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Influence of Nitrate and Ammonia on Photosynthetic Characteristics and Leaf Anatomy of *Moricandia arvensis*[1]

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ABSTRACT

The leaf anatomy and certain photosynthetic properties of nitrate- and ammonia-grown plants of *Moricandia arvensis* (L.) DC., a species previously reported to be a C_3-C_4 intermediate, were investigated. Nitrate-grown plants had a high level of malate in the leaves while ammonia-grown plants had low levels of malate. In young leaves of nitrate-grown plants, there was a diurnal fluctuation of malate content, increasing during the day and decreasing during the night. Titratable acidity remained low in leaves of both nitrate- and ammonia-grown plants.

In nitrate-grown plants, the activity of phosphoenolpyruvate (PEP) carboxylase was about 2-fold higher than in ammonia-grown plants, the latter having activity typical of C_3 species. Also, in nitrate-grown plants, the ratio of activities of ribulose 1,5-bisphosphate (RuBP) carboxylase/PEP carboxylase was lower than in ammonia-grown plants. Nitrate reductase activities were higher in nitrate- than in ammonia-grown plants and the greatest activity was found in younger leaves.

With nitrate-grown plants, during a pulse-chase experiment the label in malate, as a percentage of the total labeled products, increased from about 7% after a 10-second pulse with $^{14}CO_2$ up to 17% during a 5-minute chase with $^{12}CO_2$. The pattern of ^{14}C labeling in various metabolites suggests the primary carboxylation is through RuBP carboxylase with a secondary carboxylation through PEP carboxylase. In similar experiments, with ammonia-grown plants, the percentage label in malate was only 0% to 4% with no increase in malate labeling during the chase period. The CO_2 compensation point was lower in nitrate-grown than ammonia-grown plants.

There was no evidence of Kranz-like anatomy in either the nitrate or ammonia-grown plants. Mitochondria of bundle-sheath cells were strikingly positioned along the inner tangential wall. This might allow the chloroplasts of these cells to fix the mitochondrial photorespired CO_2 more effectively and contribute to the low CO_2 compensation point in the species. Chloroplasts of bundle-sheath cells and contiguous mesophyll cells were similar in size and structure in plants grown on different media, although chloroplast thylakoids and stromata of the ammonia-grown plants stained more intensely than those of nitrate-grown plants. In addition, irregular clusters of phytoferritin particles occurred in the chloroplasts of the ammonia-grown plants.

The results indicate that the substantial activity of PEP carboxylase, incorporation of CO_2 into malate, the high malate content, and in part the relatively low CO_2 compensation point in *Moricandia arvensis* may be accounted for by metabolism of nitrate rather than by a state of C_3-C_4 intermediacy.

Moricandia arvensis is a species having some characteristics intermediate to C_3 and C_4 plants based on its CO_2 compensation point and PEP[6] carboxylase activity (2, 3, 13, 23). Recently, Holaday et al. (20) concluded that these C_3-C_4 intermediate features did not result from the functioning of a limited C_4 cycle as proposed for *Panicum milioides* (30) primarily due to an inability to detect activity of pyruvate,Pi dikinase in leaf extracts and low activities of C_4 acid decarboxylases.

Winter *et al.* (43) reported that in some species of the family Brassicaceae, including a *Moricandia* species, the malate content increases during the day and decreases during the night. Certain plants when grown on nitrate as the nitrogen source contain considerable levels of malate in the leaves in comparison to plants grown on ammonia (8, 22, 28).

In the present study, we evaluated the leaf anatomy, nitrate reductase activity, malate content, and some photosynthetic properties of nitrate and ammonia grown plants of *Moricandia arvensis.*

MATERIALS AND METHODS

Growth of Plants. Seeds of *Moricandia arvensis* (L.) DC. were obtained from the Royal Botanic Gardens at Kew (U. K.). Plants were grown in a nonshaded glasshouse between March and December 1980. On bright days, PAR was up to 1,900 μE m^{-2} s^{-1} and maximum temperatures up to 35°C. Seedlings were established in soil. When the 3rd foliage leaf pair appeared, seedlings were transferred into 2-L pots (1 plant/pot) filled with vermiculite. Plants were divided into three groups differing in the form of nitrogen supplied. The pots were flushed at 2-d intervals with one of the following nutrient solutions. Solution A (22) contained nitrogen only in the form of NO_3 (5 mM). Solution B was a standard nutrient solution (40) and had a large excess of NO_3^- (7 mM) over NH_4^+ (1 mM). Solution C (22) contained nitrogen only in the form of NH_4^+ (5 mM). Between treatments with nutrient solution, the vermiculite was kept moist by addition of deionized

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[6] Abbreviations: PEP, phosphoenolpyruvate; RuBP, ribulose 1,5-bisphosphate; Γ, CO_2 compensation point.

H_2O and immediately before nutrient solution was applied, the vermiculite was flushed with deionized H_2O. About 2- to 3-month-old plants, which were just before the flowering stage, were used in the experiments.

Enzyme Assays. For determination of RuBP and PEP carboxylase activities, leaf discs were ground in mortar and pestle in ice-cold 100 mM Hepes (pH 7.6) containing 5 mM $MgSO_4$, 1 mM EDTA, 10 mM $NaHCO_3$, and 5 mM DTT. The ratio of fresh weight (g) to volume of extraction buffer (ml) was approximately 1:4. The homogenate was centrifuged at 10,000*g* for 1 min. Aliquots of the supernatant were desalted with a Sephadex G-25 column which was equilibrated with a buffer similar to the extraction buffer, except that the concentration of Hepes was 50 mM. PEP carboxylase was assayed by oxidation of NADH via malate dehydrogenase (41). RuBP carboxylase was assayed using a radiochemical procedure after Lorimer *et al.* (25).

For determination of pyruvate,Pi dikinase activity, leaf discs (taken from fully illuminated leaves) were ground at room temperature in mortar and pestle in a medium containing 100 mM Hepes (pH 7.6), 10 mM $MgCl_2$, 2.5 mM pyruvate, 2 mM K_2HPO_4, 1 mM EDTA, 0.5% (w/v) ascorbate, and 5 mM DTT. The homogenate was centrifuged for 1 min at 10,000*g*. The supernatant was kept at room temperature for 1 h. The crude extract was then desalted with a column of Sephadex G-25 equilibrated with 50 mM Hepes (pH 7.6), 5 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM DTT. Pyruvate,Pi dikinase was assayed using a spectrophotometric test after Hatch and Slack (17) and Sugiyama and Laetsch (38).

