-d-old).

Leaf Phosphorus and Nitrogen Content. Excised leaves were immediately frozen in liquid N_2, lyophilized, and ground with a Wiley mill to a size fine enough to pass through a 40-mesh screen.

Phosphorus content of leaves was determined in a 50-mg sample of ground leaf tissue by a colorimetric assay employing molybdivanadophosphoric acid (14). Absorbance at 420 nm was linear for concentrations of P from 0 to 0.4 mg/100 mg dry weight leaf tissue.

Total nitrogen was determined in a 25-mg sample of ground leaf tissue using the conventional micro-Kjeldahl method. Nitrate was determined in a 100-mg sample extracted in 100 ml 16 mM $CaSO_4 \cdot 2H_2O$ using a Technicon AutoAnalyzer (24). Ammonia as NH_3-NH^+_4 was quantified in a 200-mg sample extracted in 50 ml 1 N KCl using a Technicon AutoAnalyzer (29). Amino acid content of the leaf tissue was determined as described previously (25) using a Beckman 120 C Amino Acid Analyzer calibrated with commercial standards in the appropriate buffer at 570 nm (15). Soluble protein content of the leaf tissue was determined according to Bradford (4).

Incorporation of $NaH^{14}CO_3$ into the Combined Pool of Arginine Plus Urea. Activity of the *de novo* arginine biosynthetic pathway was assessed in the intact cells of the youngest fully expanded leaves (500 mg fresh leaf tissue cut into 5 × 5 mm pieces, midvein removed) by measuring the incorporation of $NaH^{14}CO_3$ (25 mM, 37.5 μCi for squash and 5 mM, 37.5 μCi for rough lemon) into arginine and urea during a 3-h incubation period in Shive's nutrient solution (25). The amounts of [guanido-^{14}C]arginine and [^{14}C]urea synthesized by the leaf tissue from $NaH^{14}CO_3$ was determined using commercial arginase and urease as described previously by Lovatt and Cheng (18).

Nitrate Reductase Activity in Cell-Free Extracts of +P and −P Leaves. Cell-free extracts were prepared from 1 g fresh leaf tissue homogenized with a Duall all-glass tissue grinder (Kontes Glassware) (citrus leaves were frozen in liquid N_2 and ground with a mortar and pestle before homogenization) in 5 ml 100 mM K-phosphate (pH 7.5) containing 1 mM EDTA, 1 mM cysteine, and 3% (w/v) casein and centrifuged at 10,000*g* for 10 min at 0°C. The supernatant fraction served as the source of enzyme. The activity of nitrate reductase was measured according to the method of Scholl *et al.* (26) in a 2-ml reaction mixture of the following composition: 50 μmol K-phosphate (pH 7.5), 20 μmol KNO_3, 0.8 μmol NADH, and 50 to 200 μl enzyme. After incubation at 30°C for 30 min, the reaction was stopped with the addition of 50 μmol/ml zinc acetate. Phenazine methosulfate (15 nmol/ml) was added as a postassay treatment to oxidize excess NADH.

Carbohydrate Content. Leaf tissue (500 mg fresh weight) was homogenized in 4.0 ml of 80% ethanol using a Polytron tissue homogenizer (PCU-2, Brinkman Instruments) at speed 6. The Polytron probe was rinsed with 2 ml 80% ethanol, which was added to the homogenate. Glucose and starch (as glucose equivalents) were quantified by a modification of the glucose oxidase-peroxidase-*O*-dianisidine method described by Hamid *et al.* (11). The assay was linear for glucose concentrations from 10 to 100 μg/ml. Samples were diluted before enzyme treatment to a concentration within this range.

Statistical Analysis. Analysis was by Student's *t* test.

