ORGANIC ACIDS OF THE COTTON PLANT[1]

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The quantities of the organic acids occurring in the leaves of cotton have been found to be comparable in amount with the proteins and often several times greater than the sugars plus starch. As in other plants (**2, 10, 18, 22**), such a mass of little understood material incites interest as to its function and lability. It has been suggested than an organic acid may be an intermediary in the photosynthetic process and also that these acids may be of significance in the initiation of reproduction. In either such role it seems doubtful that any great part of a mass constituting 10 or 20 per cent. of the dry weight of the leaves could be very active. Although a nutritional role may not be eliminated it has been common to regard the organic acids as neutralizing or buffering agents. The object of the work back of this paper has been that of gaining some insight into the functional activity of the organic acids of cotton. The behavior of these acids has been studied by means of experiments wherein such factors as moisture supply, mineral nutrition, fruitfulness, and respiration were varied.

Analytical methods

Preparatory to analysis all tissues were dried at 75° C. in a forced-draft oven and ground in a small Wiley mill to pass an 80-mesh screen.

Ash constituents were determined by A.O.A.C. official methods (**1**).

Determination of the various carbohydrate fractions and cellulose was based upon procedures described elsewhere (**6, 7**).

The ether-extraction and subsequent preparation of an aqueous solution of the organic acids from the cotton tissues was accomplished by the method of PUCHER *et al* (**15**). Total organic acids were determined by titrating aliquots of the aqueous solution between the limits of pH 7.8 and pH 2.6, using the method of VAN SLYKE and PALMER (**21**). Additional aliquots of the same solution were analyzed for citric, malic, and oxalic acid, following the procedures of PUCHER *et al* (**16**). The "unidentified" portion of the total organic acids was obtained by subtracting the sum of citric, malic, and oxalic acid from the determined value for total organic acids.

Tests were made for isocitric and *cis*-aconitic acids by the method of KREBS and EGGLESTON (**8**), using an extract of aconitase derived from frozen breast muscle of pigeon. As tested on plants known to contain isocitric and *cis*-aconitic acids the aconitase thus obtained was found to be active.

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Experimental

DISTRIBUTION AND KINDS OF ACIDS

Of the parts of the cotton plant which have been examined, higher concentrations of organic acids are found, table I, in the leaves than in other vegetative parts. Next in order come the petioles and then the stems and the roots, each of which contains about half as much as the petioles. The flower as a whole contains less organic acid than the petioles, and 13-day old bolls and mature seed kernels contain still less. Organic acids have been found by McCALL and GUTHRIE (**9**) to constitute 0.84 per cent. of the dry weight of raw cotton fiber.

In all tissues except seed kernels there was less citric than malic acid and only in the flowers did oxalic exceed malic. The "unidentified" acids exceeded malic in stems, roots, 13-day old bolls, and flowers, but not in the leaves, petioles, raw cotton fibers, or seed kernels.

TABLE I

DISTRIBUTION OF ORGANIC ACIDS IN VARIOUS PARTS OF THE COTTON PLANT. MILLIEQUIVALENTS PER 100 GRAMS DRY TISSUE

PLANT PART	CITRIC	MALIC	OXALIC	UNIDENTIFIED	TOTAL
Leaves	105.6	119.6	11.5	39.2	275.9
Petiole	22.1	70.9	21.6	40.2	154.8
Stem	7.5	27.0	9.8	28.5	72.8
Root	3.4	17.0	14.9	34.8	70.1
Flowers	14.7	17.8	27.3	48.5	108.3
13-day bolls	4.5	18.7	7.3	28.9	59.4
Seed kernels	12.5	11.2	7.8	....	30.9
Raw cotton fibers*	1.1	7.0	0.1	4.9	13.1

*Average of data by McCALL and GUTHRIE (9).

Attempts to identify the organic acids comprising the "unidentified" group were limited to tests for isocitric and *cis*-aconitic acids. In the cotton tissues examined neither of these acids was present in amounts sufficient to be demonstrated by KREBS' (**8**) micro-method.

