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THE PENNSYLVANIA STATE COLLEGE

The Graduate School

Department of Agricultural and Biological Chemistry

A STUDY OF THE NON-VOLATILE ORGANIC ACIDS
OF PENNSYLVANIA CIGAR-LEAF TOBACCO

A Thesis

by

Seymour Stanton Block

Submitted in partial fulfillment
of the requirements
for the degree of
MASTER OF SCIENCE

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Approved:

July 30 1941, W. B. Halsey
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A STUDY OF THE NON-VOLATILE ORGANIC ACIDS OF PENNSYLVANIA CIGAR-LEAF TOBACCO

Introduction

The non-volatile organic acids, malic, citric, and oxalic, make up a considerable part of the total solids of the leaf of Pennsylvania Cigar-leaf tobacco and, as has recently been shown (6), play an important role in the metabolism of the tobacco plant. It was thought, therefore, that a study of these acid constituents might help to indicate some of the factors which influence the quality and burn of the tobacco. Such a study has now been carried out on the mature and cured Pennsylvania Cigar-leaf tobacco grown under different conditions of fertilizer treatment at the experimental plots at Roseville, Pennsylvania. In this study an attempt was made to correlate the organic acid composition with the burn and quality as well as to the fertilizer treatment.

Review of the Literature

The early work on the chemical factors influencing the quality and burn of the tobacco leaf was concerned mainly with the mineral constituents and the total organic acidity. It has been only within the last decade that accurate, reliable methods for the determination of the more prominent individual acids have be-

come available. A review of the literature relating to the organic acids of the tobacco plant is given in an article by Vickery and Pucher (34). The non-volatile organic acids--malic, citric, and oxalic--constitute 80 percent of the total ether-soluble acidity (13) and nearly 20 percent of the total organic solids of the tobacco leaf tissue, a value comparable in quantity to the total protein of the tissue (23). Traces of succinic and fumaric acids, as well as some unidentified phenolic acids, have also been identified in extracts of cigar-leaf tobacco (35).

One of the first investigators to attempt to determine the chemical factors which control the burn of tobacco was Schlosing (29). He treated the leaves with solutions of various salts and found that the potassium salts of several organic acids were decidedly beneficial.

Behrens (5), some years later, claimed that a high content of potassium salts of citric, oxalic, and malic acids largely determined burning quality.

Garner (9) found that extracting tobacco with ether, petroleum ether, and alcohol did not remove the burning qualities of the tobacco but that extracting with water was deleterious to the burning qualities. In the water extract was found potassium salts of citric and malic acids while the water-extracted tobacco contained calcium ox-

alate and other calcium salts. It was also shown that extracts of poor-burning and good-burning leaves contained about the same amount of potassium, but that the inferior leaf had higher amounts of mineral acids while the superior leaf was higher in organic acids.

Nasset (15) confirmed the above work by stating that a relatively large quantity of potassium in combination with organic acids is necessary to insure good burning quality in cigar-leaf tobacco. He also pointed out that the organic acid content of cigar-leaf tobacco may vary greatly from year to year and cannot, therefore, serve as an absolute index of burning quality.

Analyzing Kentucky tobacco grown in Italy, Nuccerini (16) showed that there were higher amounts of citric and oxalic acids in leaves having ready combustibility. He was unable to show, however, any correlation between the malic content of the leaf and the burn.

Coolhaas (7) stated that oxalic, citric, and malic acids all bore a direct relationship to the burning qualities of a number of varieties of tobacco. He later (8) restricted that statement to citric acid alone. He also noticed large variations in the duration of burn in different varieties of tobacco grown under identical conditions.

In his investigations of Russian tobacco, Pyatnitskii (25, 26) found that the citric acid content is in reverse

relation to the quality of the tobacco, increasing in the inferior grades. The same relationship was observed for malic and oxalic acids, although not to the same degree.

Shmuk (31, 32) confirmed the results of Pyatnitskii and also presented evidence to show that the citric acid content increased with an increase in water supply during growth, and with an increase in fertilization.

Nasset (15) studied the effect of fertilization on the organic acid content as well as the effect of the organic acidity on burning quality. He observed that plots treated with muriate of potash produced tobacco of low organic acid content and poor burning quality while those receiving nitrate or sulfate of potash produced tobacco of relatively high organic acidity and burning quality.

Kurchatov and co-workers (12) and Psarev (18), working with high citric acid Russian tobacco, running as high as 13.3%, found that the maximum accumulation of citric acid was obtained by the use of a potash fertilizer.

Another Russian worker, Vladimirov (39, 40), working with the same type of tobacco, found that an increase in the potassium content of the nutrient medium decreased the citric acid and total acidity of the tobacco leaves.

He also found that ammonium salts as a source of nitrogen decreased the total acidity and the content of citric acid, while the use of nitrate as a source of nitrogen resulted in increased acidity and citric acid content.

Coolhaas (8), on the other hand, attributing good burning quality to the citric acid content of the tobacco, stated that ammonium sulfate treatment was satisfactory for good burning quality.

Olsen (17) showed that plants which are good producers of oxalic acid thrive on ammonium sulfate, but that less oxalic acid is found when a nitrate is used.

The effect of curing on the organic acid content of tobacco leaves was investigated by Vickery and Pucher (36), working with Connecticut shade-grown tobacco. They found that the total acidity remained unchanged during curing, while the citric acid content increased almost six times that of the fresh leaf; the oxalic acid content increased very slightly during the curing process, and the quantity of malic acid diminished about 16 percent of the original amount. Three possibilities are given for the increase in citric acid: it may have arisen as a by-product of the deamination of amino acids; it may have come from the decomposition of carbohydrates; or it may have been converted from unknown acid precursors. Later work (23), in which leaves were cultured in water solution in the dark, showed considerable decrease in the malic acid content as

the citric acid content increased. The formation of citric from malic acid by the mechanism of Knoop and Martius (10) was suggested as a reasonable hypothesis.