All enzymes were assayed at 29 to 30°C. Chl was determined in the original crude homogenate using the method of Arnon (4). In the preparation of tissue extracts (leaf and root) for the assay of nitrate reductase 1 g fresh weight tissue was added per 7 ml of extraction buffer (0.1 M phosphate buffer [pH 7.8] containing 5 mM EDTA, 1 mM reduced glutathione, and 2% [w/v] casein). The tissue was homogenized in a VirTis 60K homogenizer, speed setting 70 for 1 min, and the homogenates squeezed through four layers of cheesecloth and centrifuged at 10,000*g* for 10 min. All procedures were carried out at 0°C. The resulting supernatants were used immediately in the nitrate reductase assays.

In the assay of nitrate reductase, nitrite formed from nitrate was determined colorimetrically. Fifty μl of enzyme extract were added to 0.5 ml 0.1 M phosphate buffer (pH 7.5), 0.2 ml 0.1 M KNO_3, and 1.05 ml water. The reaction was started by the addition of 0.4 μmol NADH (final volume 2.0 ml). Tubes were incubated at 30°C for 15 min and the reaction stopped by boiling for 5 min. After cooling, 0.2 ml of 0.15 mM phenazine methosulphate was added to each tube. The tubes were kept at room temperature for 15 min, then 1 ml 1% (w/v) sulfanilamide in 3 N HCl and 1 ml 0.02% (w/v) *N*-napthylethylenediamine-HCl in H_2O were added to each tube. The resulting red color was read at 535 nm. All experiments were run with boiled enzyme controls and standards were obtained using known quantities of nitrite.

Determination of Malate and Titratable Acidity. Leaf discs were taken at dawn or dusk or at various time points during a day/night cycle and extracted with 20% (v/v) boiling ethanol for 15 min. Extracts were used for determination of L-(−)-malate after Hohorst (19). For determination of titratable acidity, extracts were titrated with 5 mM NaOH to pH 6.5.

$^{14}CO_2$ Fixation. $^{14}CO_2$ fixation experiments were performed with attached leaves. One leaf was enclosed in a 320 ml Perspex chamber and preilluminated at 1,200 μE m^{-2} s^{-1} and 30°C for 5 min. For $^{14}CO_2$ exposure at a CO_2 partial pressure, 100 μbars above ambient levels, the chamber was continuously aerated during preillumination. Previous measurements during IR gas analysis in our laboratory showed ambient levels of CO_2 between 340 to 400 μbars. The chamber was then sealed and $^{14}CO_2$, which had been liberated by acidification of a $NaH^{14}CO_3$ solution, was injected into the chamber (1.4 μmol CO_2, 58.3 Ci/mol), thereby increasing the CO_2 partial pressure by 100 μbars. The chamber was placed on a magnetic stirrer, and during exposure to $^{14}CO_2$ mixing inside the chamber was provided by a magnetic bar. For $^{14}CO_2$ exposure at low CO_2 partial pressure, leaves were preilluminated in a closed chamber. Given a CO_2 compensation point of about 30 to 60 μbar (see also "Results"), injection of 0.7 μmol of $^{14}CO_2$ increased the CO_2 partial pressure to 80 to 110 μbar.

In the pulse-chase experiments, the pulse with $^{14}CO_2$ was for 10 s and the chase with air ($^{12}CO_2$) at the same light intensity outside the chamber was for 5 min. Following a pulse with $^{14}CO_2$ with one leaf, and a pulse-chase treatment with another leaf, the tissue was killed in liquid N_2. Extracts were prepared after the method described by Hatch (16). A mixture of solid CO_2 and ethanol was added to the samples and the liquid N_2 boiled off. The leaf tissue was then crushed into small pieces with a glass rod. Samples were warmed to −20°C in a freezer. Boiling 85% (w/v) ethanol was added and the samples were placed for 2 min onto a hot plate. The tissue was then extracted 3 times with boiling 80% (v/v) ethanol and 3 times with boiling water. Procedures in which leaves were killed by direct transfer to boiling 80% ethanol or in which liquid N_2 frozen leaves were directly extracted with boiling 80% ethanol led to substantial occurrence of label in glycerate, probably due to the operation of a P-glycerate phosphatase during extraction. After extraction, an aliquot was taken to determine total ^{14}C fixed. The remainder was then dried under air and the labeled products were separated by two dimensional chromatography on Whatman 3 MM filter paper and radioautograms prepared to identify the ^{14}C-labeled products (27). Areas on the chromatogram containing labeled sucrose, serine + glycine were identified using authentic compounds. Glycerate and malate were identified using authentic [^{14}C]glycerate and [^{14}C]malate. Glycerate and malate showed very similar chromatographic behavior. These were separated by elution, and rechromatography in one dimension in butanol:propionic acid:water (117:55:78, v/v/v) for 36 h. The radioactivity in the various products was quantitated by liquid scintillation spectroscopy after placing excised labeled areas in vials containing 50 mg PPO in 10 ml toluene.

For determining the radioactivity in the insoluble fraction, a

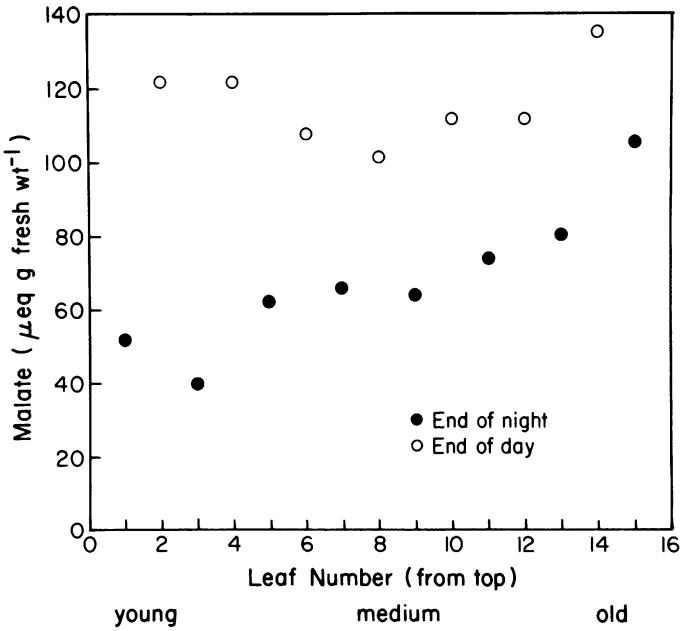


FIG. 1. Malate content in leaves of *M. arvensis* at the end of a bright day and end of the following night. Plants were kept during the summer in a nonshaded greenhouse and watered with a nutrient medium containing nitrogen in the form of 7 mM nitrate and 1 mM ammonium salt (solution B).