RESULTS

Effects of Phosphorus Deprivation on Plant Growth. Withholding P resulted in a significant reduction in shoot growth for both squash and rough lemon ($P < 0.01$; Table I). Root growth was inhibited less than shoot growth in −P rough lemon; an increase in root biomass occurred in −P squash. Root growth was significantly different between the two treatments at the 1% level. The greater sensitivity of shoot growth to P deficienty is reflected by the significantly lower shoot-to-root ratios of −P plants ($P < 0.05$). The YFE leaves of −P squash plants exhibited no symptoms of P deficiency. Mature leaves from squash plants deprived of P for 10 d exhibited burning of the leaf margin and collapsed water-soaked areas on the blade, which later became necrotic. Rough lemon seedlings deprived of P for 6 weeks exhibited no visual symptoms of deficiency other than reduced root and shoot growth. Mature leaves, but not YFE leaves, of rough lemon plants deprived of P for 12 weeks exhibited burning of the leaf margin.

Effects of Phosphorus Deprivation on Levels of Phosphorus, Total Nitrogen, Nitrate, and Ammonia. Withholding P, 10 d for squash and 12 weeks for rough lemon, reduced phosphorus levels of the YFE leaves by 70 and 50% for the two species, respectively ($P < 0.01$) (Table II).

P deficiency had no effect on the concentration of total nitrogen in the YFE or mature leaves of 5-d-old squash plants transferred from +P Shive's nutrient solution to Shive's without P for an additional 10 d (Table II). However, nitrate levels were 2-fold greater in both the YFE ($P < 0.01$) and mature leaves of −P squash plants (Table II). Ammonia levels were also significantly elevated by P deficiency in squash leaves of both ages ($P < 0.05$) (Table II). The amount of nitrate and ammonia in −P squash leaves in excess of the levels in +P leaves was for the YFE leaves 2.8 mg nitrate and 52 μg ammonia/g dry weight, and for the mature leaves, 5.5 mg nitrate and 161 μg ammonia/g dry weight.

Nitrate and ammonia levels increased in the YFE leaves of *C. limon* with increasing severity of P deficiency (Table II). After 12 weeks of P deficiency, the YFE leaves had 3- and 2-fold more nitrate and ammonia, respectively, than the YFE leaves from +P control plants ($P < 0.01$). Total nitrogen increased 30% in

Table I. *Effects of Phosphorus Deprivation on Plant Growth*

Data are the mean ± SE with the number of experiments given in parentheses.

Plant	Treatment Duration	Treatment	
		+P	−P
		Leaves + shoots	
		mg dry wt/plant	
Squash	10 d	502 ± 22	392 ± 16 (7)
Rough lemon	6 wk	176 ± 28	88 ± 9 (8)
	12 wk	802 ± 89	152 ± 20 (10)
		Roots	
Squash	10 d	44 ± 3	52 ± 4 (7)
Rough lemon	6 wk	52 ± 6	38 ± 6 (8)
	12 wk	338 ± 55	116 ± 15 (10)
		Ratio (shoot:root)	
Squash	10 d	11.6 ± 0.5	7.7 ± 0.4 (7)
Rough lemon	6 wk	3.34 ± 0.14	2.50 ± 0.22 (8)
	12 wk	2.59 ± 0.19	1.31 ± 0.08 (10)

the YFE leaves, a net increase of 8.4 mg/g dry weight, after 12 weeks of P deficiency ($P < 0.001$).

Amino Acid Content and *de Novo* Arginine Synthesis in +P and −P Leaves. The accumulation of L-arginine in both the free amino acid and protein fractions was the most significant change observed when the amino acid content of −P leaves were compared with that of the +P control plants. Free arginine and protein arginine levels were consistently greater in the leaves of −P plants compared to those of +P control plants (Table III). P deficiency either had no effect or increased the level of other amino acids in the free amino acid fraction, but consistently reduced the level of other amino acids present in the protein fraction (Table III). This suggests that arginine is being synthesized at the expense of other amino acids normally incorporated into protein. Protein content of −P leaves was 30 ± 2% (mean ± SE, $n = 8$) less than that of +P control plants, but the amount of arginine incorporated into protein in −P leaves was 1.1- to 4-fold greater.

