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A comparison between ammonium and nitrate nutrition of young sugar-beet plants grown in nutrient solutions at constant acidity. 2. Effect of light and carbohydrate supply

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Summary

The effect of light and of glucose in the nutrient medium on the release of OH^- or H^+ and ion accumulation by plants grown in NO_3 or NH_4 nutrition was studied at constant pH 5.50.

Decrease in OH^- production rate in the dark was probably caused by retarded nitrate assimilation or carbohydrate shortage in the roots. Decrease in the rate of H^+ production (NH_4 uptake) in the dark was probably due to carbohydrate depletion. Both 24-h illumination and the addition of glucose increased H^+ production during NH_4 uptake and OH^- production during NO_3 uptake. Twenty-four hours of darkness decreased the carboxylate pool on both nitrogen sources and preliminarily it was concluded that light and darkness exert more influence on the carboxylate pool than nitrogen assimilation. Relationships between organic acid, carbohydrate and nitrogen metabolism, H^+ or OH^- production and inorganic composition were considered in more detail.

Introduction

The first publication of this series (Breteler, 1973) concerned growth and composition of young sugar-beet plants in ammonium or nitrate nutrition at constant pH. Owing to the continuous neutralization of the acidity evolved in NH_4 nutrition by automatic titration, the yield of DM was only 12 % less than on nitrate medium.

With nitrate nutrition the plants contained more soluble carbohydrates, carboxylates, alkali cations and less inorganic anions, ammonium, free amides, free amino acids and total nitrogen as well as organic nitrogen than in ammonium nutrition.

The OH^- release curves showed that during a dark period of 8 h/day the rate of release of alkali to the nitrate medium fell until zero rate was attained a few hours after the beginning of the subsequent light period. Thereafter, alkali production recommenced and regained its initial rate in the light until it dropped again in the next dark period to attain zero rate early in the next light period, and so on. This was shown by the changes in the slope of the recorded OH^- production curves during the course of diurnal light and darkness.

In the NH_4 medium, the evolution of acidity continued at a measurable rate in the dark and early in the subsequent light period without interruption and regained its rate in the light.

The alkali released to the nitrate medium was no measure of the amount of nitrate used by the plants. The acidity released to the ammonium medium was for more than 85 % due to NH_4 uptake, and could be considered as a rough measure of the absorption of NH_4 during growth on the test solution. The lines of evidence supporting this are summarized below.

Consideration of the ionic balance within the whole plant showed that in NO_3 nutrition the OH^- released equals $N_{org} + S_{org} - (C–A) + 2\ NH_4$, with all terms expressed as ion equivalents of the sum of inorganic cations (C), the inorganic anions (A), the carboxylate anions (C–A), and of the nitrate converted into organic nitrogen (N_{org}) and the sulphate into S_{org}. In NO_3 nutrition, N_{org} was of the order of 2750 meq/kg DM, N_{tot} = 3150 and (C–A) about 2800 meq/kg DM. When the organic S (150) and the ammonium (25) in the plants are taken into account, the alkali released to the medium would be 150 meq for each kg DM produced by the plants, which is only 5 % of the nitrate consumption.

In NH_4 nutrition the acidity released equals $N_{org} - S_{org} + (C–A)$ because of the electroneutrality conditions of ion uptake and metabolization. The plants fed with ammonium contained 3600 meq N_{org} and 1000 meq (C–A) per kg DM, and the amount of acidity released to the medium was 4400 for each kg DM produced by the plants (S_{org}: 200, NH_4: 150). Thus the NH_4 used by the plants was numerically equal to about 85 % of the 4400 meq of acidity evolved in the medium, and the H^+ production curves were a rough measure of the course of ammonium absorption.

The aim of the present work was to investigate the effect of light and carbohydrate supply on the shape of H^+ or OH^- production curves and the chemical composition of the plants.