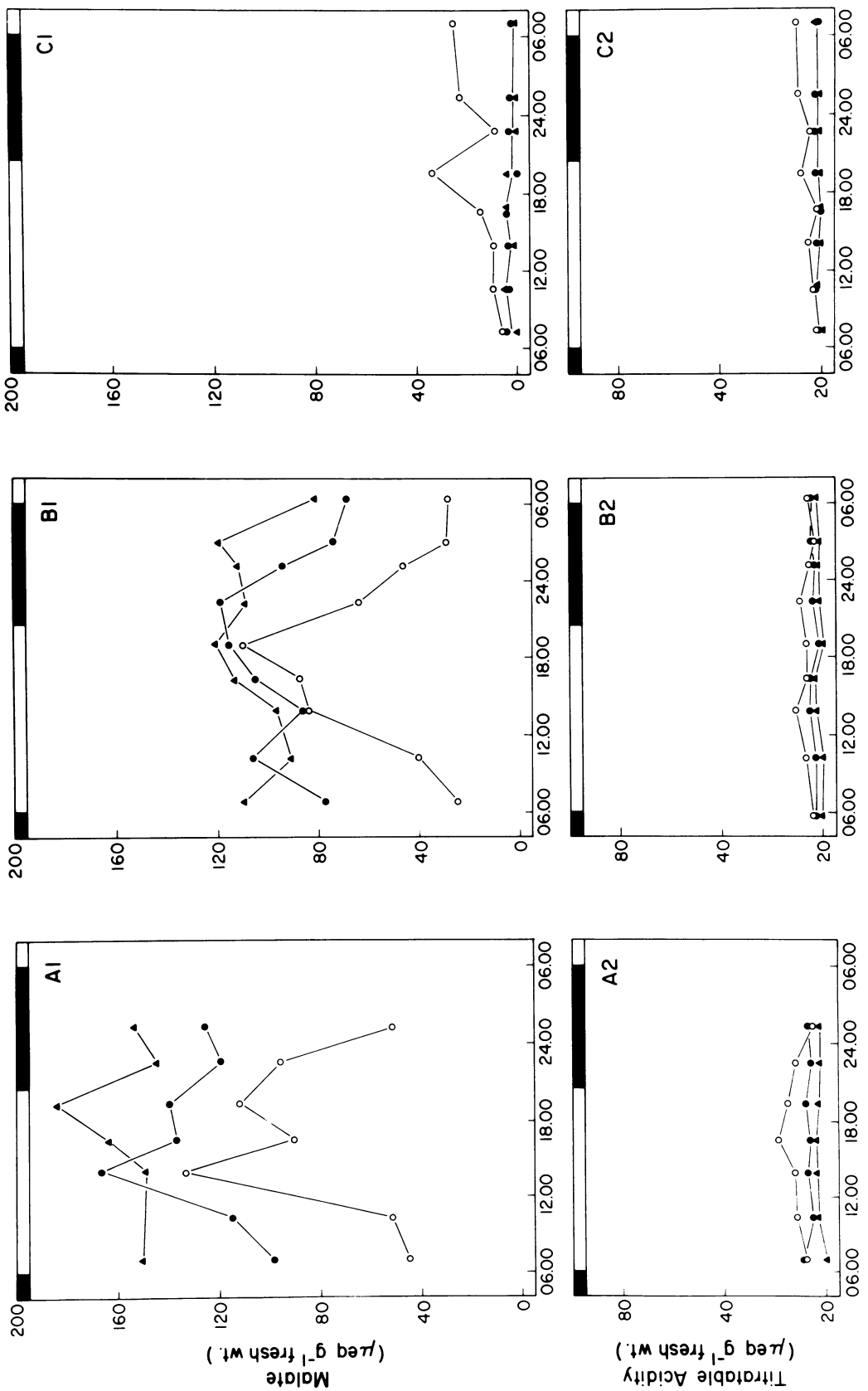


FIG. 2. Malate content (A1, B1, C1) and titratable acidity (A2, B2, C2) in *M. arvensis* during a day/night cycle. Plants were kept during the summer in a non-shaded glasshouse. (□), period with light; (■), period with darkness. (○), Young leaf; (●), middle-aged leaf; (▲), old leaf. A, plants watered with a nutrient solution containing nitrogen in the form of 5 mM nitrate, no ammonia; B, plants watered with a nutrient solution containing nitrogen in the form of 7 mM nitrate and 1 mM ammonium salt; C, plants watered with a nutrient solution containing nitrogen in the form of 5 mM ammonium salt.

0.5-ml solution of amylase (350 units), suspended in 50 mM Tris-HCl (pH 7.0), was added to each fraction. After the samples were placed in a hood for about 20 h, 1 ml 0.2 N NaOH was added to each fraction. A 0.15-ml aliquot was taken from each sample and counted by scintillation spectroscopy.

CO_2 Compensation Point Measurements. Γ was determined by extrapolation from the plot of net rate of CO_2 exchange in 21% O_2 at varying CO_2 concentrations between 20 and 150 μbar using a Barnes or Anarad CO_2 analyzer. A single middle-aged leaf (on plants normally having 10–15 leaves) of a nitrate- or ammonia-grown plant was sealed in a Plexiglas leaf chamber and illuminated for at least 20 min at 1,500 $\mu E\ m^{-2}\ s^{-1}$ prior to making measurements on CO_2 exchange (24). Separate plants were used for each replication. The leaf temperature measured with a chromel-constructed thermocouple was between 25 and 30°C.

Light and Electron Microscopy. Tissues were obtained from middle-aged leaves of medium B- and medium C-grown plants. After the same period of growth, plants grown on medium C were smaller than those grown on medium B, and had fewer leaves: 11 compared with 16. It was necessary, therefore, to sample the fifth visible leaf from above of a medium C-grown plant, and the eighth visible leaf from above of a medium B-grown plant. Both of these middle-aged leaves were fully expanded and apparently healthy.

The tissues were fixed in 6% glutaraldehyde in cacodylate buffer (pH 7.0) for 6 h at room temperature, and postfixed in 2% OsO_4 in cacodylate overnight in a refrigerator. Embedment was in Spurr's resin (37). Thin sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed and photographed with a Hitachi HU-11C microscope. For light microscopy, serial transverse and paradermal sections, 1 to 2 μm thick, were cut with glass knives, and then were stained on microslides with toluidine blue.

RESULTS AND DISCUSSION

In a previous study (43) of species representing many families grown in certain desert and coastal habitats, there was a notable increase in the malate content during the day among species of the family Brassicaceae. With *Moricandia arvensis*, also in the family Brassicaceae, we examined the malate content of leaves at the end of the day and night period at various leaf positions (Fig. 1). The malate content of the tissue ranged from about 40 to 135 $\mu eq\ g^{-1}$ fresh weight, similar to that of several species in the family Brassicaceae (43). These levels of malate are similar to those of many CAM plants during the night period (29). The malate content was higher at the end of the day than at the end of the dark period, with the greatest change occurring in the younger leaves. This fluctuation in malate content in the leaves is opposite that seen during Crassulacean acid metabolism. These plants were grown in a standard nutrient medium containing 7 mM nitrate and 1 mM ammonia (medium B).

