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Changes in production of organic nitrogen and carboxylates (C-A) in young sugar-beet plants grown in nutrient solutions of different nitrogen composition

V. J. G. Houba, F. van Egmond and E. M. Wittich

Department of Soils and Fertilizers, Agricultural University, Wageningen,
the Netherlands

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Summary

Sugar-beet plants were grown in a growth chamber in culture solutions with nitrate. After 19 days the plants were transferred to culture solutions with nitrate, or with ammonium or without nitrogen. The production of carboxylates in the plants was compared with the production of organic nitrogen.

The results expressed in equivalents can be summarized as follows:

NO_3 plants : C–A = organic N
zero-N plants : C–A > organic N
NH_4 plants : C–A < organic N

These differences are accompanied by a small increase in pH of the culture solution of nitrate plants and a sharp decrease in pH in the other treatments.

Introduction

Differences in the uptake between inorganic cations and inorganic anions are accompanied by changes in the amount of carboxylates present in plants tissues (Ulrich, 1942). The metabolic conversion of nitrates and sulphates into non-ionic organic N and S gives rise to an equivalent quantity of carboxylates in plant tissues (Dijkshoorn, 1962). Table 1 summarizes the processes which influence the carboxylate pool. When there is a substantial uptake of ions other than those mentioned in Table 1, such ions, for instance NH_4^+, should also be considered.

Since sulphate reduction is small compared with nitrate reduction the former will be neglected in the present discussion. Dijkshoorn et al. (1968) in experiments with perennial rye-grass (*Lolium perenne* L.), grown in nutrient solution, assumed that their results could be explained by Processes 1, 3 and 4 mentioned in Table 1. Van Egmond and Houba (1970), working with sugar-beet (*Beta vulgaris* L.) on complete nutrient solution, could explain their results with Processes 3 and 4 only. Process 1 does not take place in sugar-beet plants, or it was so small that it could not be demonstrated (van Egmond and Houba, 1970).

Houba (unpublished results) using data from field experiments with sugar-beet concluded that the excess of inorganic cations over inorganic anions was higher than the amount of organic nitrogen in the whole plant as soon as nitrate was depleted. This

Table 1. Processes which influence the carboxylate pool.

Process	Carboxylate
1 Uptake of $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq $\leq$ uptake of $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	decrease
2 Uptake of $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq $\geq$ uptake of $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	increase
3 Nitrate reduction	increase
4 Sulphate reduction	increase
5 Transition of NH_4^+ into organic N	decrease

points to the possibility of introducing Process 2, by replacing nitrate in the nutrient solution by chloride or sulphate. Dijkshoorn et al. (1968) used this procedure to demonstrate Process 2 in *Lolium perenne.* If nitrate is replaced by ammonium as the source of nitrogen, Process 2 (increase in carboxylate production) is followed by Process 5 (decrease in carboxylate production). Only the net change in amount of carboxylates can be determined. Dijkshoorn et al. (1968) working with rye-grass showed that with ammonium chloride as nitrogen source (and no potassium), the organic nitrogen increased rapidly but the carboxylate production stopped.

The aim of the trial described in this article was to investigate if there is any further production of carboxylates in young sugar-beet plants when the nitrate reduction is stopped due to nitrate depletion brought about by either substitution of ammonium for nitrate, or by omission of nitrogen in the supplied salts.

Experimental

Diploid sugar-beet seeds (No P2167) were sown in sand. After germination the seedlings were placed on a well-aerated nutrient solution. The composition of the nutrient

Table 2. Composition of the nutrient solutions.

Component	Concentration in meq/l		
	$+NO_3$ (a)	zero-N (b)	$+NH_4$ (c)
Na	2	2	1
K	4	3	2
Ca	2.5	2.5	1.25
Mg	4	3	2
NH_4	—	—	4
H_2PO_4	1	1	1
NO_3	6	—	—
Cl	3	3	3
SO_4	2.5	6.5	6.25

Trace elements according to Hoagland, Fe–EDTA 35 mg/l.

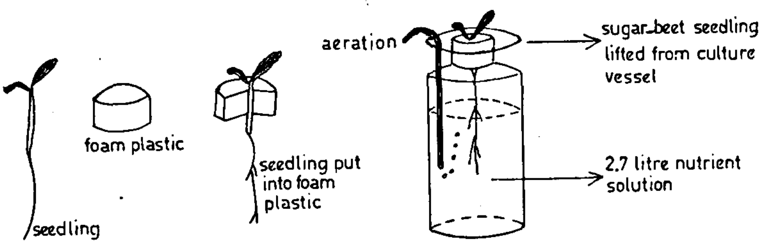

Fig. 1. Growing sugar-beet on a nutrient solution.

solution is given in Table 2a. Fig. 1 shows how the plants were held in foam plastic.