INFLUENCE OF WATER SUPPLY ON THE CITRIC ACID-MALIC ACID EQUILIBRIUM

The data reported in table II were derived from an experiment with Acala P18-c cotton conducted at Shafter, California in 1946. The plants were grown in plots irrigated weekly which showed no wilting at any time and in plots which were not irrigated after early June. The latter plants wilted severely each afternoon from early July through August and September. At the end of the season the dry plants weighed about one-fifth as much as the plants in the weekly-irrigated plot. The leaf samples were collected on July 16 and 17 and again on August 6 and 7. For each sampling there were two mid-morning and two mid-afternoon collections of

TABLE II

Influence of moisture and fruitfulness on the organic acids of cotton leaves, stems, and roots (milliequivalents per 100 grams dry weight), and on the level of sugars and sugars plus starches, and level of nitrogen

	Organic acids						Carbohydrates		Nitrogen	
	Citric	Malic	Oxalic	Uniden-tified	Total	Moisture	Sugars	Sugars plus starch	Soluble	Total
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	%	%	%	%	%
Leaves										
Treatment										
Wet										
Full load	102.4	90.5	8.8	77.5	279.2	78.6	1.11	2.81	0.63	3.28
2-boll	98.2	97.8	8.3	56.8	261.1	77.9	1.28	3.70	0.57	3.42
Dry										
Full load	44.8	142.7	12.9	67.4	267.8	71.3	.96	2.24	0.78	3.08
2-boll	50.1	143.8	13.2	68.1	275.2	70.5	.97	2.19	0.68	3.09
Mean change as a result of:										
Drought	−52.9	49.1	4.5	0.6	1.4	−7.4	−.23	−1.04	.13	−.26
2-boll	0.5	4.2	−0.1	−10.0	−5.4	−0.7	.09	.42	−.08	.07
Stems										
Treatment										
Wet										
Full load	7.7	30.9	9.1	44.0	91.7	76.8	3.92	5.07	0.33	0.78
2-boll	8.3	34.9	10.8	31.1	85.1	75.9	4.14	5.41	0.32	0.91
Dry										
Full load	5.7	22.3	14.1	46.1	88.2	66.4	7.19	10.43	0.44	0.94
2-boll	6.1	24.2	13.1	38.1	81.5	64.3	6.13	11.89	0.44	1.00
Mean change as a result of:										
Drought	−2.1	−9.7	3.7	4.5	−3.6	−11.0	2.63	5.92	.12	.13
2-boll	0.5	2.9	0.3	−10.4	−6.6	−1.5	−.42	.90	.00	.00
Roots										
Treatment										
Wet										
Full load	3.9	13.4	10.9	36.5	64.7	72.0	3.34	5.73	0.25	0.65
2-boll	3.6	12.2	10.9	33.0	59.7	70.8	3.71	6.81	0.28	0.62
Dry										
Full load	4.7	9.8	15.6	32.4	62.5	62.8	6.59	13.54	0.27	0.70
2-boll	3.1	9.8	13.8	25.3	52.0	58.4	5.05	15.58	0.29	0.72
Mean change as a result of:										
Drought	0.2	−3.0	3.8	−5.9	−5.0	−10.8	2.29	8.29	.02	.08
2-boll	−1.0	−0.6	−0.9	−5.3	−7.8	−2.8	−.58	1.56	.02	.00

three plants from each treatment. The leaves from twelve plants were composited for each treatment sample. The means of the two sets of samples are reported in table II. The leaf samples are from the middle third of the main stalk, the stem samples are the middle third of the main stalk, and the root samples are the tap and large lateral roots as lifted with a shovel.

In the leaves, table II, there was under drought a loss of 52.9 m.e. (per 100 gms. of dry sample) of citric acid and a gain of 49.1 m.e. of malic acid and also a gain of 4.5 m.e. of oxalic acid. In the stems and roots, citric acid was changed little with drought and malic acid was apparently decreased.

The increase in malic and loss of citric acid in the leaves of the dry plants occurred both in plants with a full load of bolls and, in similar proportions, in plants maintained with only two bolls each by bud and flower removal. In neither treatment did drought have a significant effect on total organic acids.

TABLE III

INFLUENCE OF MOISTURE SUPPLY ON THE ORGANIC ACIDS OF THE LEAVES OF TWO VARIETIES OF COTTON. MILLIEQUIVALENTS PER 100 GRAMS DRY WEIGHT. JULY 24, 1946

	ORGANIC ACIDS					MOISTURE	CARBOHYDRATES		NITROGEN	
	CITRIC	MALIC	OXALIC	UNIDENTIFIED	TOTAL		SUGARS	SUGARS PLUS STARCH	SOLUBLE	TOTAL
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	%	%	%	%	%
ACALA P 18-C										
Wet plot	125	109	2	27	263	79	1.41	5.36	.45	3.13
Dry plot	49	205	4	20	278	73	1.58	2.01	.86	3.11
STONEVILLE 2B										
Wet plot	115	169	6	6	296	78	1.33	4.38	.67	3.11
Dry plot	55	235	11	......	299	68	1.96	2.18	.79	2.72
Mean change as a result of drought:	–68	81	4	–7	9	–8	.40	–2.78	.27	–.21

Other leaf samples of both Acala P18-c and Stoneville 2B cotton were collected from similarly treated plots on July 24, and the organic acids in these samples were also determined. In this instance, table III, as before in table II, large reductions were found in the concentrations of citric acid in the dry-plot leaves. There were large and roughly equivalent, or greater, gains in malic acid.