The malate content and titratable acidity of leaves during a day/night cycle with plants grown under different nitrogen nutrition is shown in Figure 2. In plants watered with medium A (containing 5 mM nitrate and no ammonia) and medium B (containing 7 mM nitrate and 1 mM ammonia) there was a marked increase in malate content during the day and a decrease in malate content during the dark period, particularly in the younger leaves. However, with plants grown in medium C (5 mM ammonia and no nitrate) the malate content was very low (Fig. 2, C1). Only in the young leaves was there some increase in malate. The titratable acidity of the leaves from all treatments was quite low with little or no fluctuation between day/night periods. These results suggest that malate synthesis in the leaves is linked to assimilation of nitrate. Malic acid may be synthesized to neutralize the hydroxyl ions formed during nitrate reduction to ammonia (8, 22, 28) and to balance the residual inorganic cations. If nitrate reduction

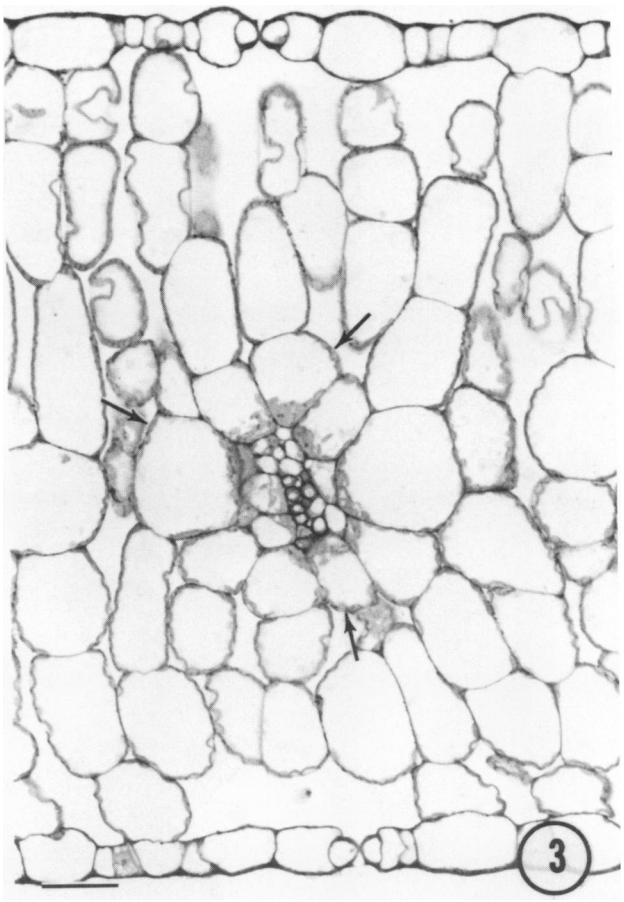


FIG. 3. Photomicrograph showing a transverse section of a portion of lamina of *M. arvensis*. Unlabeled arrows point to chloroplasts located along portions of bundle-sheath cell walls bordering intercellular spaces. Bar = 52.63 μm. × 190.

occurs in the leaves during the light period as previously suggested (6), there may be a corresponding increase in malate content. It is uncertain whether malate decreases during the dark period due to metabolism in the leaf or transport to other parts of the plants. Dijkshoorn (14) and Ben-Zioni *et al.* (9) have proposed that potassium malate, which is formed during KNO_3 reduction in shoots, may return to the roots where it is oxidized. Direct experimental evidence is missing thus far, but *Moricandia arvensis* seems to be an elegant system to test this hypothesis. Plants grown in the two different nitrate media (Fig. 2, A and B) showed similar changes in malate content. In the early phases of the study, substantial data were collected with plants grown in medium B. The subsequent experiments reported are with plants grown on medium B, hereafter referred to as nitrate-grown plants and medium C, hereafter referred to as ammonia-grown plants.

As mentioned previously, plants grown on medium B were larger than those grown on medium C; otherwise, the plants were similar in appearance. Moreover, there was no apparent difference in the structure of the leaves between medium B and medium C grown plants.

The leaves of *M. arvensis* have been variously described as being unifacial, the mesophyll consisting entirely of palisade parenchyma (2, 18); as being bifacial, the mesophyll differentiated into palisade parenchyma and spongy parenchyma (3, 13); or, as having centric mesophyll, the mesophyll cells more or less radially arranged around the vascular bundles (26). Which of these descriptions is accurate? Surprisingly, all three are applicable for the leaves examined during the present study. Depending upon the