Experimental

The plants used for the present experiments were those remaining from the previous tests (Breteler, 1973). The solutions, containing 3 meq N/litre as either ammonium or nitrate, were kept at pH 5.50 by automatic titration and the volumes of standard HCl (NO_3 medium) or NaOH (NH_4 medium) were continuously recorded. The plants that had grown in a growth cabinet at 20 °C, 70 to 80 % relative humidity, and a light period of 16 h/day from 5h00 to 21h00 at 20 000 lux for about 6 weeks on the media, were now subjected to a one-day treatment of:

a: 24 hours in the light
b: 24 hours in the dark
c: 16 hours in the light and 8 hours in the dark
d: as c but with 35 ppm glucose added to the medium.

These treatments were applied on consecutive days and ammonium and nitrate plants were treated in turn. During the treatments apparent H^+ or OH^- release was recorded. Immediately after the 24-hour treatment plants were removed and divided into tops and 'roots' the latter part comprising the hypocotyledon with beet primordium and the roots. Both parts were weighed and analysed for the main inorganic ions, nitrogen, carboxylates, water-soluble carbohydrates and some free N-compounds. Whole plant com-

position was calculated from the dry weight and composition of the plant parts.

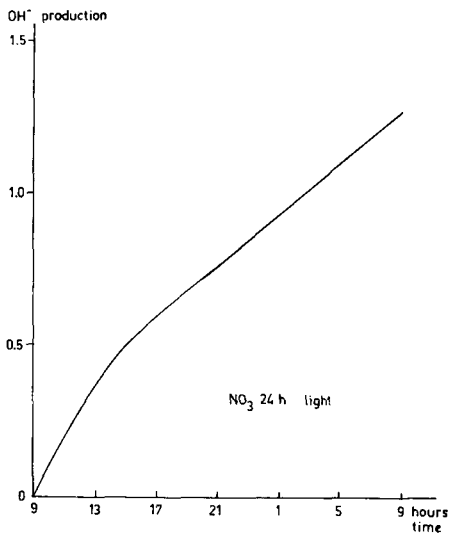
For further details on technique and analysis the reader is referred to Breteler (1973). The experiments started with 10 plants.

Results

Nitrate nutrition

Alkali release curves for the nitrate medium are shown in Fig. 1–4. Characteristics given in the subscripts of the figures refer to graphical curve analysis as described in the previous paper.

If the plants received light continuously for 24 hours (Fig. 1) the first rapid release of alkali levelled off after a few hours to a constant rate which was further maintained as shown by the straight section of the curve. If the plants stood in the dark for 24 hours (Fig. 2) the rate of OH^- release fell steadily as shown by the gradual decrease in slope of the curve. Other experiments demonstrate that the plants took longer to attain zero rate in the dark. This finding is symbolized by the dashed extention of the curve which becomes horizontal a few hours later. Fig. 3 shows the time course of alkali release under normal conditions of light and darkness. Owing to the previous dark period, evolution of OH^- had stopped, and it took a few hours light to become measurable. The curve, ascending at first, acquired a constant slope indicating a con-



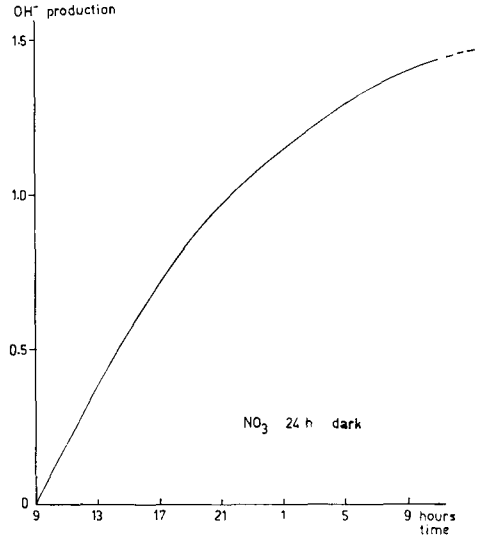
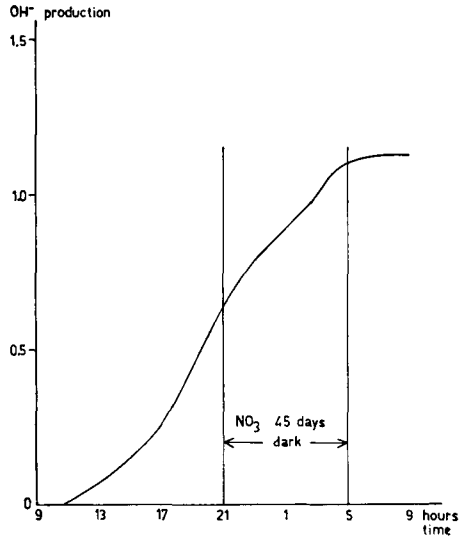