Pyatnitskii (27, 28) conducted an experiment whereby he submerged the petioles of Russian tobacco leaves in solutions of the calcium and potassium salts of various organic acids and allowed them to "cure" in the dark for two days. The leaves kept in potassium malate showed five-and-a-half times as much citric acid after "curing" as the control in distilled water. From this, Pyatnitskii concluded that the increase in citric acid in tobacco leaves during curing is at the expense of the malic acid.

Pyatnitskii's experiment confirmed the earlier work of Mikhlin and Bakh (14) who impregnated tobacco leaves with malic and other organic acids by vacuum infiltration. Malic acid was the only organic acid used which gave appreciable increases in the content of citric acid in the leaf, being two to three times that infiltrated with water alone.

Preparation of Sample

The tobacco used for analysis was grown at the Tobacco Experiment Station Farm at Roseville, Pennsylvania, and received various controlled fertilization treatments. The middle leaves of the plants were sampled, weighed, judged by experts for quality and burn, packed in dry ice,

and shipped to State College, where they were placed in an oven at 70° to 80° C. for 24 hours. The leaves were then re-weighed, the lamina or web of the leaves separated from the midribs and ground in a Wiley mill using a 40 mesh screen. The samples were then stored in sealed jars prior to chemical analysis.

Analytical Procedures

All samples were analyzed in duplicate and expressed on a moisture-free basis.

Preparation of the Organic Acid Extract:

The organic acids of the tobacco leaf were extracted according to the technique of Fucher, Vickery, and Wakeman (21). Two grams of the dried and powdered tissue was acidified to pH1.0 with sulfuric acid, mixed with 3.5 grams of asbestos, and extracted with anhydrous ethyl ether in a Soxhlet extraction apparatus for a period of 24 hours. At the end of the extraction period, 25 ml. of distilled water was added to the ether-extract, the system was thoroughly agitated to transfer the acids to the aqueous phase, and the ether was carefully distilled off. The aqueous solution was cooled in the refrigerator in order to solidify the resinous material which was then removed by filtration through a Gooch crucible. The organic acid extract was made to volume at 100 ml.

Determination of Oxalic Acid:

Oxalic acid was determined by the method of Pucher, Vickery, and Wakeman, as follows (21): a 25 ml. aliquot of the organic acid extract was acidified to congo red with 0.5 N hydrochloric acid. The precipitate the formed was allowed to settle, filtered off on asbestos in a Gooch crucible, and washed with water. A drop of methyl red was added to the clear filtrate and ammonium hydroxide was added to a faint alkaline reaction. Two to three ml. of glacial acetic acid were added, followed by 5 ml. of 10 per cent calcium chloride solution. After standing at least two hours, the calcium oxalate was filtered on asbestos in a Gooch crucible and was washed with a little very dilute ammonium hydroxide. Crucible and contents were then transferred to a 100 ml. beaker. Five ml. of 50 per cent sulfuric acid and 20 ml. of water were added; and the solution was heated to boiling and titrated while very hot with 0.02 N potassium permanganate solution.

Determination of Citric Acid:

Citric and malic acids were determined by the simultaneous method of Pucher, Vickery, and Wakeman (22). In this method, citric and malic acids are oxidized simultaneously, and pentabromoacetone which is formed from the citric acid is separated from the malic acid oxidation product by extraction with petroleum ether,

after which the acids are determined separately. The procedure that was used is as follows: a 5 ml. aliquot of the organic acid solution was diluted to 20 ml. with water and 3 ml. of 50 per cent sulfuric acid were added. The mixture was boiled gently for eight to ten minutes to expel traces of ether, cooled, and 1 ml. of saturated bromine water was added. After five minutes the solution was filtered with gentle suction through a Gooch crucible into a suction flask marked at 35 ml., and 2 ml. of 1 N potassium bromide were added. The temperature of the solution was then adjusted at 20° to 22° C. and 5 ml. of potassium permanganate, previously brought to the same temperature, were added. The mixture was allowed to stand in a water bath at 20° to 22° for 10 minutes with occasional stirring, and was then chilled to 5° to 10°C. and decolorized with three per cent hydrogen peroxide added dropwise with vigorous stirring.

The oxidation mixture was transferred to a 125 ml. pear-shaped separatory flask, and the suction flask was rinsed into the separatory flask with 25 ml. of petroleum ether used in small portions. The flask was shaken vigorously and the aqueous layer was drawn off. The petroleum ether was transferred to a second flask. The aqueous solution was shaken again with 20 ml. of petroleum ether and was then put aside for the determination of malic acid. The two petroleum ether extracts were combined and washed four times with 3 ml. of water and the

washings were added to the aqueous extract for the malic acid determination. A 3 ml. quantity of four percent sodium sulfide solution was added to the separatory flask, and the mixture was shaken vigorously. The aqueous layer, which turned reddish, was drawn off into a 50 ml. Erlenmeyer flask. The petroleum ether was then treated a second time with 3 ml. of sodium sulfide, and was washed three times with 3 to 4 ml. of water, the aqueous solutions all being combined. To this solution was added 2 ml. of 2 N sulfuric acid and a few quartz pebbles; and the solution was boiled gently for three minutes to expel hydrogen sulfide. It was then cooled to room temperature and sufficient 1.5 N potassium permanganate added to produce a red color permanent for 20 seconds. This usually took 0.8 to 1.0 ml. of permanganate. The color was discharged by the addition of a small excess of a specially prepared halogen-free hydrogen peroxide (22, 24), followed by the addition of 2 ml. of concentrated nitric acid, 3 ml. of standard silver nitrate, 1 ml of ferric alum indicator solution, and 3 to 4 ml. of ethyl ether. The flask was shaken vigorously to coagulate the silver bromide, and the solution was titrated with ammonium thiocyanate from a micro-burette to a faint salmon-pink color. Inasmuch as the conversion of citric acid to pentabromoacetone is not strictly quantitative, it was necessary to multiply

the quality of citric acid determined by a factor of 1.12.