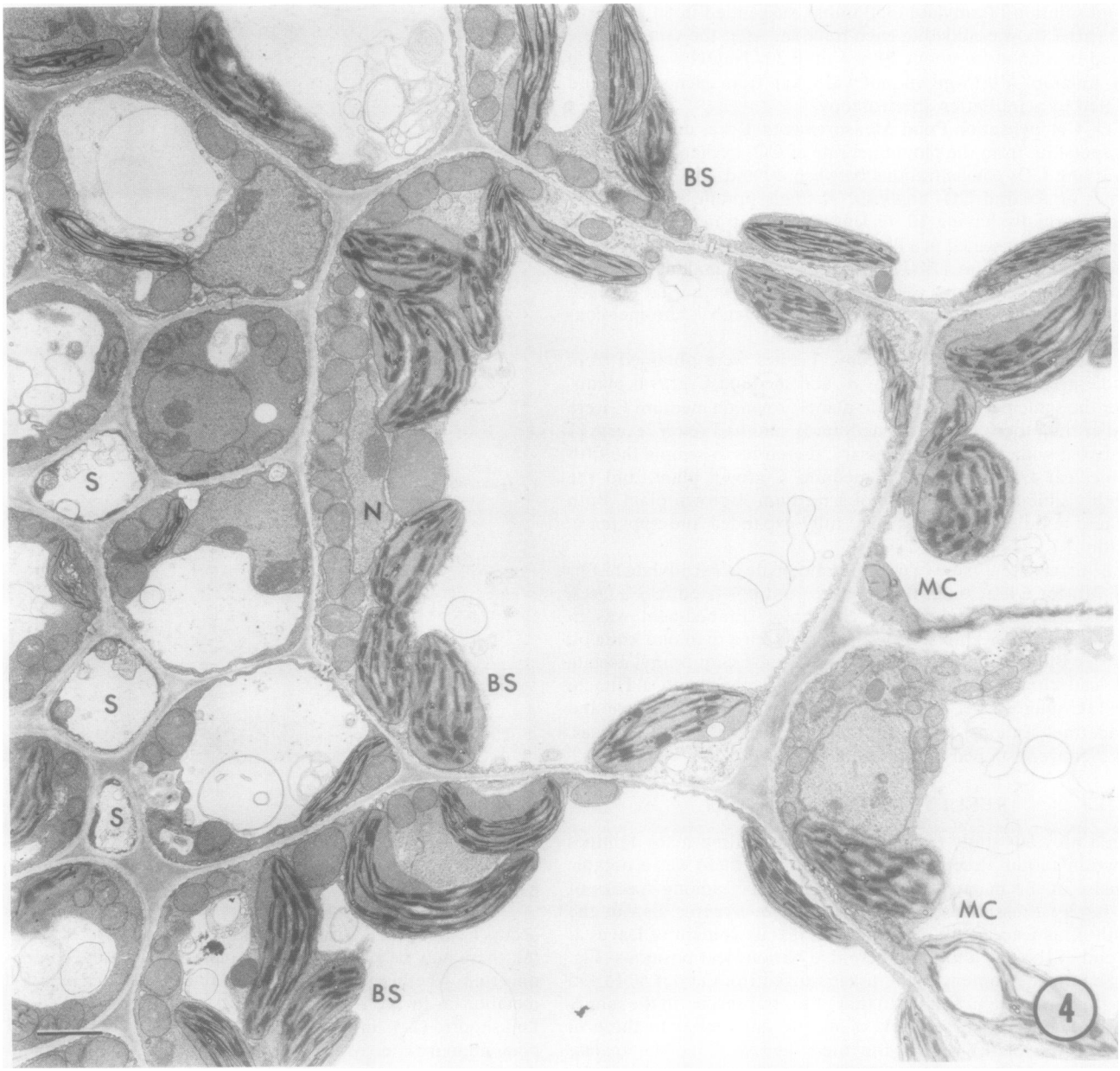


FIG. 4. Electron micrograph showing portion of vascular bundle, of neighboring bundle-sheath cells (BS), and of mesophyll cells (MC) of *M. arvensis*. Note that the chloroplasts of the bundle-sheath cells and mesophyll cells are similar in size and appearance. Chloroplasts, smaller in size than those of the bundle-sheath and mesophyll cells, also occur in the parenchymatous cells of the vascular bundle. N, nucleus; S, sieve element. Bar = 2.33 μm. × 4,300.

plane of section, the same leaf appeared either unifacial (cut parallel with the long axis of the lamina) or bifacial with centric mesophyll (cut at right angles to the long axis of the lamina).

Although it has been reported that the vascular bundles in *M. arvensis* are enclosed by a wreath of cells containing numerous, especially large chloroplasts, in the manner of Kranz cells (2), this feature was not apparent in leaves of either medium B- or medium C-grown plants. Nor were the chloroplasts exclusively distributed along the inner tangential walls of the bundle-sheath cells as depicted by Apel and Ohle (2), in their Figure 2a. Chloroplasts were found along cell walls of the bundle-sheath cells, especially along wall portions bordering intercellular spaces (Fig. 3, arrows [20]).

Both bundle-sheath cells and mesophyll cells contained numerous mitochondria and peroxisomes, and their chloroplasts were of similar size and ultrastructure (Fig. 4). In the bundle-sheath cells, the nucleus was inevitably located along the inner tangential wall in the parietal layer of cytoplasm, which also included a fair number of chloroplasts (Figs. 4 and 5). Most striking, however, were the arrays of mitochondria consistently found in the parietal layer of cytoplasm along the inner tangential walls (Figs. 4 and 5). Few mitochondria were found elsewhere in the bundle-sheath cells, whereas chloroplasts commonly were located along portions of anticlinal and outer tangential walls bordering intercellular spaces, as well as along the inner tangential walls (Fig. 3).

Obviously the leaves of *M. arvensis* do not exhibit a Kranz anatomy (13). In surface view, the vascular bundles of living leaves appear more intensely green than the intercoastal areas (2). This is due only in part, however, to the presence of chloroplasts along the inner tangential walls of the bundle-sheath cells. Also contributing to this intensity are numerous chloroplasts located in most of the parenchymatous elements—vascular parenchyma cells

