ica'. Although specific activities were not greater in the roots and petioles of 'Hybrid 424' than in 'America', 'Hybrid 424' had a greater total NO_3^- reductase activity in these tissues because of its larger size.

The qualitative and quantitative composition of bleeding sap from the 2 cultivars did not differ significantly. The proportions of NO_3-N and reduced N did not differ although it appeared that 'Hybrid 424' might transport slightly larger amounts of these constituents than those found in the bleeding sap of 'America'. No evidence was found to indicate that 'Hybrid 424' had a lesser capacity to absorb and translocate NO_3^- than 'America'. It is also clear that there was no dilution effect giving lower NO_3^- concn in 'Hybrid 424', for the total NO_3^- content in its leaves was less than that found in 'America' (see also 10).

Determination of the NO_3^- reductase activity is another factor, in addition to leaf morphology and growth rate, which will be useful in distinguishing between the tendencies of spinach cultivars to accumulate NO_3^-. The potentially higher growth rate, higher yields, higher protein contents, and lower NO_3^- contents appear to be interrelated features found in the smooth-leafed cultivar (12). Careful varietal selection based on this relationship and management of the nutritional regime (11) will virtually insure avoidance of any human health hazards connected with the consumption of spinace and should lead to production of a more nutritious product at lower costs.

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A Physiological Basis for Different Patterns of Nitrate Accumulation in Cucumber and Pea[1]

Frederick C. Olday[2], Allen V. Barker, and Donald N. Maynard
Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA 01002

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Abstract. **Cucumber (*Cucumis sativus* L.) plants accumulate more NO_3^- than pea (*Pisum sativum* L.) plants. The differences in accumulation appear to be due to differences in the abilities of the two species to reduce NO_3^- in their roots. Only 2% of the NO_3^- reductase activity of cucumber was found in its roots, whereas nearly 92% of the activity was found in the blades. In pea, NO_3^- reductase activity was more evenly distributed throughout the plant; 67% of the activity was in the blades, 18% in the roots, and the remainder in the stems and petioles. Nitrate-N comprised 80% of the N present in bleeding sap of roots of cucumber plants from which the shoots had been excised. In contrast, NO_3-N constituted only 30% of the N in the sap from pea roots, the remaining 70% of the N consisting of amino acids and amides. Asparagine or aspartic acid was the major carrier of reduced N in pea, and glutamine was the major carrier in cucumber. The differences in N transport and assimilation appear to bear considerably on plant composition and efficiency of N usage.**

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[2]Present address: College of the Atlantic, Bar Harbor, ME 04609.

Table 1. Fresh wt and nitrate concn of cucumber and pea grown on 3 levels of nitrate-N nutrition.

NO_3-N nutrition level (ppm)	Plant part	Fresh wt (g/pot)		NO_3-N concn (ppm fr wt)	
		Cucumber	Pea	Cucumber	Pea
10	Blades[z]	5.08	8.86*	72.5	7.2*
	Petioles	0.54	2.24*	19.3	6.5*
	Stems	1.28	4.10*	6.7	8.5
	Roots	8.01	23.26*	10.5	6.7
50	Blades	13.08	12.00*	80.4	16.7*
	Petioles	2.65	2.70	18.7	121.0*
	Stems	5.51	4.87*	8.5	133.0*
	Roots	19.06	17.41*	24.4	101.5*
250	Blades	31.09	14.33*	222.1	182.3*
	Petioles	10.10	3.16*	1237.1	579.5*
	Stems	18.50	5.42*	1065.6	707.7*
	Roots	25.66	16.03*	508.7	528.5

*Mean fresh wt or NO_3 concn of pea significantly different from that of cucumber at 5% level.

[z]In pea, blades include leaflets and stipules; petioles include rachii.

A previous study (10) showed that differences in concn of NO_3^- accumulated by a smooth-leafed and a savoy-leafed cultivar of spinach (*Spinacia oleracea* L.) were due to the higher NO_3^- reductase activity in the smooth-leafed cultivar. Unrelated plant species also differ in ability to accumulate NO_3^- (2, 3, 8). Striking differences were found in NO_3^- and cation composition of pea and cucumber, with the cucumber plant being the greater accumulator of K+, Ca++, Mg++, and NO_3^- (2). In that study, attempts were made to determine NO_3^- reductase activity in the tissues of pea and cucumber. The results did not seem to support the assumption that pea roots should have a high NO_3^- reductase activity to correspond with the large amounts of reduced N found in pea roots (7). It was concluded that an inhibitor of NO_3^- reductase was operative in pea roots or that the assay procedure needed modification to improve detection of NO_3^- reductase. In the present study the assay procedure for NO_3^- reductase activity in pea was refined, and attempts were made to account for the differences in NO_3^- concentrations in pea and cucumber on the basis of the distribution and activity of NO_3^- reductase in the plants and by the rates of NO_3^- uptake and assimilation by the plant roots.

Table 2. Nitrate reductase activity in cucumber and pea grown on 3 levels of nitrate-N nutrition.