Determination of Malic Acid:

Malic acid was determined by the method of Pucher, Vickery, and Wakeman (22). The aqueous solution, together with the wash fluid from the petroleum ether (obtained as described under the determination of citric acid), was diluted to a 100 ml. A 25 ml. aliquot was transferred to a 300 ml. Kjeldahl flask to which 25 ml. of water and a few quartz pebbles were added. To insure the destruction of hydrogen peroxide, about 0.5 ml. of potassium permanganate was added and the solution decolorized by the addition of 2 ml. of sodium sulfite solution. This step has been eliminated in the modifications of the methods recently published (24) by the authors. The flask was fitted with a bent distillation tube which extended into a 250 ml. wide-mouth Erlenmeyer flask charged with 10 ml. of a freshly filtered 2, 4-dinitrophenylhydrazine solution and 20 ml. of water. The end of the distillation tube was dipped beneath the surface of the reagent in the receiving flask, and the flame of a microburner was applied to the Kjeldahl flask. The receiving flask was immersed in a cold water bath and the rate of distillation was adjusted so that the receiving solution did not boil vigor-

ously during the distillation. This last procedure is contrary to the directions as given in the original paper (22), but was advised by the authors in later modifications (24, 19). The distillation was continued until the volume of solution remaining in the Kjeldahl flask was reduced to somewhat less than 10 ml., usually taking from 12 to 15 minutes. The receiving flask was then removed and the end of the distillation tube rinsed into it.

The orange precipitate was transferred as completely as possible with water to a small (No. 2) Gooch crucible furnished with an asbestos mat, and was dried for a short time at 100° to 110° C. Meanwhile the receiver was thoroughly drained, and the last traces of precipitate were taken up in hot pyridine used in several successive portions but not more than 3 to 4 ml. in all. The pyridine washings were transferred to a 25 ml. volumetric flask. The crucible was then fitted into a rubber stopper, carried on a cylindrical funnel--which is attached to a test tube equipped with a side arm---and marked at 20 ml. Boiling pyridine was added in small portions to the crucible; and the contents were gently triturated with a glass rod, after which suction was applied and the pyridine was drawn through each time. Three or four washings usually were sufficient to dissolve and transfer all the precipitate to the 25 ml. flask used for the rinsings of the receiver.

After being cooled to room temperature, the solution was made to volume with pyridine and mixed. It was then allowed to settle or was filtered, if necessary, through soft paper to remove shreds of asbestos.

Either a 2 ml. or a 5 ml. aliquot of the pyridine solution was transferred to a 100 ml. flask and 50 ml. of water were added, followed by 5 ml. of 5 N. sodium hydroxide. The solution was then diluted to the mark, and the blue color was read in a colorimeter by comparison with a standard malic acid solution. The standard malic acid solution containing 10 milligrams per 5 ml. was treated in exactly the same manner as described for the aliquot from the organic acid fraction.

Determination of Potassium:

Potassium was determined by the method outlined by Thomas (33): a 2.5 gram sample was weighed into a porcelain crucible and placed in a muffle furnace, regulated so as not to exceed a temperature of 480° C., for 24 hours. The ash was taken up with 5 ml. of dilute nitric acid and 5 ml. of dilute hydrochloric acid, filtered, and washed with hot one-percent nitric acid. The ashless filter paper and residue were replaced in the crucible and burned in the muffle furnace at a full red heat. This ash was then taken up with an equal mixture of concentrated hydrochloric and nitric acids, boiled, diluted, filtered,

and washed with hot one-percent nitric acid. The filtrate and washings were made up to a volume of 250 ml. To a 50 ml. aliquot in a porcelain evaporating dish was added 1 ml. of 50 percent sulfuric acid. The solution was evaporated to dryness on the steam bath, and the dish was then heated over a Meeker burner until the residue was white. The residue was taken up with 1 ml. of concentrated hydrochloric acid and hot water added with stirring until solution was complete. Then 5 ml. of two percent platonic chloride were added and the solution evaporated on the steam bath just to dryness. The residue was taken up with 25 ml. of acidulated alcohol (2.25 N hydrochloric acid in 80 percent alcohol) and the mixture was thoroughly stirred. After standing for a half hour, the contents of the dish were filtered through a weighed Gooch crucible, washed with 80 percent alcohol, dried at 100° C. for a half hour, and weighed.

Determination of Total Nitrogen:

Total nitrogen was determined by the official method of the Association of Official Agricultural Chemists. The Gunning modification of the Kjeldahl method to include nitrate nitrogen (1) was followed up to the point where the material was ready for digestion. After this the Kjeldahl Gunning-Arnold method (2) was followed, with the exception that copper sulfate and a selenized granule were

used instead of mercuric oxide or mercury as catalysts.