The nutrient solutions were renewed at regular intervals in order to avoid possible nutrient shortages. The trial was carried out in a growth chamber. The daily light period was 14 hours, while the temperature was 25° C during the light period and 17° C during the dark period; the relative humidity was 80 to 90%. After 19 days of growth on the culture solution with nitrate (Table 2a), 10 plants were kept on the nitrate solution (Table 2a), 18 were transferred to a solution without nitrogen (Table 2b) and 12 were transferred to an ammonium solution (Table 2c).

Plants were harvested after 19, 23, 30, 33 and 36 days from the day of transplanting from the seed bed, and divided into three sections: leaf blades (including the midribs), petioles and beet + roots. For each section, the fresh and dry weights were recorded and the material was finely ground and analysed as described by van Egmond and Houba (1970). The carboxylates were extracted using water in the presence of a H+ sulphonic acid resin. Since this treatment did not extract all the oxalates, the remaining oxalates were determined oxidimetrically with $KMnO_4$ following a double treatment of the samples with 2 *N* HCl. (In the blank determination hardly any $KMnO_4$ was used.) The pH of the nutrient solutions was measured regularly from the 19th day until the 33th day of the trial.

Results and discussion

Dry-matter production

Dry weights and contents are tabulated in Table 3.

At the end of the experiment the dry-matter production of the nitrate plants was higher than that of the plants from the other solutions. The dry-matter production of the ammonium plants fell behind that of the nitrate plants only during the last three days of the trial. The lowest dry-matter production during the whole period was obtained with plants lacking nitrogen in the culture solutions. The distribution of the produced dry matter over the various plant parts is given in Table 4. Without nitrate in the culture solution, the dry matter produced is located for a larger part in the roots.

The balance of uptake and utilization of inorganic ions

Plants with nitrate. Van Egmond and Houba (1970) already described the balance of uptake and utilization of inorganic ions by sugar-beet plants grown on a culture solu-

Table 3. Content of cations, anions and organic substances of the sugar-beet plants in meq/kg dry matter

Days after transplanting	Treatment	Dry weight (g/3 plants)	Ca	Na	K	Mg	C	H_2PO_4	NO_3	Cl
Leaf blade										
19	+ NO_3	6.6	416	371	2250	1220	4257	246	410	98
23	+ NO_3	7.8	470	466	2045	1386	4367	319	480	79
30	+ NO_3	13.8	380	441	1940	1664	4425	237	490	129
33	+ NO_3	19.4	450	498	2120	1620	4688	217	703	203
36	+ NO_3	31.1	380	375	1853	1702	4310	199	630	129
23	zero-N	7.0	456	408	1850	1184	3898	605	65	143
30	zero-N	10.4	420	320	1603	1076	3419	854	29	49
33	zero-N	10.5	546	366	1490	1314	3716	835	29	53
36	zero-N	11.9	642	425	1253	1348	3668	1080	29	86
23	+ NH_4	7.2	394	378	1700	1162	3634	808	130	87
30	+ NH_4	14.3	312	424	1370	984	3090	1015	94	80
33	+ NH_4	14.4	372	388	1210	1020	2990	1270	55	51
36	+ NH_4	17.1	382	474	929	1214	2999	1380	32	84
Petiole										
19	+ NO_3	1.4	168	233	3460	352	4213	191	1675	519
23	+ NO_3	3.2	170	271	3340	380	4161	193	1975	359
30	+ NO_3	8.2	92	257	3210	318	3877	181	1900	585
33	+ NO_3	10.2	92	268	3020	190	3570	217	1750	564
36	+ NO_3	24.1	100	231	2950	268	3549	178	1475	566
23	zero-N	2.5	142	250	2500	314	3206	314	335	572
30	zero-N	6.7	144	145	1780	212	2281	294	88	251
33	zero-N	7.4	120	210	1690	268	2288	249	72	281
36	zero-N	7.4	192	226	1740	270	2428	265	53	337
23	+ NH_4	3.3	98	240	2555	222	3115	395	720	606
30	+ NH_4	8.9	72	325	1450	172	2019	374	295	380
33	+ NH_4	12.2	82	261	1230	224	1797	442	147	257
36	+ NH_4	12.8	82	374	1190	258	1904	433	92	325
Roots and beet										
19	+ NO_3	2.2	106	110	1390	310	1916	289	630	172
23	+ NO_3	2.7	78	70	1330	264	1742	283	660	114
30	+ NO_3	7.0	60	60	1089	274	1483	244	298	123
33	+ NO_3	8.1	72	68	1130	302	1572	304	403	114
36	+ NO_3	18.4	66	54	1185	220	1525	250	395	183
23	zero-N	3.2	68	102	1040	298	1508	313	39	224
30	zero-N	6.4	52	54	688	172	966	278	13	91
33	zero-N	8.2	42	37	554	266	899	210	9	38
36	zero-N	12.4	42	34	494	266	836	221	7	43
23	+ NH_4	2.9	64	71	920	198	1255	341	84	206
30	+ NH_4	8.8	30	74	440	170	714	226	16	71
33	+ NH_4	10.3	34	56	468	168	726	289	11	58
36	+ NH_4	15.3	30	66	416	148	660	253	10	73