Fig. 1 (left) Alkali released in the nitrate medium by 10 plants (meq), during 24 hours illumination. A: 1.26 meq, α: 0.10 meq/h, δ_{21}: 0.04 meq/h. Curve characteristics like A, a and α were defined in the first article of this series (Breteler, 1973).

Fig. 2 (right) Alkali released in the nitrate medium by 10 plants (meq) during 24 hours darkness. Curve characteristics are not fully comparable with characteristics for normal dark/light production curves. B or A: 1.39 meq, a: 6.0 h; b: 32h (estimated), α: 0.10 meq/h, β: 0.06 meq/h (9–9).



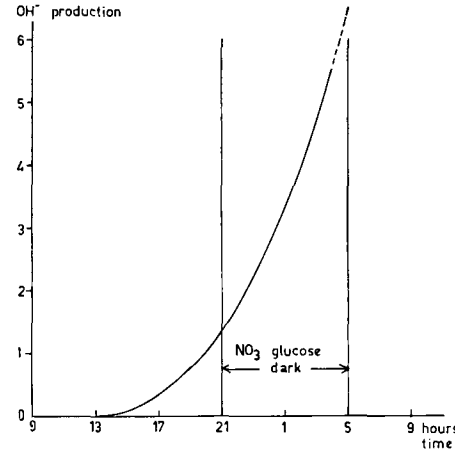


Fig. 3 (left) Alkali released in the nitrate medium by 10 plants (meq) during a normal light and dark cycle. A: 1.12 meq, B: 0.46 meq, B/A: 41 %, a: 1.8h, b: 8.8h, β: 0.06 meq/h, δ_{21}: 0.08 meq/h.

Fig. 4 (right) Alkali released in the nitrate medium by 10 plants (meq) during a normal light and dark cycle and 35 ppm glucose (added at 10h00) in the nutrient solution. A: 5.6 meq (13–4), B: 5.0 meq, B/A: 89 %, β: 0.65 meq/h, δ_{21}: 0.40 meq/h.

stant rate during the last one-third of the light period. During the subsequent dark period the curve became less steep until zero rate of alkali evolution was attained early in the next light period. With glucose added to the medium (Fig. 4), the rate of OH^- release became measurable after a few hours in the light and continued to increase into the dark period as shown by the steady increase in the slope of the curve. During the observation period of 1 day the total amount of OH^- released to the medium with glucose was 6 times greater than that released to the glucose-free medium under the same conditions (Fig. 3).

The curves obtained are not fully comparable with those of the previous tests because the plants had meanwhile increased in size.

Concentrations of the main inorganic constituents and of total and organic nitrogen in the dried plant material are listed in Table 1. The plants submitted to 24 hours of light or darkness differed by not more than about 10 % in composition except for free NH_4, Cl and SO_4 which were lower in the tops of darkened plants, Na, NO_3, A and total N which were higher and (C-A) which was lower in the roots of darkened plants. In the light unmetabolized nitrate was 12 %, in the dark it was 16 % of all the nitrate used by the plants. The increase in nitrate and the fall in (C–A) in the root tissue of the plants that received no light during the last 24 hours indicates that nitrate metabolism was retarded by darkness.

The plants exposed to 24 hours of light or darkness were analysed for carboxylates; the results are shown in Table 2. The bottom row gives (C–A) from inorganic analysis of Table 1. In the light, the plants contained more of the main carboxylic anions oxalate, malate and citrate than in the dark, and in the former treatment the sum of

Table 1 Ionic constituents and nitrogen in tops, roots and whole sugar-beet plants grown for about 6 weeks on nitrate medium at the end of a period of 24 h light and at the end of a period of 24 h darkness. Contents in meq or mmol (N)/kg DM.

