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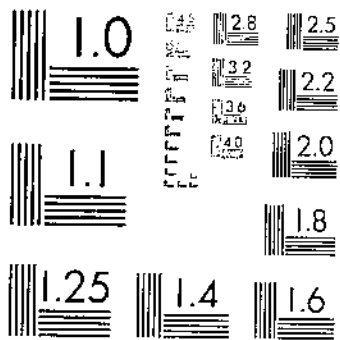
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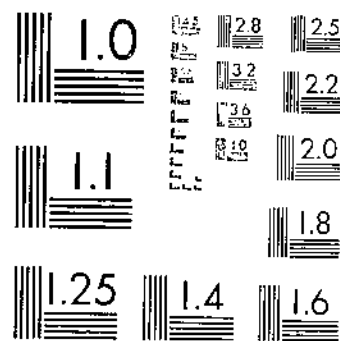
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THE NITROGEN NUTRITION OF TOBACCO¹

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CONTENTS

	Page		Page
Introduction.....	1	Influence of nitrogen supply on nutrition and metabolism.....	33
Scope and plan of the experiments.....	3	Methods of sampling and analysis.....	33
Conditions under which experiments were conducted.....	5	Recovery of fertilizer nitrogen by the crop and its distribution in the leaf and stalk.....	37
Quantities and forms of nitrogen used.....	5	Minimum nitrogen requirements.....	39
Soil of the experimental plots.....	6	Water relations in the plant.....	40
Weather conditions.....	7	Osmotic concentration of cell sap.....	43
Effects of supply of fertilizer nitrogen on crop yields.....	9	Acidity relations.....	44
Effects of increasing quantities of nitrogen.....	9	Nitrogen metabolism.....	46
Effects of form of nitrogen applied.....	15	Carbohydrate metabolism.....	50
Application of the Mitscherlich formula.....	18	Observations on oxidizing enzymes.....	51
Influence of nitrogen supply on market value of leaf.....	22	Effects of high and low topping on growth and metabolism.....	53
Relation of nitrogen supply to rate and type of growth.....	24	The nitrogen supply and the curing process.....	56
Leaf, stalk, and root growth.....	24	Chemical composition of cured tobacco as affected by the nitrogen supply.....	59
The growth rate.....	25	Interpretation of results and conclusions.....	61
Number, size, and shape of leaves.....	25	Yield and value of crop.....	61
Weight and thickness of leaf and specific gravity of leaf material.....	28	Growth relations and leaf characteristics.....	65
Time of flowering and ripening of leaf.....	30	Internal processes of nutrition and metabolism.....	66
Color of leaf.....	32	Chemical composition of cured leaf.....	71
Burning qualities of leaf.....	32	Summary.....	72
		Literature cited.....	75

INTRODUCTION

It is particularly difficult in the case of tobacco to obtain a satisfactory understanding of the functions and the effects of the essential elements in the nutrition of the plant in relation to practical culture. It is characteristic of the tobacco crop that within reasonable limits the returns realized by the grower usually depend more on the quality of the product than on the yield obtained. In this crop the leaf constitutes the valuable portion and, as is well known, foliage leaves are especially sensitive to changes in the supply of nutrients and other soil and climatic factors with respect to their morphological and histological characteristics and their chemical composition. These physical and chemical characteristics largely determine the commercial value of the tobacco leaf and, moreover, requirements as to these

¹ The investigations here reported were conducted cooperatively by the Bureau of Plant Industry and the Maryland Agricultural Experiment Station.

properties for the manufacture of cigars, cigarettes, and other tobacco products differ very widely. Finally, the physical and chemical properties of the soil, as well as the climatic conditions required for successful production of leaf tobacco suitable for one manufacturing purpose, may differ radically from those required for obtaining a product adapted to another form of manufacture.

It is known that although nitrogen is one of the essential and characteristic constituents of the living matter of the cell, the quantity absorbed and assimilated by the plant does not bear a fixed relation to the rate or the extent of growth, for this relation is subject to considerable variation in accordance with the supply of nitrogen becoming available in the soil and numerous other factors influencing nutrition and growth. It appears that considerable quantities of assimilated nitrogen may be stored in the tissues in the form of so-called "reserve protein" and related compounds. Available data show that in the tobacco plant the nitrogen content of the mature leaf on a dry basis may range from less than 2 percent to as much as 5 percent or even more, although with the higher percentages appreciable quantities of the nitrogen usually remain in the unassimilated, inorganic form. It is a matter of common observation by growers that variation in the soil supply of nitrogen is likely greatly to affect the value of the cured leaf. Evidence is presented in this bulletin which tends to show that not only does the quantity of nitrogen absorbed and assimilated by the plant affect the grade or quality of the leaf of a given type, but it is also one of the important factors in determining the type of leaf produced; that is, it may largely determine whether the leaf is primarily adapted to manufacture of cigars, cigarettes, or other finished products. In this and in certain other respects nitrogen appears to be of outstanding importance in its effects on the development of the tobacco leaf and on its properties after it has been cured.