— = not determined.
I = citric acid; II = malic acid; III = oxalic acid;
IV = succinic and malonic acid? (traces); V = fumaric acid? (traces).

(total N and organic N in mmole/kg dry matter).

SO_4	A	I *	II	III	IV	V	Total N	Organic N	Total Z (I–V)	C–A
66	820	—	—	—	—	—	3550	3140	—	3437
74	952	—	—	—	—	—	3790	3310	—	3415
73	929	—	—	—	—	—	3420	2930	—	3496
77	1200	—	—	—	—	—	3280	2577	—	3488
79	1037	—	—	—	—	—	3240	2610	—	3273
110	923	—	—	—	—	—	2320	2255	—	2975
130	1062	—	—	—	—	—	1440	1411	—	2357
109	1026	112	212	1496	44	32	1220	1191	1896	2690
122	1317	—	—	—	—	—	1034	1005	—	2351
79	1104	104	156	2132	60	48	3520	3390	2500	2530
83	1272	32	100	1232	44	48	3000	2906	1456	1818
69	1445	56	76	1124	44	40	2490	2435	1340	1545
82	1578	64	124	1176	28	24	2590	2558	1412	1421
27	2412	—	—	—	—	—	2990	1315	—	1801
18	2545	—	—	—	—	—	3030	1055	—	1616
35	2701	—	—	—	—	—	2530	630	—	1176
40	2571	—	—	—	—	—	2570	820	—	999
29	2248	—	—	—	—	—	2310	835	—	1301
33	1254	—	—	—	—	—	1380	1045	—	1952
23	656	—	—	—	—	—	966	878	—	1625
28	630	80	308	504	28	32	833	761	952	1658
49	704	—	—	—	—	—	523	470	—	1724
21	1742	160	220	792	76	40	2050	1330	1288	1372
24	1073	168	156	496	44	32	1530	1235	896	946
5	851	96	124	504	44	40	1350	1203	808	946
114	964	128	292	556	44	24	1300	1208	1044	940
6	1097	—	—	—	—	—	3035	2405	—	819
0	1057	—	—	—	—	—	2870	2210	—	685
0	665	—	—	—	—	—	2470	2172	—	818
0	821	—	—	—	—	—	2884	2481	—	751
0	828	—	—	—	—	—	2240	1845	—	697
8	584	—	—	—	—	—	1690	1651	—	924
0	382	—	—	—	—	—	1145	1132	—	584
0	257	4	10	348	46	16	972	963	424	642
0	271	—	—	—	—	—	734	727	—	565
10	641	104	58	506	26	12	2290	2206	706	614
0	313	12	26	370	18	8	1500	1484	434	401
0	358	0	58	360	10	0	1740	1729	428	368
0	336	32	62	488	22	24	1518	1508	528	324

Table 4. Distribution in % of the produced dry matter over the shoots and roots of sugar-beet plants grown with nitrate, with ammonium or without nitrogen in the culture solution.

Days after transplanting	Shoot			Root		
	$+NO_3$	zero-N	$+NH_4$	$+NO_3$	zero-N	$+NH_4$
19	79	—	—	21	—	—
23	80	75	78	20	25	22
30	76	72	73	24	28	27
33	78	68	72	22	32	28
36	75	61	66	25	39	34