Determination of Protein Nitrogen:

Protein nitrogen was determined by the method employed by a Research Department of one of the large tobacco manufacturers, as follows: one gram of tobacco was placed in a 400 ml. beaker and 100 ml. of 0.5 per cent acetic acid was added. The contents were boiled for five minutes on a hot plate and allowed to cool. The solution was filtered, the filtrate discarded, and the filter washed with hot acetic acid water. The entire mass of tobacco, including the filter paper, was transferred to an 800 ml. Kjeldahl flask and the Kjeldahl Gunning-Arnold procedure, as mentioned above, was followed.

Determination of Non-Protein Nitrogen:

The non-protein nitrogen content of the leaf tissue was calculated by subtracting the percentage of protein nitrogen from the total nitrogen of the leaf.

Determination of Calcium:

Calcium was determined by the official method of the Association of Official Agricultural Chemists (3).

Determination of Moisture:

Moisture was determined by drying the tobacco in vacuo over concentrated sulfuric acid to constant weight, according to the methods of the Association of Official Agricultural Chemists (4).

Experimental Data

TABLE I
The Citric, Malic, and Oxalic Acid
Content of Pennsylvania Cigar-Leaf Tobacco.
1938 and 1939 Crops¹

		<u>1938</u> Mature Leaf (40 Samples) %	<u>1938</u> Cured Leaf (27 Samples) %	<u>1939</u> Mature Leaf (18 Samples) %
Oxalic Acid	range	0.3-1.9	1.2-2.7	2.1-3.0
	average	1.2	2.0	2.5
Citric Acid	range	0.5-3.3	0.8-4.7	2.8-5.2
	average	1.1	3.3	3.7
Malic Acid	range	5.9-14.1	1.9-10.4	7.8-11.8
	average	10.3	6.1	9.7

¹ Samples represent various fertilizer treatments.

TABLE II

Changes in the Concentration of Citric, Malic and
Oxalic Acids of Pennsylvania Cigar-Leaf Tobacco
as a Result of Curing.

1938 Crop¹

Sample	% Oxalic Acid		% Citric Acid		% Malic Acid	
	Mature %	Cured %	Mature %	Cured %	Mature %	Cured %
17	0.91	1.50	0.65	4.37	11.61	5.92
18	1.34	1.62	0.69	3.78	11.70	5.40
19	1.02	2.02	0.52	3.59	12.20	4.54
20	1.42	2.51	1.02	3.77	9.13	6.74
22	1.41	2.55	1.04	3.36	13.02	9.06
23	1.28	2.25	1.22	3.52	13.31	7.07
24	1.53	2.32	1.13	3.60	13.42	7.19
59	1.82	2.52	1.40	2.71	11.62	5.50
60	1.57	1.97	0.73	2.54	8.66	7.99
67	1.72	1.83	0.61	4.01	10.94	9.64
68	1.38	1.65	0.52	4.15	10.01	10.47
69	1.66	1.77	0.51	3.22	10.11	8.68
72	1.71	2.11	0.84	2.69	9.64	6.71
Range	0.91- 1.82	1.50- 2.55	0.51- 1.40	2.69- 4.37	8.66- 13.42	4.54- 10.47
Average	1.44	2.05	0.83	3.49	11.18	7.30

¹Samples from Potash Series of Four Year
Rotation

TABLE III
The Citric, Malic, and Oxalic Acid Content
of Cured Pennsylvania Cigar-Leaf Tobacco
1938 Crop¹

Sample	% Oxalic Acid	% Citric Acid	% Malic Acid
<u>Poor Quality Tobacco</u>			
1	2.52	1.44	3.85
2	2.70	1.71	4.70
3	1.31	0.80	1.92
4	2.06	2.01	6.22
5	2.11	2.73	6.00
6	1.43	2.20	4.34
18	1.62	3.78	5.40
60	1.97	2.54	7.99
70	1.50	4.20	8.82
72	2.11	2.69	6.71
Range	1.31-2.70	0.80-4.20	1.92-8.82
Average	1.93	2.41	5.60
<u>Good Quality Tobacco</u>			
7	1.22	4.60	4.15
8	2.20	4.61	4.24
9	2.32	4.72	3.90
10	1.88	4.73	4.20
11	2.10	4.05	4.53
12	2.31	3.51	5.01
19	2.02	3.59	4.54
21	2.22	3.32	7.26
22	2.55	3.36	9.06
23	2.25	3.52	7.07
Range	1.22-2.44	3.32-4.73	3.90-9.14
Average	2.11	4.00	5.40

¹Samples 1-12 from Lancaster County Cigar Manufacturer, others from Four Year Rotation.

TABLE IV

Fertilizer Treatments of the Four Year

Rotation Experimental Plots

Potash Series

Plot	Formula	$(\text{NH}_4)_2\text{SO}_4$	Cotten- seed meal	NaNO_3	Soy Bean meal	urea	Super Phos- phate	K_2CO_3	KNO_3	K_2SO_4
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
17	check	30	100	40	100	14 $\frac{1}{2}$	444			0
59	check	30	100	40	100	14 $\frac{1}{2}$	444			0
18	3-8-4	30	100	40	100	14 $\frac{1}{2}$	444			83
60	3-8-4	30	100	40	100	14 $\frac{1}{2}$	444			83
19	3-8-8	30	100	40	100	14 $\frac{1}{2}$	444			166
72	3-8-8	30	100	40	100	14 $\frac{1}{2}$	444			166
20	3-8-16	30	100	40	100	14 $\frac{1}{2}$	444			333
71	3-8-16	30	100	40	100	14 $\frac{1}{2}$	444			333
70	3-8-20	30	100	40	100	14 $\frac{1}{2}$	444			420
22	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444			250
69	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444			250
23	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444	30	231	0
68	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444	30	231	0
24	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444	197		0
67	3-8-12	30	100	40	100	14 $\frac{1}{2}$	444	197		0

Applications at rate of 1000 lbs with
10 tons of manure per acre

TABLE V

The Relation of Fertilizer Treatment to the Burn,
Quality and Concentration of Certain Constituents
of Pennsylvania Cigar-Leaf Tobacco.