	24 h light			24 h dark		
	tops	roots	whole plant	tops	roots	whole plant
K	1902	1410	1801	1848	1460	1769
Na	610	152	514	698	196	594
NH_4	46	19	40	15	22	16
Mg	1006	356	872	901	336	783
Ca	311	126	273	351	138	307
C	3875	2063	3500	3813	2152	3469
Cl	340	313	334	212	285	227
SO_4	110	4	87	61	26	54
H_2PO_4	257	295	267	296	336	304
NO_3	382	183	341	464	584	488
A	1089	795	1029	1033	1231	1073
N_{tot}	2910	2570	2840	3067	3230	3101
N_{org}	2482	2368	2459	2588	2628	2597
(C–A)	2786	1268	2471	2780	921	2396

Table 2 Carboxylates and (C–A) in tops, roots and whole sugar-beet plants grown for 6 weeks on nitrate medium at the end of 24 hours of light or dark. Contents in meq/kg DM.

	24 h light			24 h dark		
	tops	roots	whole plant	tops	roots	whole plant
Fumarate	20	16	19	16	0	13
Succinate	24	24	24	20	8	18
Malonate	20	36	23	24	20	23
Oxalate	2138	936	1889	1858	760	1631
Malate	240	124	216	108	36	93
Citrate	468	360	446	140	272	167
Total	2910	1496	2617	2166	1096	1945
(C–A)	2786	1268	2471	2780	921	2396

Table 3 Total water-soluble carbohydrates, glucose, fructose and sucrose content (% of DM) in the tops of plants grown on nitrate medium at the end of 24 hours of light or dark.

	24 h light	24 h dark
Glucose	1.09	1.34
Fructose	0.50	1.01
Sucrose	3.11	2.21
Total	7.9	5.2

Table 4 Ammonium, free amides and amino acids in tops, roots and in whole plants grown on nitrate medium at the end of 24 hours of light or dark. Contents in meq NH_4/kg DM of mmol/kg DM of amides and mmol N/kg DM of amino acids and amides.

	24 h light			24 h dark		
	tops	roots	whole plant	tops	roots	whole plant
NH_4	46	19	40	15	22	16
Glu. NH_2	22	13	20	42	18	37
Asp. NH_2	22	9	19	25	7	21
Glu. NH_2 + Asp. NH_2	44	22	39	67	25	58
Amino acids and amides	179	71	144	203	56	163

the carboxylates determined directly as organic acids exceeds the value of (C–A), suggesting the presence of free acids within the tissue.

The tops of the plants were also analysed for glucose, fructose, sucrose and total water-soluble sugars (Table 3). Glucose and fructose contents were raised in the dark, while the sucrose content was lower. Metabolic interconversion, consumption, and drainage of sugars from the tops may be involved and the data give no evidence for depletion of reducing sugars in the dark. Not enough root material was left for analysis, so that no data are availigle on contents in the whole plant.

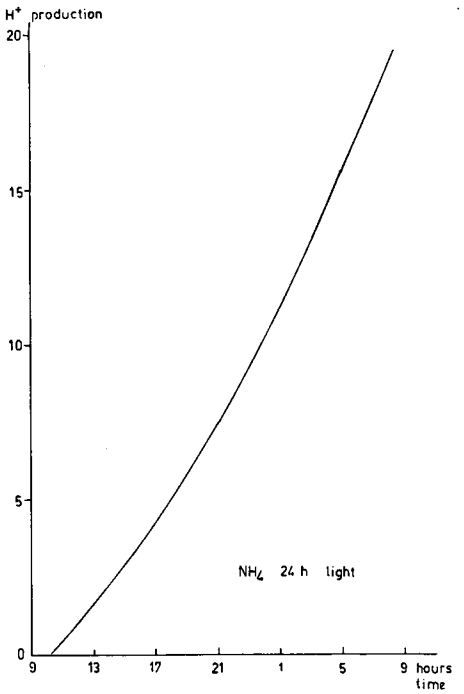
Concentrations of some of the free nitrogenous compounds in the tissues are listed in Table 4. Glutamine concentrated to a higher level in the dark, whereas free ammonium was higher in the light. In mint plants, Rabson & Steward (1962) observed the lowest concentration of free amino acids at the end of the light period of the day. Prianishnikov (1951) associated low NH_4 levels with higher sugar levels within the tissues which is not confirmed by the present data (Tables 3 and 4).