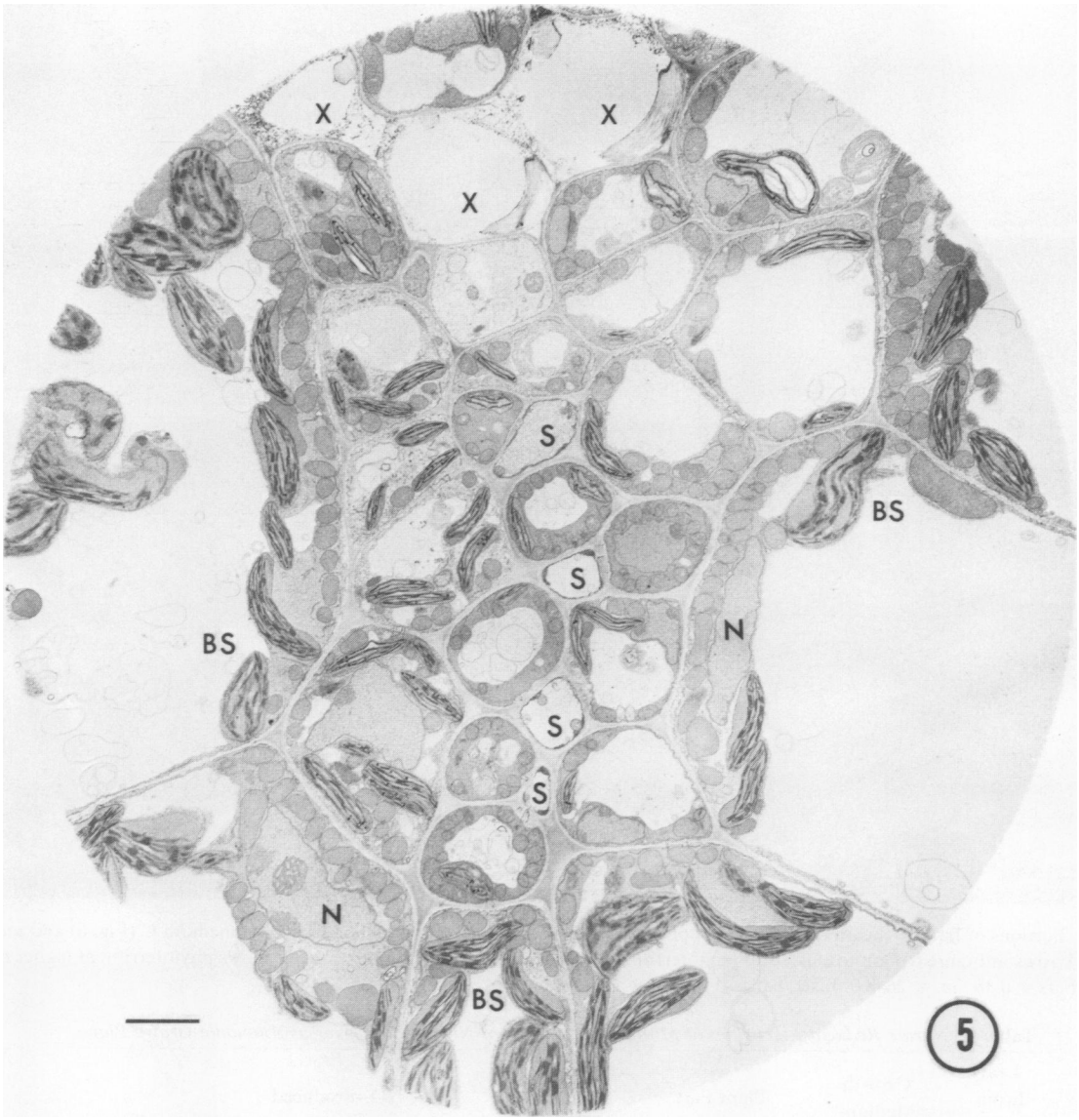


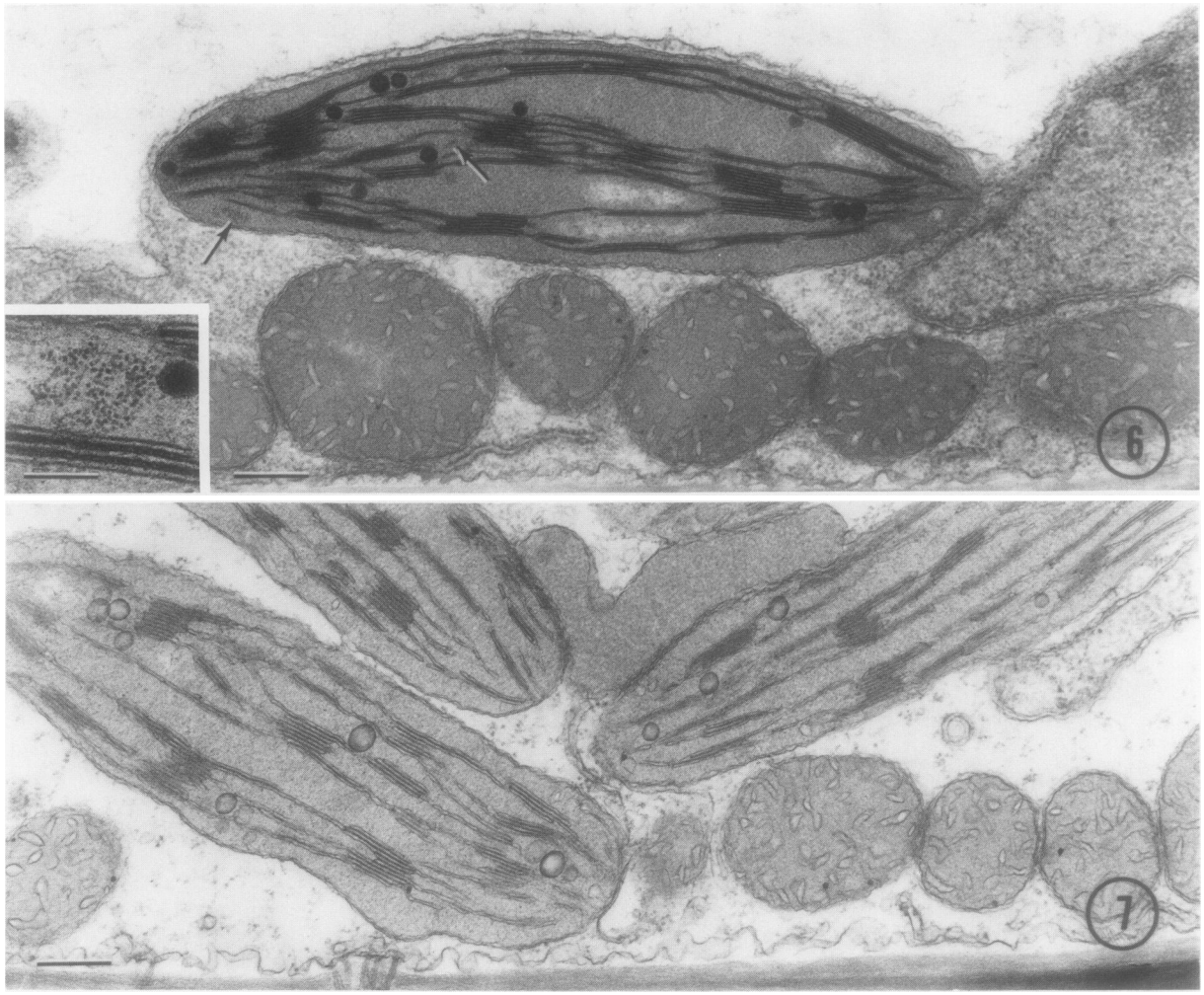
FIG. 5. Electron micrograph of vascular bundle and portions of contiguous bundle-sheath cells of *M. arvensis*. BS, bundle-sheath cell; N, nucleus; S, sieve element; X, xylem element. Bar = 4.54 μm. × 2,200.

and companion cells—of the vascular bundles (Figs. 4 and 5). (Figs. 3–5 are micrographs of plants grown on medium C, but these micrographs are representative of plants grown on either medium B or C.)

The only differences encountered between the leaves of medium B- and medium C-grown plants were apparent only with the electron microscope. Both the chloroplast thylakoids and stroma, and the mitochondrial matrices of medium C-grown plants consistently stained more intensely than those of medium B-grown plants (compare organelles in Figs. 6 and 7). Moreover, irregular clusters of phytoferritin particles, which also were clearly discernible in unstained material (a criterion used in the identification of phytoferritin), were encountered in the stroma of medium C (ammonia only) chloroplasts, but not in those of medium B (nitrate plus ammonia) chloroplasts. We have no ready explanation for these differences.

Phytoferritin is generally found in the stroma of developing and senescent chloroplasts, and it has been interpreted as a nontoxic store of iron that can be used for the synthesis of chloroplast components or that represents a breakdown product associated with disruption of chloroplast structure and photosynthetic activity (12, 35, 36, 39). Phytoferritin rarely has been reported in mature chloroplasts (34). The chloroplasts of both the medium B- and medium C-grown plants were fully differentiated, with well-developed grana and stroma thylakoids. To our knowledge, this represents the first report of a possible relationship between the presence or absence of phytoferritin and differences in mineral nutrient media.