The analyses reported in table III were made on one-third of the 110 to 140 leaves (blades only) collected from each treatment. The second and third aliquots—taken leaf by leaf in turn in the order of collection—were placed respectively between wet and dry towels and allowed to stand in the laboratory for 24 hours before being dried in the oven. The data from these

TABLE IV

Organic acids, carbohydrates, and nitrogen in cotton leaves from wet and dry plots. Also leaves from same collections after standing between either wet or dry towels for 24 hours. Shafter, California, July 24, 1946. Organic acids in milliequivalents per 100 grams dry weight. Other constituents on per cent. of dry weight

	Organic acids					Moisture	Carbohydrates		Nitrogen	
	Citric	Malic	Oxalic	Unidentified	Total		Sugars	Sugars plus starch	Soluble	Total
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	%	%	%	%	%
Acala P18-C										
Wet plot										
Original	125	109	2	27	263	79	1.41	5.36	.45	3.13
Wet towel	126	94	1	34	255	81	.60	1.33	.57	3.15
Dry towel	108	124	6	4	242	69	1.14	1.23	1.20	3.23
Dry plot										
Original	49	205	4	20	278	73	1.58	2.01	.86	3.11
Wet towel	60	175	8	28	271	76	.35	.55	.92	2.98
Dry towel	50	220	10	......	275	68	.69	.79	.88	3.14
Stoneville 2B										
Wet plot										
Original	115	169	6	6	296	78	1.33	4.38	.67	3.11
Wet towel	129	142	13	33	317	79	.58	1.27	.83	2.89
Dry towel	121	154	10	27	312	66	1.66	2.18	1.12	3.10
Dry plot										
Original	55	235	11	......	299	68	1.96	2.90	.79	2.72
Wet towel	66	205	8	19	298	72	.98	1.73	.72	2.92
Dry towel	54	239	10	......	297	61	1.23	1.40	1.06	2.71
Means										
Original	86	180	6	14	284	74	1.57	3.66	.69	3.02
Wet towel	95	154	8	29	285	77	.63	1.22	.76	2.99
Dry towel	83	184	9	8	282	66	1.18	1.40	1.07	3.05

24-hour samples, together with a repetition of the data from the original samples, are reported in table IV.

Comparing first the wet-towel and dry-towel samples, we find a further confirmation of the moisture relation brought out in tables II and III, *i.e.* with the reduction in moisture supply there was a loss of citric acid and a gain in malic acid in each of the paired data of each of the two cotton varieties. The gain in malic averaged 30 m.e. and the loss in citric averaged only 12 m.e. But in these 24-hour samples there was also a loss of 21 m.e. of ''unidentified'' acid.

The loss of citric acid and gain in malic acid with moisture reduction represents, apparently, a reversible reaction. This is shown when the ''original'' samples are compared with the ''wet towel'' samples. All of the paired data between the wet towels show that while there was a gain in citric and a loss in malic acid, the average loss in malic acid was 26 m.e. and the average gain in citric was 9 m.e. But over this 24-hour period there was also a gain of 15 m.e. in the ''unidentified'' acid.

The foregoing data thus show that whether under conditions of growth and respiration in the field or under conditions of respiration in the laboratory there is a reversible interchange, though necessarily indirect, between citric and malic acids. As moisture is lost the change is toward malic and as it is gained the change is toward citric. The 24-hour results also show that the ''unidentified'' acids are involved in the reaction, *i.e.* the reaction did not go to completion in 24 hours. Instead, a part of the precursor, or derivative, of the citric acid required to equal the gains, or losses, in malic was apparently still included in the ''unidentified'' acids at the end of 24 hours.

The conversion of malic to citric acid has been observed previously by others under other conditions, but the reverse of the reaction—citric to malic—may not have been noted heretofore. The malic to citric transformation has been recorded (**13**) when tobacco was cultured in the dark and also when tobacco was supplied with potassium malate (**11**).