Ammonium nutrition

For the ammonium medium the H^+ production curves are shown in Fig. 5 to 8. During a light period of 24 hours the acidity released to the medium rose to 2 meq H^+ plant^{-1} day^{-1} (Fig. 5). During 24 hours in the dark the acidity released to the medium (Fig. 6) is less than one half of that in the light, and the curve relating the H^+ release and the time in the dark levels off to zero rate within 24 hours. Fig. 7 is the H^+ production curve of plants exposed to the normal light-dark cycle. Owing to the previous dark period H^+ production took several hours before becoming measurable. If the same conditions were applied to plants grown on a NH_4 medium containing glucose, the amount of acidity evolved during 24 hours was greater (Fig. 8) and there was no reduction in the rate of its evolution during the 8-h dark period as observed in the absence of glucose (Fig. 7). Similar to alkali release to the nitrate medium, the addition of glucose resulted in an uninhibited rate of H^+ release during the dark period.

Considering Fig. 5 to 8, one should realize that the plants differed in size from one day to another.

The carboxylate and (C–A) contents in the tops are listed in Table 5. It seems that in the tops of the plants supplied with ammonium, some free organic acids accumulate after 24-hours illumination. Although analytical errors may be involved, it is striking that this also occured in nitrate nutrition (Table 2). Accumulation of free carboxylic



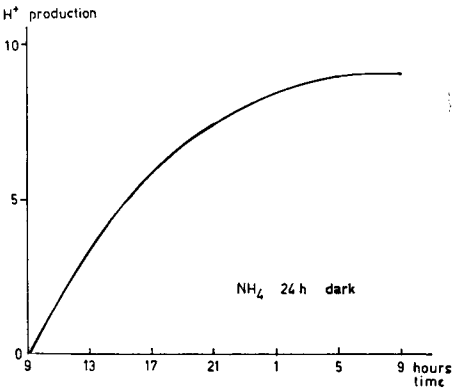
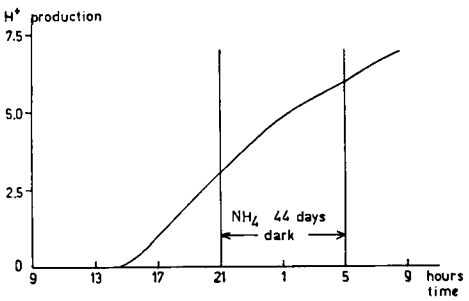


Fig. 6 (above) Hydrogen ion production in the ammonium medium by 10 plants (meq) during 24-hours darkness. B or A: 9 meq, a: 3.5 h, b: 21 h, α: 0.85 meq/h, β: 0.38 mey/h.

Fig. 5 (left) Hydrogen ion production in the ammonium medium by 10 plants (meq) during 24-hours illumination. A: 20 meq, α: 0.55 meq/h, δ: 0.85 meq/h.



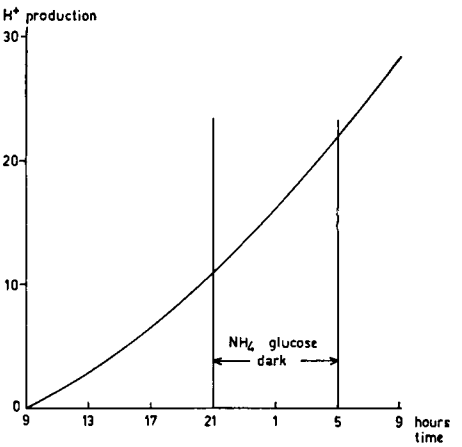


Fig. 7 (left) Hydrogen ion production in the ammonium medium by 10 plants (meq) during a normal light and dark cycle. A: 7 meq, B: 3 meq, a: 2.3 h, β: 0.38 meq/h, δ_{21}: 0.50 meq/h.