Activity of the pathway for the *de novo* biosynthesis of arginine increased dramatically during P-deficiency: 2-fold in squash and 10-fold in rough lemon ($P < 0.01$ for all treatments; Table IV). The incorporation of $NaH^{14}CO_3$ into urea was not significantly increased during P deficiency. In each case, [^{14}C]urea constituted less than 5% of the combined pool of [^{14}C]arginine plus [^{14}C] urea synthesized by −P leaves. The significantly greater accumulation of NH_3-NH_4^+ in leaves from −P rough lemon plants

Table II. *Effect of P Deprivation on Concentrations of Phosphorus, Total Nitrogen, Nitrate, and Ammonia in Leaves of Squash and Rough Lemon*

Data are the mean ± SE with the number of experiments given in parentheses. When the number of experiments for +P and −P treatments is unequal, the number of experiments with +P plants is given first, followed by the number with −P plants.

Plant Leaf Tissue		Treatment Duration	Treatment Conditions	
			+P	−P
			Phosphorus (mg/g dry wt)	
Squash	YFE[a]	10 d	7.2 ± 0.2	2.0 ± 0.1 (11)
Rough lemon	YFE	6 wk	2.3 ± 0.4	1.4 ± 0.1 (3)
	YFE	12 wk	2.1 ± 0.2	1.0 ± 0.1 (3; 4)
			Total nitrogen (mg/g dry wt)[b]	
Squash	YFE	10 d	49.2 ± 1.6	46.5 ± 1.0 (14)
	Mature		34.5 ± 4.1	38.3 ± 1.9 (3)
Rough lemon	YFE	6 wk	30.4 ± 0.1	26.9 ± 2.5 (3)
	YFE	12 wk	29.4 ± 0.6	37.8 ± 0.3 (5; 4)
			Nitrate (μg/g dry wt)	
Squash	YFE	10 d	2496 ± 328	5227 ± 461 (9)
	Mature		4050 ± 750	9550 ± 500 (2)
Rough lemon	YFE	6 wk	910 ± 269	1325 ± 425 (3; 2)
	YFE	12 wk	1086 ± 105	3658 ± 546 (5; 4)
			NH_3-NH_4^+ (μg/g dry wt)	
Squash	YFE	10 d	660 ± 58	712 ± 70 (5)
	Mature		396 ± 33	557 ± 26 (3)
Rough lemon	YFE	6 wk	785 ± 35	939 ± 189 (3; 2)
	YFE	12 wk	796 ± 108	1411 ± 78 (5; 4)

[a] Leaves approximately 5-d-old for squash and 21-d-old for rough lemon.

[b] Total nitrogen was determined by the Kjeldahl method and does not include nitrate which is given in the table.

Table III. *Protein, Free, and Total Amino Acid Levels in +P and −P Squash and Rough Lemon Leaves*

Each value represents the mean of 3 to 5 experiments. The average difference between experiments was ± 20%; number of experiments is given in parentheses.

Plant	Treatment Duration	Amino Acid Fraction: Protein		Free		Total	
		+P	−P	+P	−P	+P	−P
		Arginine					
		μmol/g dry wt					
Squash	10 d	109.7	120.1	0.9	5.2	110.6	125.3 (5)
Rough lemon	6 wk	62.8	112.2	1.6	12.3	64.4	124.5 (3)
	12 wk	54.2	200.1	5.5	54.6	59.7	254.7 (3)
		All other amino acids except arginine					
Squash	10 d	2059.9	1881.6	70.2	67.6	2130.1	1949.1 (5)
Rough lemon	6 wk	1131.5	921.8	237.1	255.9	1368.8	1177.7 (3)
	12 wk	1049.6	628.3	196.7	335.6	1246.2	963.9 (3)

Table IV. *De Novo Arginine Biosynthesis in Young, Fully Expanded +P and −P Leaves from Squash and Rough Lemon*

Data are the mean ± SE with the number of experiments given in parentheses.