The fact that major interconversions are here found to occur between citric and malic (evidently through the unidentified fraction) without material alteration of the total organic acid is evidence of a reversible equilibrium. The data of VICKERY *et al* (**25**) on the malic-to-citric acid reaction correspond fairly well with the Martius and Knoop hypothesis. This reaction proceeds to oxalacetic acid, to pyruvic acid, to a condensation product, and finally to citric acid. The present writers are without an opinion as regards the reversibility of this chain. In the 24-hour experiment the gain in ''unidentified'' as well and in citric acids equalled nearly the loss in malic. Also in the reverse reaction the loss of ''unidentified'' acids, added to the losses of citric (possibly preceding the full loss in citric) made up for gains in malic. This relation suggests that the ''unidentified'' acids are deserving of more attention than they sometimes receive. And, also, that the key to the intermediate acids between malic and citric may be found in the "unidentified" group.

AMMONIUM VERSUS NITRATE NITROGEN ON BASE AND ORGANIC ACID ACCUMULATION

The organic acids have for many years been regarded as the great neutralizers or buffers of the Plant Kingdom. Recent papers by ULRICH (**20**) and by PIERCE and APPLEMAN (**10**) have dealt with phases of the inorganic cation-anion and organic acid relation. In other papers CLARK (**4**) and PUCHER *et al* (**12**), VICKERY *et al* (**23**) and WADLEIGH and SHIVE (**26**) have interested themselves in the influence of ammonium versus nitrate nitrogen on the organic acids. It has seemed to the writers, as well as to others undoubtedly, that the foregoing subjects might very well be closely related: *i.e.*, that the differences found in the quantity of organic acid might be in response to differences in total base and anion accumulation rather than to specific effects of the ammonium and nitrate ions. With this viewpoint the major inorganic cations and anions were included in the leaf analyses of the experiment reported in this section, table V.

A group of cotton plants (Stoneville 2B doubled haploids) was planted and thinned one to a pot in the greenhouse in tall four-gallon sand-filled stone jars and grown for 35 days on half-strength Hoagland's solution. Iron was supplied as magnetite mixed with sand. The pots were then taken out-doors (May 8) and divided into five groups of five plants each. One of these groups was cropped at this time for leaf analyses and weights. The other four groups were grown for an additional 20 days during which time they were differentially supplied with single salts plus boron and manganese. The four treatments were 8 m.e. per liter of the following: (1) $(NH_4)_2SO_4$, (2) NH_4Cl, (3) $Ca(NO_3)_2$, and (4) $NaNO_3$. Each of these solutions was supplied in sufficient excess to produce about 50 per cent. of drainage.

Although the plants made about three-quarters of their total growth during the last 20 days while supplied with single salts, table V, there was at the end of 20 days no evidence of any decline or deficiency. The ammonium sulphate plants were somewhat larger than those of the other treatments and they were especially deep green.

The salts carried by the stems and roots, together with those supplied by the tap water, contributed substantially to the maintenance of the initial mineral concentrations in the leaves. However, there were losses; among these is potassium but it remained sufficiently high in the leaves of all treatments for plant needs.

Between the two ammonium salts—and also between the two nitrate salts—there was little difference in the accumulations of total cations or of total inorganic anions. Likewise, the excess of cations over anions, and the concentrations and kinds of organic acids were similar within pairs. Possible exceptions are the extra amounts of "unidentified" organic acid in the Cl

TABLE V

INFLUENCE OF AMMONIUM AND NITRATE NITROGEN ON THE ORGANIC ACIDS OF COTTON LEAVES. MILLIEQUIVALENTS PER 100 GRAMS DRY WEIGHT*

TREATMENT	DRY WT. OF LEAVES	CATIONS					ANIONS					EXCESS OF CATIONS OVER INORGANIC ANIONS	ORGANIC ACIDS					FREE ORGANIC ACID
		CA	MG	K	NA	TOTAL	CL	NO_3	PO_4	SO_4	TOTAL		CITRIC	MALIC	OXALIC	UNIDENTIFIED	TOTAL	
END INITIAL 35 DAYS																		
Hoagland's Solution	19.3	198	45.6	77.2	7.8	329	11.3	23.6	24.5	71.9	131	198	63.1	56.6	12.7	119.6	252	54
END FINAL 20 DAYS																		
$(NH_4)_2SO_4$	98.5	174	34.0	29.7	10.4	248	11.4	9.5	23.9	86.5	131	117	40.5	79.1	15.1	58.3	193	76
NH_4Cl	78.5	174	33.6	26.2	8.7	243	54.9	12.2	26.2	25.2	119	124	42.2	73.3	16.0	83.5	215	91
$Ca(NO_3)_2$	82.5	197	37.4	36.9	9.3	281	23.3	12.3	25.6	22.9	84	197	55.3	96.0	17.6	88.1	257	60
$NaNO_3$	78.5	189	40.5	40.0	15.1	285	23.6	14.3	27.3	23.5	89	196	61.7	112.8	17.8	64.7	257	61

* All solutions were made up with tap water containing in m. e. per liter: Ca 0.2, Mg 0.1, Na 2.2, Cl 0.7, and SO_4 0.2. For the initial solution salts were added to give in addition: Ca 5.0, Mg 2.0, K 3.0, NO_3 7.5, PO_4 1.5, and SO_4 2.0.

and Ca leaves—but this is not especially important here. The main point is that neither SO_4 versus Cl nor Ca versus Na had any very great effects on the accumulation of the constituents mentioned.