Fig. 8 (right) Hydrogen ion production in the ammonium medium by 10 plants (meq) during a normal light and dark cycle and 35 ppm glucose (added at 09h00) in the nutrient solution. A: 28.5 meq, B: 11.0 meq, B/A: 39 %, α: 0.62 meq/h, β: 1.38 meq/h, δ_{21}: 1.25 meq/h.

Table 5 Carboxylates and (C–A) in the tops of sugar-beet plants grown for 6 weeks on ammonium medium at the end of 24 hours of light or dark. Content in meq/kg DM.

	24 h light	24 h dark
Fumarate	32	0
Succinate	8	0
Malonate	20	4
Oxalate	932	612
Malate	68	60
Citrate	64	56
Total	1124	732
(C–A)	936	1056

Table 6 Total water-soluble carbohydrates, glucose, fructose and sucrose content (% of DM) in tops and roots and in whole plants grown on ammonium medium at the end of 24 h light, 24 h dark and after 24 h exposure to a medium containing glucose.

	24 h light			24 h dark	Glucose		
	tops	roots	whole plant	tops*	tops	roots	whole plant
Glucose	0.34	0.40	0.35	0.31	1.13	0.81	1.07
Fructose	0.30	0.32	0.31	0.24	1.34	0.59	1.21
Sucrose	3.22	5.42	3.61	2.97	8.12	5.66	7.70
Total	5.8	7.8	6.1	5.2	14.5	8.3	13.4

* No material was left for analysis of the roots

acids occurs in the dark in Crassulacean plants (Astruc, 1903; Pucher et al., 1947; Somers, 1951; Bruinsma, 1958). Changes in the carboxylate pool are completely covered by changes in the oxalate content.

The water-soluble sugar contents of the plants of Fig. 5, 6 and 8 are shown in Table 6. The effect of the 24-hours exposure to the medium containing glucose is clearly re-

Table 7 Ammonium, free amides and amino acids in tops, roots and in whole plants grown on ammonium medium at the end of 24 hours of light or dark. Contents in meq NH_4/kg DM or mmol/kg DM of amides and mmol N/kg DM of amino acids and amides.

	24 h light			24 h dark		
	tops	roots	whole plant	tops	roots	whole plant
NH_4	145	122	141	198	63	174
Glu. NH_2	145	135	143	170	103	158
Asp. NH_2	48	24	44	42	34	41
Glu. NH_2 + Asp. NH_2	193	159	187	212	137	199
Amino acids and amides	460	545	476	474	334	466

flected in higher contents of glucose, fructose, sucrose and total water-soluble sugars. Twenty-four hours of darkness decreased the content of these compounds in the tops compared with 24-hours light on the preceeding day. In nitrate nutrition the content of the reducing sugars, glucose and fructose, in the tops increased in the dark.

Contents of free ammonium ions, amides and amino acids are listed in Table 7. In the light NH_4 ions accumulate in the roots as compared with plants grown 24 hours in the dark and it seems that the same is true for free amino compounds. As far as the amides are concerned there also is a trend for a decrease in the roots and an increase in the tops after the plants have been 24 hours in the dark, especially for glutamine. The sum of free amino acids and amides behaved similarly in nitrate nutrition (Table 4).

Discussion

In the Introduction it was recalled that 85 % of the environmental acidification in ammonium media was caused by the uptake of NH_4 ions. The other 15 % came from the uptake of non-nitrogenous cations over anions. H^+ release equals the sum of NH_4 uptake and (C–A) production when organic S is neglected and the (C–A) is exclusive of free NH_4 ions. From plant analysis it appeared that $N/[N + (C–A)]$ remained nearly constant under the present test conditions so that H^+ release curves roughly represent NH_4 uptake curves.