Potash Series
Four Year Rotation
Mature Leaves. 1938 Crop.

Sample	Oxalic Acid %	Citric Acid %	Malic Acid %	Quality	Burn	K %	Total N %	Protein N %	NPN %
17	0.91	0.65	11.61	F	F	4.20	3.08	1.71	1.37
59	1.82	1.40	11.62	P	P	2.02	2.44	1.30	1.14
18	1.34	0.69	11.70	F	F	4.77	3.17	1.76	1.41
60	1.57	0.73	8.66	P	P	3.04	2.16	1.28	0.88
19	1.02	0.52	12.20	G	G	4.72	2.88	1.70	1.18
72	1.71	0.84	9.64	P	F	3.22	2.12	1.38	0.74
20	1.42	1.02	9.13	F	G	5.12	2.87	1.59	1.28
71	1.40	1.11	9.12	F	F	3.67	2.21	1.35	0.86
70	1.00	0.72	8.16	P	F	4.54	2.43	1.44	0.99
22	1.41	1.04	13.02	G	G	4.69	2.44	1.03	1.41
69	1.66	0.51	10.11	F	F	4.46	2.39	1.43	0.96
23	1.28	1.22	13.31	G	F	4.22	2.77	1.13	1.54
68	1.38	0.52	10.01	F	F	4.79	2.24	1.37	0.87
24	1.53	1.13	13.42	F	F	3.98	2.52	1.17	1.34
67	1.72	0.61	10.94	F	F	4.13	2.87	1.59	1.28
<u>Average</u>	1.40	0.85	10.84			4.10	2.57	1.42	1.15
<u>Av. of Good</u>	1.24	0.93	12.84			4.54	2.70	1.29	1.38
<u>Av. of Fair</u>	1.42	0.76	10.75			4.39	2.67	1.50	1.17
<u>Av. of Poor</u>	1.53	0.93	9.52			3.20	2.29	1.35	0.94

TABLE VI

Fertilizer Treatments of the Four Year
Rotation Experimental Plots.

Nitrogen Series

Plot	Formula	Manure tons/acre	Carrier of N	(NH ₄) ₂ SO ₄ lbs.	Cotten seed meal lbs.	NaNO ₃ lbs.	Soy Bean meal lbs.	Urea lbs.	Super Phos- phate lbs.	KNO ₃ lbs.	K ₂ SO ₄ lbs.
1	3-8-12	10	Mixed	30	100	40	100	14½	444		250
48	3-8-12	10	Mixed	30	100	40	100	14½	444		250
3	3-8-12	10	Cottonseed Meal		500				444		250
44	3-8-12	10	Cottenseed Meal		500				444		250
10	6-8-12	None	Cottenseed Meal		1000				444		250
52	6-8-12	None	Cottenseed Meal						444		250
4	3-8-12	10	Urea					71	444		250
11	6-8-12	None	Urea					143	444		250
5	3-8-12	10	Soybean Meal				500		444		250
12	6-8-12	None	Soybean Meal				1000		444		250
7	3-8-12	10	KNO ₃						444	231	38
49	3-8-12	10	KNO ₃						444	231	38
14	6-8-12	None	KNO ₃			170			444	273	
56	6-8-12	None	KNO ₃			170			444	273	
8	3-8-12	10	(NH ₄) ₂ SO ₄	150					444		250
15	6-8-12	None	(NH ₄) ₂ SO ₄	300					444		250
57	6-8-12	None	(NH ₄) ₂ SO ₄	300					444		250
13	6-8-12	None	NaNO ₃			400			444		250

Applications at rate of 1000 lbs. per acre

TABLE VII

The Relation of Fertilizer Treatment to the Burn
Quality, and Concentration of Certain Constituents
of Pennsylvania Cigar-Leaf Tobacco.

Nitrogen Series
Four Year Rotation
Mature Leaves. 1938 Crop

Sample	Oxalic Acid %	Citric Acid %	Malic Acid %	Quality	Burn	K %	Total N %	Protein N %	NPN %
1	0.41	1.41	6.32	G	G	4.04	3.43	1.93	1.50
48	1.42	1.20	9.14	VP	P	2.75	2.55	1.33	1.32
3	0.82	1.29	11.35	F	G	5.27	3.15	1.82	1.33
44	1.03	1.52	14.12	F	F	3.36	2.63	1.01	1.02
10	1.00	2.71	12.90	P	P	3.39	2.61	1.28	1.33
52	1.04	1.28	10.12	F	F	3.79	3.00	1.80	1.20
4	0.30	0.91	10.43	F	F	4.88	3.30	1.88	1.42
11	1.30	1.28	7.65	P	F	3.47	2.91	1.25	1.66
5	0.43	1.06	5.92	G	G	4.78	2.96	1.85	1.13
12	1.12	0.94	9.64	P	P	2.83	2.32	1.34	0.98
7	0.92	0.55	7.41	F	F	5.92	3.17		
49	0.73	0.52	14.10	G	G	5.07	3.23	1.82	1.41
14	0.95	1.47	9.32	F	G	4.00	3.11	1.90	1.21
56	1.21	3.26	10.35	F	F	3.75	3.23	1.41	1.82
8	0.83	0.81	7.67	G	G	4.17	3.00	1.73	1.27
15	0.83	1.34	9.78	F	F	3.59	3.04	1.80	1.24
57	1.10	1.12	10.47	VP	P	2.91	2.23	1.16	1.07
13	1.90	1.33	10.41	G	G	3.44	3.30	1.89	1.41
<u>Average</u>	0.96	1.33	9.80			3.97	2.95	1.65	1.28
<u>Av. of good</u>	0.86	1.03	8.88			4.44	3.18	1.84	1.34
<u>Av. of fair</u>	0.89	1.43	10.36			4.32	3.08	1.80	1.24
<u>Av. of poor</u>	1.19	1.45	9.96			3.07	2.52	1.27	1.25