Turning, on the other hand, to comparisons between the ammonium leaves and the nitrate leaves, it is found, in round numbers, that the ammonium leaves had nearly 40 m.e. (per 100 gms. of dry tissue) less of total bases and nearly 40 m.e. more of inorganic acids than did the nitrate leaves. These marked differences give rise to a 75 m.e. greater disparity between the cations and inorganic anions in the nitrate leaves than in the ammonium leaves. The question now arises—was this greater disparity in the nitrate leaves made up by an extra accumulation of organic acids? The next to the last and last columns (''Total'' and ''Free Organic Acids'') of table V show that the disparity was compensated and with organic acids to spare.

The differences between the ammonium leaves and nitrate leaves in what is here designated as the ''free organic acids''—*i.e.* the organic acids found over and above those required to balance the excess of determined inorganic bases over the determined inorganic acids—are not very large; the four values are respectively 76 and 91 m.e. for the two ammonium salts and 60 and 61 m.e. for the two nitrate salts. Cotton (**5**), in contrast with some other plants (**17**), accumulates very little ammonium ion; otherwise an explanation might be afforded for the greater ''free organic acid'' in the plants on the ammonium salts.

Returning to one of the original motives for the experiment, there was the question—does the substitution of ammonium nitrogen for nitrate nitrogen constitute a direct and immediate cause for a profound change in the organic acid regimen of the plant? Those who think so can point to the loss of 50 m.e., or 20 per cent., in the total organic acid when the plants were supplied with ammonium salts. On the other hand, those, like ourselves, who favor the neutralizing or buffering interpretation of organic acids will maintain that with a lessened difference between cations over inorganic anions from any cause, in this instance the substitution of ammonium, less organic acid is needed to maintain the characteristic acidity of the species and less will be found. By this latter interpretation ammonium versus nitrate would be regarded only as one of perhaps a number of factors that can set in motion a chain of events that leads to a similar result. Occasional plants like the cantaloupe (**10**), on the other hand, add to the difficulty of fully accepting any explanation for the behavior of organic acids as being entirely general.

The cotton plant is shown by this experiment to be capable of increasing greatly its store of organic acids to bring about a balance in the sums of bases and acids and thereby maintain its characteristic excess of organic acid over and above the inorganic cation-anion difference.

RESPIRATION AND TRANSLOCATION

If, beyond their role as neutralizing agents, the organic acids have a nutritional significance, either as intermediates in the formation of other compounds or in respiratory activity, it might be expected that these acids would undergo substantial change during periods of prolonged darkness. Only a minor change in total acid is accredited (**13**) to excised tobacco leaves during prolonged darkness, but there was a notable shift from malic to citric. In excised rhubarb leaves (**14**) light was without effect; these leaves lost as much malic and other acids in light as in the dark. Whether grown in light or dark, plants from Narcissus bulbs (**24**) differed little in their organic acids after 28 days. On the other hand—whatever the physiological significance—marked diurnal variations have been found to occur in the organic acids of the succulent plants, the amount of organic acid being increased with reduced temperatures and with increased CO_2 of the atmosphere (**3**).

Little is known about the translocation of the organic acids largely because of difficulty of concluding in most instances whether the acids are actually moved about in the plant or disappear in one tissue while being coincidentally synthesized elsewhere. The results of PLATNITSKY (**11**) are direct in showing an accumulation of citric acid in excised tobacco leaves when the petioles were placed in a solution of potassium malate. In this instance the translocation to the leaves was presumably via dead tracheal elements rather than via the phloem. One of the major interests in the subject must center in the question of phloem transport and whether these acids are subject to polar movement analogous to the transport of the sugars.

VICKERY, *et al.* (**24**) suggest that tracer isotopes would be helpful in this problem but it is not clear how the technique could be applied to differentiate between tracheal and phloem movements. The experiment undertaken by the writers, table VI, endeavors to throw light on this subject by comparing during a period of darkness the losses of organic acids from detached cotton leaves with the simultaneous losses from attached leaves. The losses from the detached leaves are assigned to respiration and it is presumed that the additional loss from attached leaves can be accredited to translocation. The behavior of the organic acids as regards respiration and translocation is compared in these respects with that of carbohydrates.