NO_3-N nutrition level (ppm)	Plant part	Nitrate reductase activity			
		Specific (nmoles NO_2^-/hr/g fr wt)		Total (nmoles NO_2^-/hr/plant fr wt)	
		Cucumber	Pea	Cucumber	Pea
10	Blades[z]	0	0	0	0
	Petioles	0	0	0	0
	Stems	0	0	0	0
	Roots	17	20	34	118*
50	Blades	3678	346*	12162	1039*
	Petioles	152	365	101	243
	Stems	312	311	436	378
	Roots	201	142*	958	618*
250	Blades	16486	2267*	128304	8077*
	Petioles	1706	782*	4247	616*
	Stems	1118	890	5211	1203
	Roots	365	526	2343	2107

*Mean specific or total nitrate reductase activity of pea significantly different from that of cucumber at 5% level.

[z]In pea, blades include leaflets and stipules; petioles include rachii.

Table 3. Major nitrogenous constituents of bleeding sap collected from cucumber and pea.

Species	NO_3-N nutrition level (ppm)	Nitrogenous constituent				
		Amino-N	Amide-N[z]	NH_3-N	NO_3-N	Total
		μgN/hr/plant				
Cucumber	10	0.79	1.33	0	11.86	13.98
	50	5.89	10.89	0	64.42	81.20
	250	10.61	27.38	0	139.44	177.43
		% of total N				
	10	6	9	0	85	100
	50	7	14	0	79	100
	250	6	15	0	79	100
		μgN/hr/plant				
Pea	10	1.20	0.25	0	0.61	2.06
	50	4.08	2.81	0	3.35	10.24
	250	10.62	42.08	0	15.27	67.97
		% of total N				
	10	58	12	0	30	100
	50	40	27	0	33	100
	250	16	62	0	22	100

[z]gln for cucumber and gln + asn for pea.

Materials and Methods

Plant material. Cucumber cv. Marketmore and pea cv. Frosty were used in this study to correspond to the cultivars used earlier (2). Plants were cultured in the greenhouse or growth chamber under conditions previously described (10). Plants were harvested at 4 weeks of age. Growth of plants for a longer period of time resulted in flowering. Since uptake and assimilation of N by roots of plants is altered or curtailed by flowering and fruiting (5, 11), harvest at 4 weeks avoided this problem. Fresh and dry wt were determined for roots, stems, petioles, and blades.

Plant analyses. Nitrate concn was determined on the dried plant tissue using the methods of Humphries (6) and expressed on a fresh wt basis. Sap collection and analytical procedures were identical to those used previously (9). Assay for NO_3^- reductase activity was as previously described (9) except that for maximum activity in pea the extraction medium was 10^{-3} M in cysteine.

Results

Plant growth. Cucumber plants grown on 10 ppm NO_3-N were small and chlorotic. With 50 ppm of NO_3-N nutrition, plants increased in size, but their leaves remained uniformly light green. The largest growth occurred with 250 ppm NO_3-N nutrition with no evidence of chlorosis. Pea plants with 10 ppm NO_3-N nutrition were small and chlorotic with considerable marginal necrosis of lower leaves. Except for a paler green color of the upper leaves of pea plants grown on 50 ppm NO_3-N, no differences in appearance were observed between those grown on 50 ppm and 250 ppm NO_3-N. Increasing NO_3-N in the nutrient solution resulted in an overall increase in fresh wt of cucumber shoots and roots and pea shoots (Table 1). Shoot growth was enhanced more than root growth in cucumber, and with pea a growth restriction of roots occurred with increases in NO_3-N nutrition. The average dry matter content in blades, petioles, stems, and roots was 11.2, 4.8, 4.7, and 3.8% of the fresh wt for cucumber and 11.1, 8.7, 8.2, and 4.3% for pea, respectively.

Nitrate concentrations. In general, NO_3^- concn in cucumber and pea increased with increases in NO_3^- level in the nutrient solution (Table 1). The principal site of NO_3^- accumulation seemed to vary with the level of NO_3-N nutrition supplied; however, at saturating levels of NO_3-N nutrition (2), in this

Table 4. Analysis of the nitrogenous fraction of bleeding sap collected from cucumber and pea showing individual amino acids.

Sap component	μgN/plant/hr in cucumber			μgN/plant/hr in pea		
	NO_3-N nutrition level			NO_3-N nutrition level		
	10 ppm	50 ppm	250 ppm	10 ppm	50 ppm	250 ppm
ala	0	0	0	0.002	0	0
val	0.069	0.301	1.482	0.015	0.016	0.457
gly	0	0	0	0	0	0
ile	0.043	0.219	0.944	0.006	0.015	0.244
leu	0.015	0.106	0.377	0.006	0.012	0.274
pro	0.005	0.017	0.215	0.004	0.025	0.094
thr	0.039	0.175	0.833	0.013	0.073	0.696
ser	0.008	0.047	0.352	0.001	0.007	0.061
met	0.006	0.043	0.083	0	0	0
homoser	0	0	0	0.026	0.355	4.164
phe	0.001	0.012	0.075	0	0.006	0.075
asp	0.024	0.126	0.463	1.002	3.063	2.063
glu	0.330	2.631	0.838	0.030	0.266	0.043
tyr	0	0	0.204	0	0.004	0.070
lys	0.150	1.004	4.742	0.027	0.050	2.038
his	0.057	0.383	0	0.040	0.052	0
arg	0.042	0.816	0	0.029	0.106	0.341
gln	1.330	10.891	27.377	0.103	0.816	4.575
asn	0	0	0	0.142	1.992	37.506
NH_4^+	0	0	0	0	0	0
NO_3^-	11.856	64.424	139.444	0.610	3.350	15.266