The nitrate reductase activity in nitrate-grown plants was substantially higher than in ammonia-grown plants (Table I). As reported for many plants (7) the highest levels of extractable nitrate reductase activity were measured in the young expanding



FIGS. 6 and 7. Portions of bundle-sheath cells, showing appearance of chloroplasts and mitochondria, from medium C (Fig. 6) and medium B (Fig. 7) grown plants. Arrows in Figure 6 point to clusters of phytoferritin particles in stroma of chloroplast. Inset shows phytoferritin at higher magnification. Figures 6 and 7, bars = 0.38 μm, × 26,400. Inset, bar = 0.10 μm, × 100,000.

Table I. *Nitrate Reductase Activities of Moricandia arvensis of Nitrate-Grown versus Ammonia-Grown Plants*

Leaf From Top	Growth Medium	Plant Part	Area per Leaf	NO_2-Produced		
			cm^2	$nmol\ s^{-1}\ leaf^{-1}\ or\ root^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$	$nmol\ s^{-1}\ g^{-1}\ fresh\ wt$
2	$+NO_3^-$	Young leaf	12.9	4.7	3.6	14.6
5		Middle leaf	49.8	15.5	3.1	6.5
9		Old leaf	25.2	5.9	2.3	3.6
		Root		0.3		1.4
1	$+NH_4^+$	Young leaf	5.1	0.93	1.8	7.2
3		Middle leaf	14.1	2.1	1.5	3.7
5		Old leaf	9.0	0.23	0.26	0.5
		Root		0		0

leaves compared with the older expanded leaves of nitrate-grown plants (Table I). The levels measured in young expanding leaves of *M. arvensis* reported here are high in comparison with previously reported values for other species. For example, Schrader *et al.* (31) and Sherrard and Dalling (33) report a range of extractable nitrate reductase activity from a variety of nitrate-grown crop plants (C_3 and C_4) between 0.6 and 8.6 nmol nitrite produced per g fresh weight per s, using 2% or 3% (w/v) casein in the extraction media. The measurement of substantial levels of nitrate reductase activity in leaves of ammonia-grown plants of *M. arvensis* may be the result of some nitrification which was difficult to avoid with vermiculite as the growing medium. Schrader *et al.* (32) reported low levels of nitrate reductase activity in leaves of corn grown with ammonia in hydroculture where no conversion of ammonia to nitrate could be detected. No nitrate reductase activity could be detected in the roots of ammonia-grown plants, whereas activity

Table II. *RuBP Carboxylase and PEP Carboxylase Activities in Nitrate- and Ammonia-Grown Plants of Moricandia arvensis*

Values in parentheses refer to desalted extracts; other values refer to nondesalted extracts.

Growth	Leaf Age	Enzyme Activity				RUBPC:PEPC
		RuBPC	PEPC	RuBPC	PEPC	
		$\mu mol\ mg^{-1}$	$Chl\ min^{-1}$	$\mu mol\ m^{-2}\ s^{-1}$		*ratio*
NO_3^-	Young	22.2 (22.7)	2.0 (1.8)	93.5 (95.6)	8.6 (8.0)	11.0 (12.3)
	Middle	11.2 (10.0)	1.4 (1.4)	64.3 (57.8)	8.2 (8.0)	7.9 (7.2)
	Old	8.5 (8.2)	1.5 (2.0)	47.5 (46.0)	8.4 (11.0)	5.7 (4.2)
NH_4^+	Young	18.7 (18.9)	1.4 (1.5)	87.0 (87.9)	6.6 (6.9)	13.3 (12.6)
	Middle	15.7 (16.5)	0.69 (0.76)	106.5 (111.7)	4.7 (5.2)	22.7 (21.6)
	Old	10.3 (8.2)	0.62 (0.61)	71.5 (56.8)	4.3 (4.2)	16.6 (13.5)

Table III. *Percentage Distribution of Label among Products of $^{14}CO_2$ Fixation during a Pulse-Chase Experiment with Young and Middle-Aged Leaves of Nitrate-Grown and Ammonia-Grown Plants of Moricandia arvensis*

Pulse with $^{14}CO_2$ (100 μbars above ambient levels) was for 10 s while the chase in air ($^{12}CO_2$) was for 5 min.

Leaf Age Time	Nitrate-Grown				Ammonia-Grown			
	Young		Middle		Young		Middle	
	10 s	5 min	10 s	5 min	10 s	5 min	10 s	5 min
Insoluble	5.0	22.5	11.8	12.0	3.2	26.2	2.9	19.1
Soluble								
PGA[a]	10.9	1.9	28.0	1.9	40.2	1.3	43.8	1.0
SMP[b]	37.6	0.6	33.0	3.4	25.5	2.4	27.1	1.9
SBP[c]	0	0	1.9	0.1	5.6	0	7.1	0.4
Other P-esters	4.4	1.3	1.9	0.5	0.9		1.3	0
Total P-esters	52.9	3.8	64.8	5.9	72.2	3.7	79.3	3.3
Malate	6.7	16.0	0.9	6.5	3.9	1.5	0.9	0.1
Aspartate	9.3	2.9	0.5	1.3	3.7	0	2.1	0
Glycine + serine + glutamate	15.7	31.4	7.1	33.9	5.4	33.6	7.8	18.2
Sucrose	0.5	20.1	3.3	33.9	0	32.2	2.8	58.7
Others	9.9	3.3	11.6	6.5	11.6	2.8	4.2	0.8
Total soluble	95.0	77.5	88.2	88.0	96.8	73.8	97.1	81.1

[a] 3-P-glycerate.
[b] Sugar monophosphates.
[c] Sugar bisphosphates.

was readily detected in nitrate-grown plants. Although activities in the ammonia-grown plants are relatively high per unit leaf area and per unit fresh weight, these activities would be low if expressed per total plant (dry weight of tops of nitrate-grown plants was 1.6–2.3 g and of ammonia-grown plants 0.2–0.6 g). The high levels of nitrate reductase in young leaves of nitrate-grown plants (Table I) further suggests a link between nitrate reduction and the large diurnal fluctuation of malate of the young leaves (Fig. 1; Fig. 2, B1).

Since formation of malate in leaves in the light of *M. arvensis* may be linked to PEP carboxylase, the activities of this enzyme were compared with activities of RuBP carboxylase in nitrate *versus* ammonia-grown plants. Activities of PEP carboxylase were higher particularly in middle-aged and older leaves (which represent more than 80% of the total leaf biomass per plant) of nitrate-grown plants than in leaves of ammonia-grown plants on a Chl or leaf area basis (Table II). The activities of the enzyme in nitrate-grown plants are similar to those previously reported by Bauwe and Apel (5) and are about 2-fold higher than activities normally found in C_3 plants, trends also reported by Holaday *et al.* (20). The activities of the enzymes in middle-aged and old leaves of the ammonia-grown plants are similar to those typically reported in C_3 species. (In a second experiment with a new plant population, we observed as much as 3-fold higher levels of PEP carboxylase activity in middle-aged leaves of nitrate-grown plants compared to ammonia-grown plants; data not shown). In addition, the ratio of RuBP carboxylase:PEP carboxylase is substantially higher particularly in middle-aged and older leaves of ammonia-grown plants than in nitrate-grown plants. This suggests PEP carboxylase may have a relatively greater role in carbon assimilation in the nitrate-grown than in the ammonia-grown plants.