TABLE VIII

The Relation of Fertilizer Treatment to the Burn,
Quality, and Concentration of Certain Constituents
of Pennsylvania Cigar-Leaf Tobacco.

Nitrogen Series
Four Year Rotation
Mature Leaves. 1939 Crop.

Sample	Oxalic Acid %	Citric Acid %	Malic Acid %	Quality	Burn	K %	Total N %	Protein N %	NPN %
1	2.13	3.07	11.64	P	F	1.61	4.33	2.58	1.75
48	2.24	3.79	8.68	P	P	2.20	4.46	1.97	2.49
3	2.50	3.34	11.77	P	F	2.20	3.92	2.32	1.60
44	2.70	3.02	9.45	P	P	2.23	4.29	2.54	1.75
10	2.98	5.15	11.00	P	P	1.55	3.74	2.39	1.35
52	2.54	3.97	8.51	P	P	1.86	4.09	1.95	2.14
4	2.74	3.07	10.97	P	P	2.15	3.46	2.14	1.32
11	2.26	4.71	9.74	P	P	1.89	4.02	2.48	1.54
5	2.40	3.11	10.90	F	F	2.09	3.79	2.30	1.49
12	2.13	4.58	9.45	VP	VP	1.95	4.19	2.50	1.69
7	2.63	3.51	8.93	P	P	2.62	4.78	2.70	2.08
49	2.35	2.80	8.94	P	P	2.03	4.14	2.77	1.37
14	2.67	4.42	8.65	VP	VP	1.33	5.08	2.84	2.24
56	2.49	4.51	9.33	P	VP	1.37	5.12	2.80	2.32
8	2.35	3.79	7.90	VP	VP	2.10	4.60	2.66	1.94
15	2.55	3.86	7.80	VP	VP	1.86	4.57	2.72	1.85
57	2.23	3.15	10.55	VP	VP	1.34	4.60	2.83	1.77
13	2.05	3.51	9.46	VP	VP	1.47	4.90	2.91	1.99
<u>Average</u>	2.44	3.74	9.65			1.88	4.34	2.55	1.74
<u>Av. of Poor</u>	2.51	3.64	9.96			2.03	4.12	2.44	1.61
<u>Av. of Very Poor</u>	2.35	3.97	10.45			1.63	4.72	2.73	1.94

TABLE IX

The Citric, Malic, and Oxalic Acid Content
of Pennsylvania Cigar-Leaf Tobacco as Influenced
by Fertilizer Treatment and System of Rotation
Mature Leaves. 1938 Crop.

<u>Rotation</u>	<u>Sample</u>	<u>Formula</u>	<u>Oxalic</u> <u>Acid</u> <u>%</u>	<u>Citric</u> <u>Acid</u> <u>%</u>	<u>Malic</u> <u>Acid</u> <u>%</u>
3	23	3-8-8	1.02	0.80	12.16
4	19	3-8-8	1.02	0.52	12.20
4	72	3-8-8	1.71	0.84	9.64
3	22	3-8-12	1.26	0.87	11.85
4	22	3-8-12	1.41	1.04	13.02
4	68	3-8-12	1.38	0.52	10.01
3	26	3-8-16	1.19	0.81	11.42
4	20	3-8-16	1.42	1.02	9.13
4	71	3-8-16	1.40	1.11	9.12
3	31	6-8-12	0.77	1.40	13.70
4	10	6-8-12	1.00	2.71	12.90
4	15	6-8-12	0.83	1.34	9.78

TABLE X

The Ratio of Potassium to Nitrogen and of Oxalic Acid
to Potassium as Related to Quality
of Pennsylvania Cigar-Leaf Tobacco.
Mature Leaves. 1938 and 1939 Crops.

		Nitrogen: Potassium	Potassium: Oxalic
Potassium Series 1938	Average of good	3.67	0.60
	Average of fair	3.09	0.61
	Average of poor	2.00	0.78
Nitrogen Series 1938	Average of good	5.17	0.72
	Average of fair	4.85	0.71
	Average of poor	2.58	0.82
Nitrogen Series 1939	Average of poor	0.81	2.06
	Average of very poor	0.69	3.02

TABLE XI

The Influence of Fertilizer Treatment on the Oxalic Acid Content
and Quality of Pennsylvania Cigar-Leaf Tobacco.

Immature Samples. 1939 Crop

Sample	Manure tons	Fertilizer lbs./acre	Formula	Quality	Per Cent Aug. 26	Oxalic Acid Sept. 4
105	5 mushroom 5 barnyard	1000	3-8-12	good	2.88	2.15
106	"	1000	3-8-12	good	2.80	1.79
114	"	1000	3-8-16	good	2.71	2.03
204	10 fresh	1000	6-8-0	fair	2.62	2.77
209	10 "	1000	6-8-8	fair	2.19	2.36
216	20 "	500	6-0-0	fair	2.72	2.77

TABLE XII

The Incidence of Wildfire as Related
to the Oxalic Acid Content
of Pennsylvania Cigar-Leaf Tobacco.