Plant	Treatment Duration	Treatment Conditions		
		+P	−P	
		nmol $NaH^{14}CO_3$ incorporated into arginine plus urea per g fr wt tissue during 3-h incubation		*−P/+P ratio*
Squash	10 d	35.44 ± 3.07	77.63 ± 6.76 (7)	2.19
Rough lemon	6 wk	14.49 ± 2.63	148.77 ± 13.04 (6)	10.27
	12 wk	14.64 ± 3.22	161.21 ± 16.74 (3)	11.01

Table V. *De Novo Synthesis and Accumulation of Arginine*

Plant Treatment and Duration		Amount of Arginine in −P Leaves Minus amount in +P Leaves[a]	Amount of arginine Synthesized *de novo* in −P Leaves Minus Amount Synthesized in +P Leaves[b]	Percent of Excess Arginine in −P YFE Accounted for by *de Novo* Synthesis
		µmol/g dry wt		%
Squash	10 d	14.7	16.9	100
Rough lemon	6 wk	60.1	67.7	100
	12 wk	195.0	73.9	38

[a] Values in this column were calculated by subtracting µmol total arginine present in Table III for +P leaves from that given for −P leaves.

[b] Values in this column were calculated by subtracting the nmol $NaH^{14}CO_3$ incorporated into arginine plus urea per g fresh wt during the 3-h incubation presented in Table IV for +P leaves from that given for −P leaves, multiplying the difference by 24 h/d and then by 5 d, the age of the youngest fully expanded leaf of squash, or by 21 d, the age of the youngest fully expanded leaf of rough lemon. The product was multiplied by 10 g fresh wt per 1 g dry wt for squash, or by 3 g fresh wt per 1 g dry wt for rough lemon.

at 12 weeks *versus* 6 weeks ($P < 0.001$; Table II) suggests that the rate of arginine synthesis in this tissue is probably insufficient to prevent ammonia from accumulating during the additional 6 weeks of P deprivation.

The amount of arginine that accumulated in leaves of −P plants in excess of the amount in +P plants was accounted for by the difference in the rate of activity of the *de novo* arginine biosynthetic pathway in +P and −P leaves (Table V). This was especially true for the earlier stages of P deficiency.

Nitrate Reductase Activity. The higher nitrate and ammonia levels observed during P deficiency suggested that nitrate reductase activity may be increased in the leaves of −P plants. Nitrate reductase activity was 2-fold greater in leaves from −P squash plants compared to that of +P control plants ($P < 0.01$) (Table VI). The increase in nitrate reductase activity was equal in magnitude to the increase in the rate of *de novo* arginine biosynthesis (Table IV). Despite the increase in nitrate reductase activity, nitrate levels were more than 2-fold greater in leaves of −P squash plants (Table II). Nitrate reductase activity in rough lemon was very similar in both +P and −P leaves regardless of the length of P deprivation (Table VI).

Table VI. *Nitrate Reductase Activity in the Leaves of +P and −P Squash and Rough Lemon Leaves*

Data are the mean ± SE with the number of experiments given in parentheses.

Plant	Treatment Duration	Treatment Conditions	
		+P	−P
		µmol NO_2 formed/mg protein·h	
Squash	10 d	0.495 ± 0.02	1.041 ± 0.07 (3)
Rough lemon	6 wk	0.264	0.268 (1)
	12 wk	0.234	0.177 (2)

Table VII. *Carbohydrate Levels in Leaves of +P and −P Squash*

Data are the mean ± SE with number of experiments given in parentheses.

Carbohydrate	+P	−P
Glucose (mg/g fresh wt)	3.22 ± 0.48	1.72 ± 0.39 (5)
Starch (mg glucose equivalents/g fresh wt)	11.68 ± 1.08	28.68 ± 3.51 (5)

Carbohydrate Levels. The reduced growth rate and accumulation of ammonia in −P plants suggested the possibility that these plants were carbohydrate depleted. We determined the starch and glucose content of leaves from +P and −P squash plants. Starch was 2.5-fold greater ($P < 0.01$) and glucose 50% less ($P < 0.05$) in −P leaves (Table VII), consistent with the well-known inability of P-deficient plants to degrade starch which results in high starch accumulation and low glucose availability.