The analyses, table VI, are of three sets of leaves, (1) fresh leaves gathered at 2 P.M., (2) excised leaves from the same plants after standing in the dark for five days with their petioles in distilled water, and (3) leaves which remained attached to the plants during the same five-day period in the same room. The temperature varied between 28° and 30° C.

Twelve Stoneville 2B plants were grown, one to a pot, in a manured soil in four-gallon jars until May 8 in the greenhouse. By this time the plants had started to flower and they were taken outdoors until used on May 20. All floral buds were removed during the May 8–20 period to increase the store of carbohydrates and thereby equip the plants and their leaves to withstand better the prolonged period of darkness.

As an aid to the collection of samples that would be uniform as regards the number of leaves of various size, age, and position all but the youngest

TABLE VI

EFFECT OF RESPIRATION (DETACHED LEAVES) AND RESPIRATION PLUS TRANSLOCATION (ATTACHED LEAVES) ON CARBOHYDRATES AND ORGANIC ACIDS DURING FIVE DAYS IN DARK. ALL VALUES RELATIVE TO DRY WEIGHT

		CARBOHYDRATES (GRAMS)						ORGANIC ACIDS (MILLIEQUIVALENTS)				
	DRY WEIGHT	HEXOSES	SUCROSE	STARCH	SUM	HEMICELLULOSE	CELLULOSE	CITRIC	MALIC	OXALIC	UNIDENTIFIED	TOTAL
INITIAL:												
Per 100 grams		1.04	1.04	25.62	27.70	9.40	4.74	82.0	57.6	10.2	37.3	187.1
Per 10 leaves	12.45	.13	.13	3.19	3.45	1.17	.59	10.2	7.17	1.27	4.65	23.29
RELATIVE, PER LEAF:												
Initial	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5 days detached	.79	2.23	1.30	.39	.47	.77	1.03	.59	.66	1.02	1.89	.89
5 days attached	.71	.31	.31	.07	.08	.83	1.03	.69	.57	.97	1.27	.78

leaves were systematically marked, starting with the lowest healthy main-stalk leaf, with a white, yellow, or green tag taken in rotation from one plant to the next. The leaves of each tag color on six of the plants provided A samples and those on the other six plants the replicate B samples. The A and B plants during the 12-day period were in parallel rows between a pair of greenhouses. As one measure of sample uniformity, the dry weights of the A and B leaves were respectively 1.20 and 1.29 grams per leaf in the original collection, .96 and 1.00 grams per leaf after having been detached with their petioles in distilled water for five days in the dark, and .87 and .90 grams after having remained attached for five days in the dark.

Three inches of petiole were left on each leaf as a part of all samples, the petioles of the leaves to be detached for five days in the dark having been cut back to this length under water. The distilled water in the beakers was changed daily and the pH compared with that of distilled water which had stood alongside. There was no evidence of a change in pH to indicate movement of organic acid from the petioles to the water.

As shown by table VI, the detached leaves lost 21 per cent. of their original dry matter during five days in the dark. The attached leaves lost this amount and an additional 8 per cent. was translocated, *i.e.,* the three relative weights were 1.00, .79, and .71, respectively. Per ten attached leaves there was a loss of 3.62 grams in dry weight.

The sum of the sugars and starch was reduced to 47 per cent. of the original weight by respiration alone (the detached leaves) and to 8 per cent. of the original weight by translocation plus respiration (the attached leaves). Of the 3.62 grams total loss in dry weight of attached leaves, 3.17 grams are thus accounted for by the loss of sugars and starch.

There was a substantial loss in hemicellulose by respiration during the five-day period but there is no indication of any translocation within this group of constituents.

The changes in cellulose, if any, were well within the limits of error.

The total organic acids contrast sharply with starch and sugar in losses by both respiration and translocation. Whereas 92 per cent. of the sum of sugars and starch of the attached leaves disappeared during five days in the dark there was during the same period a disappearance of only 22 per cent. of the total organic acid from the same leaves. From the detached leaves only half as great a loss in organic acids occurred indicating that respiration and translocation were each responsible for the disappearance of 11 per cent. of the initial organic acid. Proportionately these losses were about the same in the A and B samples, as follows:

Group	Original	Detached	Attached
A	1.00	.91	.83
B	1.00	.86	.75

Irrespective of the various other physiological activities that may have accompanied respiration and translocation during the dark periods the data suffice to show that relative to labile carbohydrates, the organic acids are nearly inert in both respiration and translocation.. The changes in sums of starch and sugar as regards both respiration and translocation were in the order of five times as great as those of the organic acids.