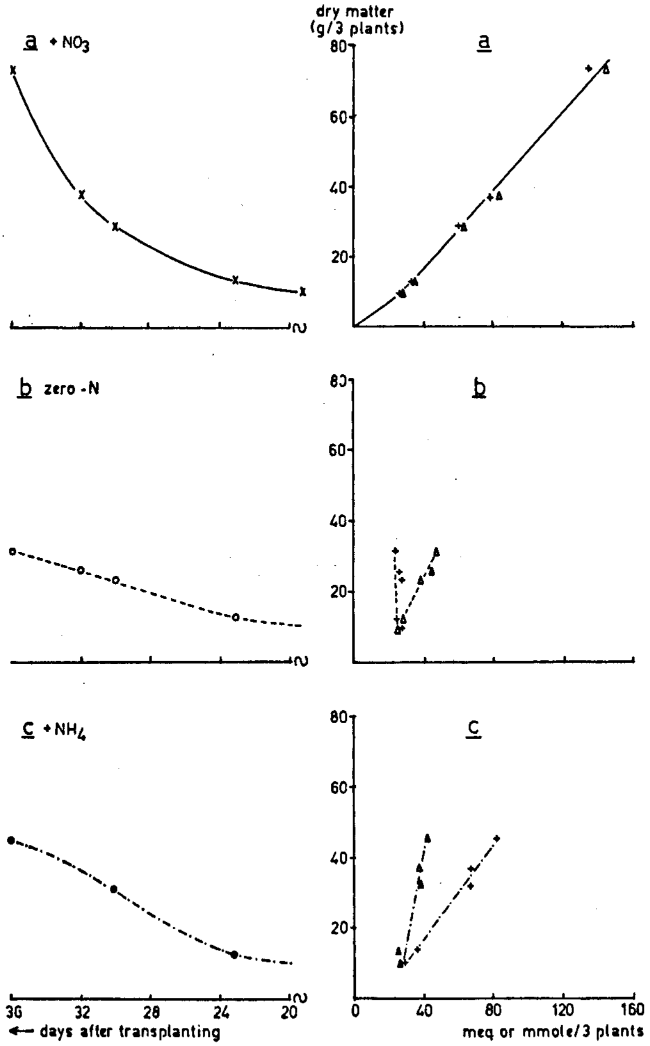

Fig. 2. Production of dry matter, organic N and C–A in sugar-beet plants grown on culture solutions of different nutrient composition.
● = $+NH_4$; × = $+NO_3$;
o = zero-N;
\+ = organic N; △ = (C–A).

Table 5. Dry matter, (C–A), organic N and carboxylates per three plants.

Days after transplanting	Treatment	Dry matter (g)	C–A (meq)	Organic N (mmole) *	Carboxylates (meq)
19	$+NO_3$	10.2	27.1	27.9	—
23	$+NO_3$	13.7	33.7	35.2	—
30	$+NO_3$	28.9	63.5	60.7	—
33	$+NO_3$	37.7	83.9	78.4	—
36	$+NO_3$	73.7	146.1	135.4	—
23	zero-N	12.7	28.7	23.7	—
30	zero-N	23.4	39.0	27.7	—
33	zero-N	26.1	45.8	26.1	30.4
36	zero-N	31.6	47.6	24.4	—
23	$+NH_4$	13.4	24.5	35.2	24.3
30	$+NH_4$	32.0	28.0	65.7	32.6
33	$+NH_4$	36.8	37.4	67.4	33.5
36	$+NH_4$	45.2	41.3	82.3	45.6

* Sometimes meq NO_3^- reduced.

tion with nitrate as nitrogen source. In their experiment, the production of carboxylates was equal to the production of organic nitrogen. The data of the nitrate-fed plants of the present trial are in agreement with these results (Table 5 and Fig. 2a).

Plants without nitrogen feeding. On replacing NO_3^- in the nutrient solution by SO_4^{2-}, there will be no further production of organic nitrogen as soon as the pool of nitrate in the plant is consumed. This is shown by Table 5 and Fig. 2b. However carboxylate production continues (after NO_3^- depletion) at a lower rate (Fig. 2a and 2b) compared with production of carboxylates by nitrate reduction. The production of carboxylates (after NO_3^- was depleted) must be explained with Process 2 of Table 1. The conclusion that this process is operative is evident from whole plant analysis and from the increase in H^+ concentration in the nutrient solution during the growth of the plants (Fig. 3).

The change in pH was so fast that the nutrient solution had to be changed every 30–40 hours. The organic-salt[1] production according to Process 2 takes place in the roots because the exchange of H^+ ions against other cations absorbed from the nutrient solutions takes place across the root-solution boundary. The carboxylate production resulting from nitrate reduction occurs (to a large extent) in the leaves of the sugar-beet. This conclusion is based on the presence of very high amounts of nitrate ions in the petiole. Assuming that a large part of the oxalates, which constitute about 80% of the total amount of carboxylates, are not mobile within the plant (van Egmond and Houba, 1970) a change in distribution of the carboxylates is to be expected when Process 2 increases and Process 3 of Table 1 decreases.