Mature Leaves. 1939 Crop¹

Sample	Wildfire* Infection	Oxalic Acid	Potassium: Oxalic	Nitrogen: Potassium
1	1	1.71	2.2	1.0
2	0	2.50	1.5	1.0
3	2	2.19	1.4	1.3
4	0	2.48	1.7	1.1
5	1	2.30	1.8	1.0
6	1	2.57	1.3	1.2
7	0	1.95	1.9	1.0
8	1	1.89	1.6	1.4
9	0	1.29	2.7	1.1
10	3+	2.17	2.2	0.9
11	3	2.89	1.1	1.3
12	1	2.99	1.4	1.0
13	1	2.37	1.8	1.3
14	3	2.00	2.1	0.9
15	2	2.12	1.9	1.1
16	1	2.27	1.9	1.0

* Wildfire Infection

- 0 No infection
- 1 Trace
- 2 Appreciable Amount
- 3 Pronounced

¹ Samples represent different sections of Lancaster County

Discussion

Composition and Range:

In order to establish the percentage composition of citric, malic, and oxalic acids normally encountered in mature and cured Pennsylvania cigar-leaf tobacco, the range and average composition of the samples analyzed are given in Table I. No consideration of fertilizer treatment or condition of the tobacco leaves is taken in the data presented in Table I.

It is evident from this table that malic acid is the predominant organic acid in the leaf of Pennsylvania cigar-leaf tobacco, whereas citric and oxalic acids are present in smaller quantities. This order is the same in numerous other American tobaccos, both cigar and cigarette (41); it does not hold, however, for some Russian tobacco which contains as much as 13.3 per cent of citric acid (18).

It is also evident from this table that the amount of the acids varies considerably in different leaves. This variation has been noted by other workers but its origin has not been established. Vichery et al (32) have found that the malic acid of shade-grown tobacco varies between 10.6% and 16.5%, the citric acid between 0.9% and 3.5%, whereas the oxalic acid is relatively constant at 2.5%. Kolesnik (11) reports a variation in

the citric acid content of Russian tobacco from 3.6% to 11.8%.

A difference between organic acid content of the cured and mature leaves as well as between the mature leaves of the 1938 crop and the 1939 crop is brought out in Table I. These differences are more obvious in some of the other tables and shall be presented more fully in the discussion to follow.

Changes during Curing:

A group of thirteen samples was analyzed before curing and after curing for three months so that the changes taking place during the curing period might be revealed (see Table II). A number of these samples were analyzed for calcium before and after curing. The average ratio of oxalic acid to calcium before curing was 0.37, after curing it was 0.40; citric acid to calcium before curing was 0.27, after curing 0.70; malic acid to calcium before curing was 2.6, after curing 1.5. The difference in these ratios between the mature and cured leaves shows that the changes in the acid content of the leaf during curing are real and not due merely to changes in the weight of the leaf.

A comparison of the acid content of the mature and cured leaves shows that the paramount changes

taking place during curing are the increases in oxalic and citric acids and the corresponding decrease in malic acid. The changes in malic and citric acids are the most significant and confirm the findings of Vichery and Pucher (36) and other workers (25, 26, 14).

Quality of Cured and Mature Leaves:

Table III presents the citric, malic, and oxalic acid content of the best and poorer grades of cured tobacco studied. The samples were selected without regard for the fertilizer treatment or growing conditions. An examination of this table shows first: the better quality contains considerably more citric acid than the poor quality tobaccos, although the amount of oxalic and malic acids is nearly the same in both grades; second, the better quality tobacco is much more uniform in composition than the poorer grade which is very irregular.

In Tables V and VII are given the average organic acid composition of the good, fair, poor and very poor samples receiving different fertilizer treatments. Table VIII gives the same information as Table VII except that Table VIII represents the 1939 crop whereas Table VII represents the 1938 crop. Inasmuch as the climatic conditions for the two years were so radically different, the series are not averaged together but are

considered as three separate sets. This consideration shows that an increase in the quality of the tobacco follows closely a decrease in the quantity of oxalic acid and less closely of citric acid. The malic acid content does not seem to be at all related to the tobacco quality. When the ratio of potassium to oxalic acid, as shown in Table X is considered, its correlation with the quality is accentuated. The potassium/oxalic acid ratio also follows closely the nitrogen/potassium ratio, but whereas the nitrogen/potassium ratio is indirectly related to the quality, the ratio of potassium to oxalic acid is directly related and gives a wider spread.

The burn, in practically all cases judged, was so close to the value given to the quality of the leaf that it is not considered separately in this discussion.

Effect of Fertilization:

To investigate the effect of fertilization on the organic acid content of the tobacco leaf, four series of differently fertilized tobacco plants were sampled and analyzed. These are the potash series in which the chief variable is the potassium treatment of the plot, the nitrogen series in which nitrogen has been supplied in different forms and quantities, a comparison of three and

four year rotation plots receiving the same fertilizer treatment, and a comparison of a balanced fertilizer treatment with an unbalanced treatment.

The fertilizer treatment of the potash series is shown in Table IV whereas a chemical analysis of the tobacco samples from the fertilized plots is shown in Table V. The N-P-K formulae vary from 3-8-4 to 3-8-20 and the potassium is supplied as the carbonate, nitrate and sulfate. An inspection of Table V does not reveal any pronounced effect of the fertilizer treatment on the organic acid content as there is as much variation in tobacco from plots receiving the same treatment as in the case of tobacco from different plots.