Similarities between P Deficiency and Ammonia Feeding. The petioles of young fully expanded leaves from P-sufficient squash and rough lemon plants were immersed in aerated solutions of 10 to 50 mM NH_4Cl for up to 15 h. The ammonia content of the leaves increased with time to a maximum of 6.5 mg/g dry weight tissue treated with 50 mM NH_4Cl for 15 h. *De novo* arginine biosynthesis increased with increasing concentration of exogenously supplied ammonia or with increasing length of treatment (Table VIII). Accumulating ammonia resulted in visible leaf symptoms that were remarkably similar to those of P deficiency.

DISCUSSION

The results of this study confirm our previous report (25) that the accumulation of arginine during P deprivation was due to

Table VIII. *De Novo Arginine Biosynthesis in Ammonia-Treated Young, Fully Expanded Leaves from P-Sufficient Squash and Rough Lemon*

The petioles of YFE leaves from P-sufficient plants were immersed in aerated solutions of NH_4Cl. Data are the mean ± SE with the number of experiments given in parentheses for all treatments for which more than two experiments were conducted.

Ammonia Concentration	Duration and Treatment	Squash	Rough Lemon
mM	*h*	*nmol $NaH^{14}CO_3$ incorporated into arginine plus urea/g fresh wt tissue during 3-h incubation*	
None		4.0 ± 0.7 (5)	9.3 ± 1.4 (5)
30	3	8.7 ± 1.8 (3)	25.3 ± 1.2 (3)
10	15	14.7 ± 1.4 (3)	39.5
50	3	33.6	47.6
50	15	47.9	55.8

increased *de novo* biosynthesis. In addition, this study established that increased activity of the arginine *de novo* biosynthetic pathway was an early response to P deficiency (within 10 d in squash and 6 weeks in rough lemon) of plants of two diverse families. It is of interest to note that in rough lemon nitrogen metabolism, including nitrate and ammonia accumulation and increased arginine biosynthesis is altered before any visible symptoms characteristic of P deficiency are observed. We have demonstrated that this metabolic response to low P fertilization is not restricted to the woody species, *Citrus* and *Poncirus* of the Rutaceae (25) but is also exhibited by a non-woody annual of the Cucurbitaceae.

Further, results obtained in this study clearly demonstrate (a) that ammonia accumulates during P deficiency and (b) that the amount of accumulated ammonia increases with the increasing severity of P deprivation (Table II). The source of ammonia appears to be two-fold (a) conversion of accumulating nitrate (Table II) and (b) degradation of amino acids normally incorporated into protein (Table III). Finally, the results are consistent with the hypothesis that the increased activity of the arginine *de novo* biosynthetic pathway during P deficiency provides a mechanism for detoxifying leaf tissue of excess ammonia. This interpretation is supported by the results of the ammonia-feeding experiments. The incorporation of $NaH^{14}CO_3$ into arginine plus urea increased as the concentration of exogenously supplied ammonia or length of exposure increased (Table VIII).

Total nitrogen did not increase in the leaves of squash plants deprived of P for 10 d, nor in 6-week-old rough lemon plants. However, total nitrogen levels increased in leaves of rough lemon plants after 6 additional weeks of low P fertilization. This result is consistent with previous reports of arginine accumulation and increased levels of total nitrogen during an extended period of P deficiency (1, 23). Nitrate levels increased sooner and to a greater extent than ammonia. Levels of ammonia would be expected to be low during the early stages of P deficiency since increased activity of the *de novo* arginine pathway is an early response to low P nutrition. Increased levels of alcohol-soluble N have been reported for P-deficient (10) species, but reports of ammonia levels during P deficiency are lacking. Early in P deficiency, arginine accumulating in young developing leaves can be completely accounted for as a product of increased *de novo* synthesis. The failure of *de novo* synthesis to account for all of the arginine accumulating in leaves of rough lemon plants deprived of P for 12 weeks suggests the remobilization of arginine from older senescing leaves to young developing leaves.