The ratio: amount of carboxylates in the shoot to that in the root decreases more in the zero-nitrogen plants than in the nitrate plants (Table 6). The carboxylate content in the roots of the zero-nitrogen plants, however, is lower than in the nitrate

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[1] Organic salt is used here instead of carboxylate to emphasize that the production of the $RCOO^-$ could take place in every plant part but that the salt formation (exchange of H^+ towards cations) will take place in the roots.

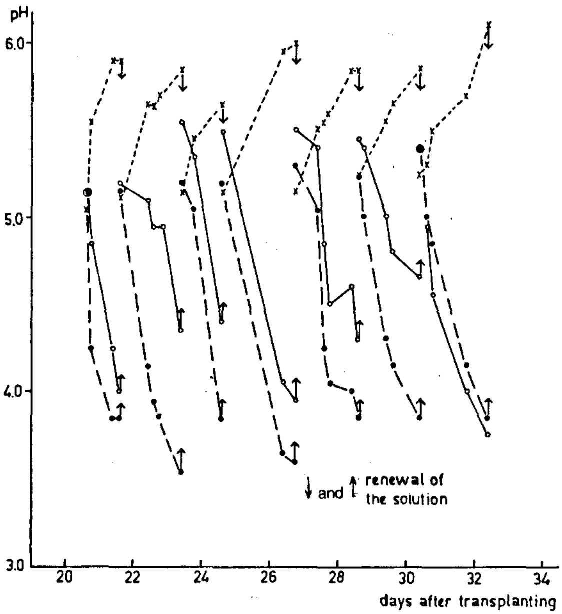

Fig. 3. Variations in pH of culture solutions caused by sugar-beet plants growing in a solution with nitrate (x----x), without nitrogen (o——o) and with ammonium (● — — ●).

plants (Table 3), because of accumulation of the produced dry matter in the roots of the zero-nitrogen plants.

When carboxylates are considered as an end product of the nitrate reduction, the percentage of carboxylates in dry matter is an interesting figure. Assuming a mean equivalent weight for the carboxylates of ± 50 g, the percentage of carboxylates in the dry matter (Table 7) of + NO_3 plants is about 4 % higher than of the zero-N plants.

Plants fed with ammonium. In this case (Table 5 and Fig. 2c) the production of organic nitrogen per unit dry-matter is almost equal to that of nitrate-fed plants (compare Fig. 2a and 2c). The carboxylate production (C–A) being equal to carboxylates, Table 5) however falls to a very low rate compared with the other two treatments. This difference in production of C–A and organic N was also shown by Dijkshoorn

Table 6. Ratio of total amounts carboxylates in the shoots to carboxylates in the roots in sugar-beet plants grown with and without nitrate nitrogen in the culture solution.

Days after transplanting	Treatment	
	+ NO_3	zero-N
19	14.1	14.1
23	17.2	8.7
30	10.5	9.6
33	12.8	7.7
36	10.4	5.8

Table 7. Percentage of carboxylates in total dry matter of sugar-beet plants grown on culture solutions with NO_3, without nitrogen and with ammonium, respectively.

Days after transplanting	Treatment		
	+ NO_3	zero-N	+ NH_4
19	13.3	13.3	13.3
23	12.3	8.9	9.1
30	12.0	8.4	4.4
33	12.0	8.8	5.0
36	10.0	7.6	4.7

et al. (1968) for rye-grass. For the NO_3 plants the level of carboxylates is 2000 meq, for the zero-N plants 1000 meq and for the NH_4 plants 500 meq/kg dry matter (these data are calculated from the slopes of the lines in Fig. 2a, 2b and 2c). The decrease in pH in the culture solution during the growth of the plants (Fig. 3) is very pronounced for the ammonium solution. Based on this high H^+-exchange one should expect a higher carboxylate production in the ammonium plants than in the plants without nitrogen. The low carboxylate production of only 500 meq/kg dry matter is associated with the process of NH_4^+ incorporation into organic nitrogen (Process 5 of Table 1). Due to the low carboxylate production the percentage of carboxylates in the dry-matter (Table 7) is lowest in the ammonium treatment.

Conclusions

Sugar-beet plants on nutrient solutions of different nitrogen composition can be characterized as follows:
NO_3 plants : C–A = organic N
zero-N plants : C–A > organic N
NH_4 plants : C–A < organic N

The differences in H^+ production are in good agreement with the above-mentioned characterization.

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