Extensive field experimentation in the form of plot tests of the conventional type concerning the nitrogen requirements of the tobacco crop has been carried on in the United States and in foreign lands, but it does not seem necessary for present purposes to review the results obtained. Experiments of this type, when properly planned and carried out, undoubtedly are of great practical value in determining the most profitable usage of nitrogen as a fertilizer for a given type of tobacco under a given set of conditions. From their nature, however, such experiments usually have only a limited more or less local application and cannot be expected to supply adequate information on the more fundamental features of the nitrogen nutrition of the tobacco plant. As far as is known, no comprehensive investigation has been undertaken previously relative to the effects of differences in the quantity of nitrogen made available to the plant upon the physical and chemical properties of the leaf and the internal processes of nutrition and metabolism through which these effects on leaf characteristics are produced.

In addition to the effects of differences in the quantity of nitrogen made available to the plant, there is also the important problem of analyzing the relative effects of inorganic and organic sources or forms of nitrogen on the yield and on the properties of tobacco leaf. There has long been a belief on the part of tobacco growers that organic sources of nitrogen such as cottonseed meal, fish, and slaughterhouse

products are essential as constituents of the fertilizer for production of high quality in certain types of leaf. In this connection it may be noted, however, that in a recent investigation Beaumont and his associates (5)² found that any special virtue which cottonseed meal may possess as a fertilizer apparently is not based upon any peculiar forms of nitrogen it contains. The comparative effects of organic and inorganic forms of fertilizer nitrogen are not dealt with in this bulletin. Considerable attention is given to the relative effects of ammonia and nitrate forms of nitrogen so far as concerns growth relations, and limited consideration also is given to different ammonia salts, such as the sulphate and the chloride. However, the effects of differences in the quantity rather than in the form of the nitrogen are especially emphasized.

With respect to interpretation of the results of technical nutritional studies relating particularly to nitrogen, such as are included in this bulletin, it is unfortunate that so little is known about the composition and properties of the different proteins and other complex nitrogen compounds functioning in nutrition, metabolism, and growth processes. A principal difficulty is that methods are not available for separating and estimating the specific nitrogen compounds in question. It is hardly possible at present, therefore, to arrive at even an approximately complete analysis of the mode of action of increasing quantities of nitrogen made available to the plant. However, some of the effects on internal processes have been measured, and their apparent relationship to the growth and the properties of the leaf have been established.

SCOPE AND PLAN OF THE EXPERIMENTS

In the present investigations the two primary objectives were: (1) To obtain practical information about the quantity of fertilizer nitrogen required for best results with respect to yield and market value of the Maryland type of tobacco, also the comparative fertilizing value of some of the new, synthetic forms of nitrogen for the tobacco crop; and (2) to make a fairly comprehensive physiological and biochemical study of the effects of varying the nitrogen supply on the growth and development of the plant, particularly its leaves, and on the associated internal processes of metabolism and nutrition. To attain the first-named objective, field-plot tests of the usual type were conducted for a period of years at Upper Marlboro, Md. The original, more extensive series of plot experiments, was begun in 1919 and was continued for 11 years. Several additional treatments were begun subsequently to 1919. The plots were approximately one twentieth of an acre, with a space of 1½ feet separating them, and every fourth plot was used as a control. The treatments were not duplicated in the usual fashion but instead, at harvest time, each plot was separated transversely into two sections of equal area, and the yields were obtained separately on the duplicate sections. The rows were 34 inches apart, and the plants were spaced 34 inches in the row. All fertilizer was applied in the drill just before transplanting except that beginning in 1925 one half of the nitrogen was applied as a top dressing at the second cultivation on the plots receiving 80 pounds of nitrogen per acre. A Maryland broadleaf variety of tobacco was used, and approved

² Italic numbers in parentheses refer to Literature Cited, p. 76.

methods of cultivating, harvesting, and curing, conforming to standard practice in southern Maryland, were used throughout.

All plots in the primary series received a uniform basal fertilizer treatment supplying 60 pounds of phosphoric acid (P_2O_5) and 40 pounds of potash per acre. In 1919 the phosphoric acid was supplied in the form of 16 percent superphosphate but thereafter precipitated bone, a dicalcic phosphate containing about 40 percent of phosphoric acid, was substituted. The potash was derived from high-grade sulphate. Evidence of magnesium deficiency having appeared on the plots, ground limestone containing 20 percent of magnesia was applied uniformly at the rate of a ton per acre in the fall of 1922 and of 1924. In 1924, through error, a low-grade muriate of potash containing 38 percent of potash was applied, instead of ammonium sulphate, to the plots regularly receiving the latter form of nitrogen. Consequently, in that year these plots received an excess of potash but no nitrogen at all. Departures from the basal treatment in the supplementary plots later added are considered in connection with the description of quantities and forms of nitrogen employed in the tests.