Table VI gives the fertilizer treatments of the plots of the nitrogen series and Table VII gives the chemical analysis. Nitrogen was supplied to the plots in numerous organic and inorganic forms. Two formulae were used: 3-8-12 with 10 tons of manure per acre, and 6-8-12 with no manure added. Inspection of Table VII shows again that although there is a great deal of variation in the acid content, it can not be attributed to any particular treatment as there is as much variation in plots receiving the same treatment as from those getting different fertilizer treatment. A comparison of the average

values of the nitrogen and potash series shows the potash series to be higher in oxalic and malic acids and lower in citric acid. The reason for these differences is not clear. It must be pointed out that the fertilizer treatments were not always reflected in the composition of the leaf as can be seen in Tables V and VII.

To see if the system of rotation influences the organic acid content of the tobacco leaf, a few samples of the three year rotation series were compared with samples of the four year rotation series receiving the same fertilization. The data in Table IX indicate that the system of rotation has very little effect on the non-volatile organic acid composition of the tobacco plant, since in all cases given the percentage of acid in the three year rotation falls within or near the range found in the four year rotation.

Table XI shows the influence of a balanced fertilizer treatment as against an unbalanced treatment on the oxalic acid content during the growing season. The "100" series represents the balanced fertilization whereas the "200" series represents the unbalanced treatments. Analysis of samples taken August 26 after a period of dry hot weather showed the samples of the "100" series with a higher oxalic acid content than existed in the "200" series at that time. Growing conditions between August 26 and September 4 were very favorable and analysis of leaves

sampled on September 4 showed that the per cent of oxalic acid in the "100" series had dropped considerably since August 26 while that in the "200" series had increased somewhat. A subsequent examination for quality of the leaf showed those of the "100" series were all judged as "good" whereas those of the "200" series were judged as "fair."

Effect of Climatic Conditions:

The study of the effect of climatic conditions upon the organic acid constituents was made by comparing samples grown on the same plots receiving identical fertilizer treatments for two consecutive years. The first year, 1938, was a particularly good year for tobacco growing and produced a good crop: the rainfall from May through August exceeded 15.5 inches and was spread evenly throughout the growing season. The second year, 1939, was a very poor year: the rainfall for the same months of 1939 was almost 11 inches but much of it came in heavy storms at the end of the growing season, the weather before having been characterized by excessive heat and drought. The chemical analyses, shown in Tables VII and VIII, reveal the average oxalic acid content to be two and a half times greater in 1939 than in 1938, the average citric acid content to be almost three times as large in 1939 as in 1938, while the average malic acid content

is about the same for both years. The increase in oxalic and citric acid approximate the increases in these acids from mature to cured tobacco and perhaps indicate that the 1939 crop may have undergone a period of starvation in the field somewhat similar to the starvation during curing. One difference is evident and that is that the malic acid is reduced considerably during curing while it apparently was not affected by the severe weather conditions existing during 1939.

Table XI, already mentioned under the effect of fertilization, also gives an indication of how the climatic conditions may affect the acid content of the tobacco leaf. Up to the latter part of August, 1939, the plants endured the severe weather conditions described above, while after that time the plants received heavy rainfall. In the period of one week, between August 26 and September 4, the per cent of oxalic acid of the "100" series fell considerably, similar to the experimental data of Vickery and Pucher (37) in their study of the organic acid content of the tobacco leaf during the period of growth.

Incidence of Wildfire:

The 1939 tobacco crop, as well as being poor in quality, contained a great number of Wildfire infected

plants, whereas the 1938 crop was comparatively free of the disease. Since the oxalic acid content of the 1939 crop was so markedly different from that of the 1938 crop and the ratios of potassium to oxalic acid and nitrogen to potassium were so closely related to the quality, it was thought that an investigation of these values might lead to a better understanding of the conditions involved in this disease. For this investigation a series of 16 samples representing tobacco grown by different farmers in different localities in Lancaster County and showing different degrees of Wildfire infection were used. The data in Table XII shows no correlation of any of the above mentioned values with the incidence of Wildfire infection. It may be assumed, however, that although these values are not directly correlated with the incidence of Wildfire, the difference in acid content between the 1938 and 1939 crops does indicate an indirect relationship between these constituents and the diseased condition. The factors directly connected with the susceptibility of the plant to Wildfire may perhaps be illuminated by a more detailed analysis directly before and after the outbreak of the disease.

Summary

1. The citric, malic and oxalic acid content of mature Pennsylvania cigar-leaf tobacco for a period of two years was determined. The percentage of citric acid varied from 0.5 to 5.2, malic acid from 5.9 to 14.1, and oxalic acid from 0.3 to 3.0.
2. It was found that during the curing period the oxalic and citric acids increased in quantity whereas the malic acid decreased.
3. The better quality cured tobacco was found to have a greater amount of citric acid whereas the quantities of malic and ^{oxalic} ~~citric~~ acid were approximately the same. With regard to the mature leaves, the better grade of tobacco was characterized by a lower percentage of oxalic and citric acids than was found in the poorer grade. The ratio of potassium to oxalic acid was found to be directly related to the quality of the samples analyzed.
4. No influence on the citric, malic, or oxalic acid content of the tobacco leaf could be found by varying the fertilizer treatment or the system of rotation.
5. The 1939 crop, which grew in a season of hot dry weather and unevenly distributed rainfall, contained a

considerably higher percentage of oxalic and citric acids than was present in the 1938 crop, which was raised during a season favorable to tobacco growing.

6. No direct relationship was found between the oxalic acid content or the ratio of potassium to oxalic acid and the incidence of the Wildfire disease of tobacco.

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