It may be noted in table VI that oxalic was the only acid whose amount per leaf remained unchanged during the five-day period. This suggests that this acid in cotton, as in so many other plants, is largely in the form of inactive calcium oxalate. SCOTT *et al.* (**10**) have made the noteworthy observation that in orange leaves, prior to leaf drop, accumulated starch is replaced by accumulations of ensheathed calcium oxalate crystals.

Another opportunity for evaluating respiratory activity in cotton leaves is provided by table IV. These Acala and Stoneville leaves were gathered in mid-afternoon in wet and dry plots in the field in California and aliquots placed between wet and dry towels for 24 hours. Although in 24 hours these leaves lost an average of 21 per cent. of the sum of sugars and starch, there was no change whatever in the total organic acids. It would seem accordingly that the organic acids in cotton leaves may not be respired until the labile carbohydrates have largely disappeared or until some derangement of the vital activities of the protoplast has occurred.

CARBOHYDRATE-NITROGEN-ORGANIC ACID RELATIONS

Both carbohydrate and nitrogen data are reported in conjunction with the organic acids in tables II, III, and IV, and carbohydrate data in table VI. This has been done with the object of providing an opportunity to search for variations in any of these constituents which might be related to changes in the organic acids. The fact that we have failed to find consistent or important correlations between these data may serve to emphasize other relations, such as differences between inorganic acids and bases or shifted pH levels, as being more directly responsible for variations in total organic acid.

It is usually assumed that the carbohydrates are the precursors of the organic acids and some investigators believe that the organic acids are in turn the precursors of the amino acids. Variations in the concentration of one of these might or might not be reflected by variation in another. The formation of organic acids from carbohydrates, or of amino acids from organic acids, can presumably proceed only through the agency of enzymes. The conditions that increase or decrease enzymatic activity or determine the direction of an equilibrium reaction are often poorly defined and now customarily thought of as being related to the formation and activity of the hormones. The fact that transformations in plant constituents can occur one at the expense of another, and that several such changes can occur simultaneously, often from currently synthesized products, adds to the complexity of differentiating cause and effect. Having found that the organic acids tend to be relatively inert as regards respiration and translocation, the changes from or to other classes of compounds are probably too minor or too gradual for clear recognition of the sequence of events.

Conclusions and summary

Organic acids, on the basis of dry weight, have been found to be present in the cotton plant in successively lower concentration in the leaves, petioles, flowers, stems, roots, young bolls, seed kernels, and lint. Leaves of cotton frequently contain 20 per cent. of organic acid on the basis of dry weight.

Malic acid is usually present in greatest amount and tends to constitute 30 to 40 per cent. of the total organic acid.

Without materially changing the concentration of total organic acid, drought was found to increase malic and decrease citric. This transformation of critic to malic is found to be a reversible reaction and apparently proceeds through some intermediate acid or acids which are included in the unidentified group.

Leaves of cotton plants cultured on nitrate nitrogen had a greater sum of Ca, Mg, K, and Na, but a lesser sum of NO_3, Cl, SO_4, and PO_4 than did plants on ammonium nitrogen. This great increase in bases relative to inorganic anions was found to be compensated by an extra accumulation of organic acid. The result provides for the conclusion that a major role of the organic acids in cotton is as equalizing agents for differences in the sums of the inorganic acids and the bases. An excess of organic acid was found over and above that required to compensate for the insufficient inorganic acids. This excess undoubtedly contributed to the maintenance of the 5.00–5.60 pH characteristic of cotton leaves.

Other than for prolonged respiration, a change in the inorganic cation-anion balance was the only condition that was found in cotton to bring about a material change in the concentration of total organic acid.

Comparisons of the disappearance of the carbohydrates and organic acids from excised and attached leaves during five days in the dark point to the conclusion that the respiration and translocation of organic acids proceeds only about one-fifth as rapidly as does that of sugars. The carbohydrates were largely exhausted during the five days. It may be that respiration of organic acids does not occur until the labile carbohydrates are nearly exhausted. As evidence of this it was found in another experiment that there was no loss of organic acid in an initial 24-hour period during which a large store of labile carbohydrates was maintained.

Consistent correlations were not found between variations in the concentrations of the organic acids and the sugars, starch, or soluble and insoluble nitrogen.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analyses. 4th ed. 1935.