case 250 ppm, NO_3^- concn were greatest in stems and petioles followed by roots and blades. At 250 ppm of NO_3-N nutrition, NO_3^- accumulation by cucumber stems, petioles, and blades exceeded that of pea, but NO_3^- accumulation of roots was not significantly different between the plants.

Nitrate reductase activity. An overall increase in NO_3^- reductase activity occurred as NO_3^- concn in the nutrient solution increased (Table 2). This increase in activity appears to be directly related to the NO_3^- concn in the individual tissues (cf. Tables 1 and 2). With low levels of NO_3^- nutrition, the roots seem to be very important in reduction of NO_3^-. In cucumber, NO_3^- reductase activity increased markedly in the shoots but less in the roots as the NO_3^- concentration of the nutrient solution increased. Thus at high levels of NO_3-N nutrition, the shoot, mainly leaf blades, is the principal site of NO_3^- reduction. In pea leaf blades, reductase activity did not approach that of cucumber blades, and the enzymatic activity seemed to be more evenly distributed over the plant. Roots continued to play an important role in NO_3^- assimilation in the pea. At the 250 ppm level of NO_3-N nutrition, 92% of the total NO_3^- reductase activity of cucumber was found in the blades with less than 2% being found in the roots. With pea at this same level of nutrition, 67% of its NO_3 reductase activity was centered in the blades and 18% was found in the roots.

Sap analysis. As the NO_3-N concn in the external medium increased, the total amount of N exported from the root system increased (Table 3). This increase was due largely to a dramatic rise in the volume of sap exudated as the level of NO_3-N nutrition increased. In the bleeding sap of cucumber, NO_3^- was the major nitrogenous constituent at all levels of NO_3-N nutrition. Glutamic acid (glu) and glutamine (gln) constituted the major fraction of the reduced N, and no asparagine (asn) or NH_3 was present (Table 4). In pea, NO_3^- accounted for only a third or less of the total N in the bleeding sap (Table 3). No NH_3 was detected, but asn was present in very large quantities especially at the 250 ppm level of NO_3-N nutrition (Table 4). Glutamine was present in lesser quantities than asn and became relatively less important as the level of NO_3-N nutrition increased. At the 250 ppm level of NO_3-N nutrition, asn constituted 55% of the total N in the xylem exudate. Lysine, homoserine (homoser), and aspartic acid (asp) were other important nitrogenous constituents of xylem sap of pea. Lysine and homoser increased, but asp decreased in absolute and relative amounts as the external NO_3-N supply increased. Homoserine was not detected in cucumber sap.

Discussion

Nitrate accumulates in both cucumber and pea plants whenever the external NO_3-N supply is high. Cucumber accumulates much more NO_3^- than pea, and most of the NO_3^- accumulation is in the stems and leaf petioles. The power to assimilate NO_3^- appears to be centered in the leaf blades for cucumber with very little NO_3^- reductase activity in the roots. Pea plants, on the other hand, have an efficient NO_3^- reducing system in their roots as well as in their shoots. This difference in distribution of NO_3^- reductase activity appears to account for the differences in the amounts of NO_3^- accumulated by these plants. Analysis of the bleeding sap revealed that 80 to 85% of the total N moving out of cucumber roots was NO_3-N with 15 to 20% of the total N being amide and amino-N. In pea, only 20 to 30% of the exuded N was NO_3 and about 70% was amide and amino-N. The presence of large quantities of asparagine in pea xylem exudate implies that NH_3 was readily formed in pea roots (1). Other amino acids related to aspargine, *i.e.,* lys, homoser, and asp, were also abundant in pea sap. The differences in composition of sap confirm the higher assayed NO_3^- reductase activity in pea roots than in cucumber roots. The results of this study help to explain findings (2) which showed differences in cationic composition between pea and cucumber shoots. Since peas translocate more reduced N upward from roots, they would be expected to translocate lesser quantities of cations than cucumber (4). Pea shoots showed a higher protein content than cucumber shoots (9). Thus, it is apparent that NO_3^- assimilation in the roots has widespread effects on plant metabolism, affecting not only the distribution of NO_3^- in the plants but also their cationic distribution and protein composition. These results indicate that because of its greater assimilation of N in the roots pea utilizes NO_3-N more efficiently than cucumber, resulting in less NO_3^- and more protein in pea.

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