Rabe and Lovatt (25) suggested that some metabolic events might specifically be spared from the effects of P deficiency until death of the cell is imminent. This appears to be the case during P deficiency. The *de novo* arginine biosynthetic pathway is expensive in terms of ATP and carbon, which causes additional stress to a plant that is "functionally" carbohydrate depleted. Thus, during P deprivation this pathway would seem prone to inhibition. This is not the case, adding strength to the argument that the *de novo* arginine pathway serves to detoxify P-deficient leaf tissue of accumulating ammonia. The symptoms of P deficiency seem to be identical to those of ammonia toxicity. In both cases, lesions appear on the leaf blade as darkened, water-soaked areas which later become necrotic. The leaf margin appears burned (3). Furthermore, in this study, we demonstrated that the symptoms and metabolic changes associated with P deficiency could be induced through ammonia feeding. While ammonia is known to be toxic to plants, we are not aware that an upper threshold value for leaf ammonia concentration has been determined for any plant species.

Previous research (9, 13, 16, 20) has emphasized the accumulation of asparagine and glutamine during ammonia feeding. However, studies which included measurement of the changes in the levels of additional amino acids reported that arginine accumulated in response to ammonia feeding: approximately 4-fold for intact tomato plants growing in different media (20) to as much as a 10-fold increase in newly synthesized arginine for citrus leaves fed $^{15}NH_4$ (13). In addition, it should be noted that glutamine is the source of nitrogen for the synthesis of carbamoylphosphate. It is through the synthesis of carbamoylphosphate that the radiolabeled carbon of $NaH^{14}CO_3$, the substrate in our assays, is incorporated into the guanido-carbon of arginine.

The accumulation of proline and putrescine is regarded as a general response of vascular plants to salinity, osmotic or water stress (2, 27). Of specific interest is the fact that arginine metabolism is a common denominator between proline and putrescine synthesis. There are several reports in the literature that suggest that arginine is the source of the increased levels of proline associated with salinity and water stress (2, 22). The conversion of arginine to ornithine plus urea is reported to provide ornithine for the synthesis of proline through the glutamic-γ-semialdehyde, pyrroline-5-carboxylate, proline pathway. Arginine would appear to be the major substrate for the synthesis of putrescine, both of which accumulate during osmotic stress, pH stress and NH^+_4 feeding since the level of arginine decarboxylase, but not ornithine decarboxylase, increases dramatically during each stress (7, 27). The inceased availability of arginine as a substrate for both proline and putrescine synthesis is consistent with the increased flow of nitrogen through the *de novo* arginine biosynthetic pathway and with the role of the *de novo* arginine pathway in ammonia metabolism. Whether the increased activity of the arginine pathway represents a specific mechanism to provide proline and putrescine during stress or results from the detoxification of ammonia is not known.

Most macro- and many micronutrient deficiencies alter amino acid metabolism. Arginine and the arginine pathway intermediates, ornithine and citrulline accumulate in leaves during K, P, Mg, S, Fe, Mn, Cl, and Cu deficiencies in a variety of vascular plant species (1, 5, 6, 8, 10, 12, 15, 19, 21, 23, 24). We propose that during these mineral nutrient deficiencies, nitrate and ammonia accumulate in leaves due to the reduction in growth that occurs during most mineral nutrient deficiencies resulting in ammonia accumulation and subsequent removal through *de novo* arginine biosynthesis. Notable exceptions are Mo deficiency, which inhibits nitrate reductase activity, and N deficiency.

The results of our research taken in light of the literature cited above suggest the possibility that any stress causing glucose depletion and/or reduced shoot growth may result in ammonia accumulation early in the stress. We are currently testing this hypothesis.

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