2. BENNET-CLARK, T. A. Organic acids of plants. Ann. Rev. Biochem. **6**: 579–594. 1937.
3. BONNER, W., and BONNER, J. The role of carbon dioxide in acid formation by succulent plants. Amer. Jour. Bot. **35**: 113–117. 1948.
4. CLARK, H. E. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**: 5–24. 1936.
5. COLLINS, E. R., and RIGLER, N. E. Effect of fertilizers on some nitrogenous and other constituents of the cotton plant as separated by electrodialysis at different stages of growth. Soil Sci. **44**: 217–229. 1937.
6. EATON, F. M., and RIGLER, N. E. Influence of carbohydrate levels and root-surface microfloras on Phymatotrichum root rot in cotton and maize plants. Jour. Agr. Res. **72**: 137–161. 1946.
7. ERGLE, D. R., and BLANK, L. M. A chemical study of the mycelium and sclerotia of *Phymatotrichum omnivorum*. Phytopath. **37**: 153–161. 1946.
8. KREBS, H. A., and EGGLESTON, L. B. Micro-determination of isocitric and *cis*-aconitic acids in biological materials. Biochem. Jour. **38**: 426–437. 1944.
9. MCCALL, E. R., and GUTHRIE, J. D. The organic acid content of raw cotton fiber. Isolation of l-malic and citric acid from cotton fiber. Jour. Amer. Chem. Soc. **67**: 2220–2221. 1945.
10. PIERCE, E. C., and APPLEMAN, C. O. Role of ether-soluble organic acids in the cation-anion balance in plants. Plant Physiol. **18**: 224–238. 1943.
11. PLATNITSKY, M. P. Effect of some organic acid salts on storage of citric acid by leaves of tobacco plants. Compt. Rend. (Dok). Acad. des Sci. U. R. S. S. **29**: 55–58. 1940.
12. PUCHER, G. W., LEAVENWORTH, C. S., GINTER, W. D., and VICKERY, H. B. Studies in the metabolism of crassulacean plants. The effect upon composition of *Bryophyllum calycium* of the form in which nitrogen is supplied. Plant Physiol. **22**: 205–227. 1947.
13. ————, WAKEMAN, A. J., and VICKERY, H. B. The metabolism of the organic acids of the tobacco leaf during culture. Jour. Biol. Chem. **119**: 523–534. 1937.
14. ————, ————, and ————. The organic acids of rhubarb (*Rheum hybridum*). III. The behavior of the organic acids during culture of excised leaves. Jour. Biol. Chem. **126**: 43–54. 1938.
15. ————, VICKERY, H. B., and WAKEMAN, A. J. Determination of the acids of plant tissue. II. Total organic acids of tobacco leaf. Ind. Eng. Chem., Anal. Ed. **6**: 140–143. 1934.
16. ————, ————, and ————. Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acids. Ind. Eng. Chem., Anal. Ed. **6**: 288–291. 1934.

17. ————, ————, and ————. Relationship of the organic acids of tobacco to the inorganic basic constituents. Plant Physiol. **13**: 621–630. 1938.

18. Ruhland, W., and Wolf, J. Metabolism of carbohydrates and organic acids in plants. Ann. Rev. Biochem. **5**: 485–512. 1936.

19. Scott, F. M., Schroeder, M. R., and Turrell, F. M. Development, cell shape, suberization of internal surface and abscission in the leaf of the Valencia orange, *Citrus sinensis*. Bot. Gaz. **109**: 381–411. 1948.

20. Ulrich, A. Metabolism of non-volatile organic acids in excised barley roots as related to cation balance during salt accumulation. Amer. Jour. Bot. **28**: 526–537. 1941.

21. Van Slyke, D. D., and Palmer, W. W. Studies of acidosis. XVI. The titration of organic acids in urine. Jour. Biol. Chem. **41**: 567–585. 1920.

22. Vickery, H. B., and Pucher, G. W. Organic acids of plants. Ann. Rev. Biochem. **9**: 529–544. 1940.

23. ————, ————, Wakeman, A. J., and Leavenworth, C. S. Chemical investigations of the tobacco plant. VIII. The effect upon the composition of the tobacco plant of the form in which nitrogen is supplied. Connecticut Agr. Exp. Sta. Bull. 442. 1940.

24. ————, ————, ————, and ————. Chemical investigations of the metabolism of plants. I. The nitrogen nutrition of *Narcissus Poeticus*. Connecticut Agr. Exp. Sta. Bull. 496. 1946.

25. ————, ————, ————, and ————. Chemical investigation of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and in darkness. Connecticut Agr. Exp. Sta. Bull. 399. 1937.

26. Wadleigh, C. H., and Shive, J. W. Organic acid content of corn plants as influenced by pH of substrate and form of nitrogen supplied. Amer. Jour. Bot. **26**: 244–248. 1939.