The labeling of products of $^{14}CO_2$ assimilation in a pulse-chase type of experiment was compared with young (Y) and middle (M) aged leaves of nitrate- and ammonia-grown plants (Table III). After 10 s exposure to $^{14}CO_2$ at 100 μbars above ambient levels, primary labeled products were P-esters in both nitrate- and ammonia-grown plants. During the 5-min chase in air, the labeling in P-esters decreased, whereas label in sucrose, insolubles, and glycine + serine + glutamate increased. This general distribution of label in a pulse-chase experiment is typical of that of C_3 plants

(10, 15). In the nitrate-grown plants, the percentage label in malate after 10 s of $^{14}CO_2$ fixation was 7% or less; and this increased during the chase period up to 16% in young leaves. In ammonia-grown plants, the percentage label in malate was very low after 10 s exposure to $^{14}CO_2$ with no increase in the percentage label in malate during the chase period. These results indicate that relatively more label from $^{14}CO_2$ fixation goes into malate in nitrate- than in ammonia-grown plants. This is consistent with the higher malate content in the nitrate-grown than in the ammonia-grown plants (Fig. 2). The increase in label in malate during the chase period in nitrate-grown plants is directly opposite to the situation in C_4 plants where malate is a primary initial product which decreases in label during the chase period. The labeling pattern of malate in the nitrate-grown plants of *M. arvensis* suggests that the primary carboxylation is through RuBP carboxylase. The P-glycerate formed through RuBP carboxylase may in part serve as a precursor for synthesis of PEP which is then used in a secondary carboxylation through PEP carboxylase. As reported by Holaday *et al.* (20), no activity was detected for pyruvate,Pi dikinase in leaf extracts of *M. arvensis.* The apparent absence of this enzyme, the low activity of the C_4 acid decarboxylases (20), and failure of label in malate to decrease in the chase period suggests a C_4 cycle like that of C_4 plants does not function in *M. arvensis.* The distribution of label among the products including malate and the pattern of change during the chase period was very similar for plants exposed to either high $^{14}CO_2$ (100 μbars above ambients) (Table III) or about 100 μbar $^{14}CO_2$ (data not shown). These results indicate that as the CO_2 concentration is decreased below atmospheric levels, CO_2 fixation through PEP carboxylase does not significantly increase as has been suggested in studies with *Panicum milioides*, another species having some characteristics intermediate to those of C_3 and C_4 plants (21).

If the relatively low CO_2 compensation point previously reported for *M. arvensis* (2, 3, 5, 20, 23) is related to PEP carboxylase activity and malate synthesis, the nitrate-grown plants might be expected to have a lower Γ than the ammonia-grown plants. During the course of the study, two sets of data were collected on Γ of middle-aged leaves of nitrate- and ammonia-grown plants. On the first set determined at the time of the pulse-chase experiments, the average values of Γ was 32 μbars for the nitrate-grown plants and 42 μbars for the ammonia-grown plants (determined June 1980 with three replications per treatment). The second set of plants had an average value of Γ of 49 μbars for the nitrate-grown plants and 57 μbars for the ammonia-grown plants (determined December 1980 with three replications per treatment). It appears that growth under nitrate may result in Γ values 7 to 10 μbars lower than growth under ammonia. In the literature, values of Γ for *M. arvensis* vary from 14 to 50 μbar (2, 3, 5, 20, 23) and presumably all studies included nitrate as a source of nitrogen nutrition. Further research is needed to determine the basis for the variation in this species which in part may depend on leaf age, time of day and season, and nitrogen nutrition. In addition, the effect of nitrogen nutrition on malate content and Γ should be measured in other *Moricandia* species. *M. foetida* (5) and *M. moricandioides* (1) are reported to have Γ similar to C_3 plants while the Γ of *M. spinosa* (1) is reported to be intermediate to C_3 and C_4 plants. It should be noted that Cresswell and coworkers have found both C_3 and C_4 plants to exhibit a lower Γ with nitrate than with ammonia as the nitrogen source (see review and citations in Ref. 11). In addition Yamada and Ikeda (44) have found the CO_2 compensation point of tomato plants was 20 μbars lower with nitrate than with ammonia as the nitrogen sources. The extent to which nitrogen nutrition influences Γ may vary among species.

M. arvensis has been classified previously as a C_3-C_4 intermediate type plant primarily due to its relatively low Γ. However, in the present study, carbon assimilation in *M. arvensis* was found to be very similar to that of C_3 plants. No evidence was obtained that a C_4 cycle functions to any degree or that there is any tendency for Kranz-like anatomy (distinctive wreath-like, thick-walled bundle-sheath cells with numerous chloroplasts) as has been recently suggested to account for the C_3-C_4 intermediacy of *Panicum milioides* (30). C_3 plants including *Moricandia* species which lack Kranz anatomy may have bundle sheath cells with or without chloroplasts.

Thus, other possibilities must be considered for the variable and generally lower Γ in *M. arvensis* than in other C_3 species. One possibility is that this species is more effective in fixing photorespired CO_2 than most other C_3 species. During photorespiration, CO_2 is released by glycine decarboxylation in the mitochondria. The magnitude of the CO_2 compensation point is dependent on the loss of photorespired CO_2 from the leaf. In *M. arvensis*, the location of mitochondria along the inner tangential walls of bundle-sheath cells, internal to the location of chloroplasts was particularly striking. This may increase the potential for chloroplasts to fix the photorespired CO_2 and reduce the level of Γ. This mechanism of conserving photorespired CO_2 and lowering Γ would not require a linkage to PEP carboxylase and malate synthesis.

It is also possible that malate synthesis linked to nitrate assimilation can contribute to a lowering of the CO_2 compensation point in *M. arvensis* due to partial assimilation of CO_2 through PEP carboxylase. A recent study suggests when a CAM plant is illuminated in the normal dark period CO_2 is fixed through both PEP and RuBP carboxylases resulting in a very low CO_2 compensation point in comparison to that of C_3 photosynthesis (42). Thus, in some cases, the CO_2 compensation point may be altered through differences in the relative *in vivo* activity of the two carboxylases without requiring a differential compartmentation between two photosynthetic cell types as in C_4 plants.

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