Since H^+ production in Fig. 5–8 measures NH_4 absorption, the following conclusions are valid. In 24 hours of light (Fig. 5) or with glucose in the rooting medium (Fig. 8) the rate of NH_4 uptake increased with time and eventually reached a high rate of 2–3 meq plant^{-1} day^{-1}. In the dark (Fig. 6) the rate of NH_4 uptake decreased and finally stopped completely. Compared with the large effect on acidity evolved of light and darkness during the day of treatment, the effect on the soluble carbohydrate content of the tops of the plants after these treatments (Table 6) was small. The effect of glucose during the normal light/dark cycle (Fig. 7 and 8) on H^+ evolved and on soluble sugars was, however, of comparable magnitude.

To understand the shape of the H^+ and OH^- release curves of these tests and those previously described some explanations are possible. For nitrate plants the decrease in rate of alkali evolution was not due to a decrease in carbohydrates or reducing sugars in the tops (Table 3). Another cause would be a reduced rate of nitrate metabolization. The time needed to restore OH^- release early in the light period may be explained by assuming that the nitrate uptake depends on the nitrate content of the plant and on the nitrate reductase activity. This implies that NO_3 uptake is the main factor influencing the OH^- production. Three to four hours induction time for nitrate reductase activity is not unlikely (Beevers et al., 1965). The first few hours after 05h00 in Fig. 4 could have supplied evidence on whether carbohydrate shortage or nitrate reduction were involved in the decrease of the rate of OH^- release early after the dark period, but unfortunately the measurements could not be continued into the subsequent light period. Besides this it is questionable whether the nitrate reductase behaves similarly in plants so different in carbohydrate content.

To explain the shape of the H^+ production curves it is useful to consider the soluble carbohydrate content of the plants (Table 6). Restoration of a sufficient soluble carbohydrate level for the normal rate of H^+ release during the light period should then take place within about $1^1/_2$ hours after the start of the light period.

On exposure to 24 hours of light, both OH^- and H^+ release tended to reach a con-

stant rate, while glucose addition resulted in a rate still increasing at the end of the test period, indicating relationship between absorbed sugar and extra H^+ or OH^- release.

As discussed in the first publication there is a difference in the reaction of H^+ and OH^- release upon light or carbohydrate supply. In the present experiments glucose added to the media stimulated OH^- release more than H^+ release (Fig. 3, 4, 7 an 8), while during 24 hours of darkness OH^- production continued many hours longer than H^+ production (Fig. 2 and 6).

Carboxylate contents of the plants increased in the light and decreased in the dark on both N forms. One could expect an increase in the carboxylate pool of NO_3 plants in the light, since increased nitrate assimilation creates more carboxylates. The light-stimulated increase in the carboxylate content of the tops of NH_4 plants was unexpected.

Data in literature on the effect of illumination on the carboxylate metabolism are often not consistent (Ulrich 1926; Ruhland & Wetzel, 1926) except for species like *Bryophyllum, Kalanchoë* and *Crassula* which have been investigated extensively (Pucher et al., 1947, 1949; Somers, 1951; Bruinsma, 1958). The form in which N was supplied was usually nitrate and sometimes it was not mentioned.

Astruc (1903) distinguished the titratable sap acidity from the combined acid (ash alkalinity) content. Diurnal changes in crassulacean plant acids concern the free acids, with the carboxylates constant. In the present experiments carboxylate anions vary somewhat more than (C–A) values. Crassulacean acid metabolism is restricted to succulent species. In the leaves of rhubarb and tobacco plants a steady loss of organic acids in long continued darkness and an increase after 25 hours constant illumination at 20 °C has been described by Bennet-Clark (1949). These data agree in the treatments and responses with the results of the present experiment. However, no attention was paid to the influence of the N form on the organic acid (anion) content by the cited authors, although the effect of nitrogen from NO_3 or NH_4 on the carboxylate content was already recognized in the 19th century by Pfeffer (1881), Wehmer (1891) and others, as cited by van Tuil (1965). A preliminary conclusion is that changes in the magnitude of the carboxylate pool are more affected by light and darkness than by nitrogen assimilation.

Acknowledgment

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