The principal feature of the physiological and biochemical studies was an attempt to trace through the growing period, the subsequent period of so-called "ripening" of the leaves, and the curing of the harvested product, the effects of varying the amount of the nitrogen supply on various features of growth and development of the plant with special reference to the leaves, and on the internal processes involved. Consideration was given also to effects of the nitrogen supply on the physical and chemical characteristics of the cured leaf in relation to the type, grade, and quality of the product. The physiological observations were concerned mainly with the relative development of leaf, stalk, and root; the rate, amount, and character of growth of leaf and stalk; the time of flowering and the progress of the ripening process; visible symptoms of nitrogen deficiency in the plant; and the combustibility of the cured leaf. In the biochemical studies attention was given to the recovery of fertilizer nitrogen by the plant and its distribution in the plant parts; the minimum nitrogen requirements of the crop; water, osmotic, and acidity relations in the leaf; nitrogen and carbohydrate metabolism; oxidizing enzymes; and the chemical composition of the cured leaf and its significance. Brief consideration also was given to the effects of high and low topping on leaf metabolism. Though the data are far from complete in all particulars, they serve as a whole to throw considerable light on the effect of the nitrogen supply on growth and metabolism, and on the nutrition of the plant and the resultant characteristics of the cured leaf affecting its value for manufacturing purposes.

In technical plant-nutrition studies it is common practice to employ cultures in sand or in nutrient solutions in order to secure better control of conditions, but such methods are not well adapted for work with tobacco which involves detailed study of development and chemical composition of the leaf in relation to quality. In seeking a physiological approach to such tobacco-fertilizer problems as were under investigation, it is believed that, with proper choice of soil type and the other necessary precautions, conditions more or less similar to those applying to sand cultures can be attained in open field cultures. This plan has the important advantage that the results obtained are much more likely to be directly applicable to conditions of commer-

cial culture. In some instances results so obtained may need to be checked by means of pot tests, but in the present case the field plots used in the previously mentioned practical tests and other similar plots supplied substantially all of the material required for the physiological and biochemical observations. Because of the disturbing effects of fluctuating weather conditions and of other variable factors under field conditions, detached observations of a physiological or biochemical nature may not be conclusive, but usually this uncertainty is readily overcome by making a series of observations at intervals, always under strictly comparable conditions and extending over a considerable period. This method also is particularly helpful in guarding against misleading results, due to differences in age or stage of development of different plants or parts of the individual plant.

CONDITIONS UNDER WHICH EXPERIMENTS WERE CONDUCTED

QUANTITIES AND FORMS OF NITROGEN USED

Nitrate of soda was employed at rates to supply 10, 20, 30, 40, and 80 pounds of nitrogen per acre. These quantities of nitrogen in combination with the basal supply of phosphoric acid and potash along with the control treatments are equivalent to 1,000 pounds of fertilizer per acre having the formulas 0-6-4, 1-6-4, 2-6-4, 3-6-4, 4-6-4, and 8-6-4, respectively. The outcome of the tests indicates that there would have been some advantage in including in this group a rate of 60 pounds of nitrogen per acre. Further information on the quantity of nitrogen required for best results also was provided in applying the other forms of nitrogen used at two or more different rates.

In the original series, in addition to nitrate of soda, cyanamide and ammonium nitrate were used in quantities to supply 20 and 40 pounds of nitrogen per acre; and ammonium chloride and ammonium sulphate were employed at rates to furnish 20, 40, and 80 pounds of nitrogen per acre.

Beginning in 1921, tests with urea and the commercial product known as Ammo-Phos in comparison with ammonium sulphate, applied at rates to supply 20 and 40 pounds of nitrogen per acre were undertaken on a nearby field having a lighter and somewhat less productive soil. Since the Ammo-Phos contained about 47 percent of phosphoric acid and only 11.5 percent of nitrogen, an additional ammonium-sulphate plot was included which received double the normal application of 60 pounds per acre of phosphoric acid in the form of precipitated bone and the nitrogen was applied at the 40-pound rate. Ground limestone was applied to these plots as in the original series.

In 1923, a test of Urea-Phos, a combination of urea and phosphoric acid, involving plots receiving nitrogen from this source at the rates of 20 and 40 pounds per acre, was added at the front end of the original set of plots. The Urea-Phos contained 45 percent of phosphoric acid and no additional phosphate was used in this experiment.

In 1925 a test of monoammonium phosphate in conjunction with nitrate of potash was added, although the primary object in this instance was to include a highly concentrated fertilizer supplying only the three nutrients, nitrogen, phosphorus, and potassium, rather than to conduct a test with ammonium phosphate as such. The materials were used in proportions to furnish approximately 40 pounds of nitro-

gen, 60 pounds of phosphoric acid, and 90 pounds of potash per acre. For comparison, a treatment was employed in which one half the nitrogen was derived from cottonseed meal and the other half from nitrate of soda; the phosphoric acid from 16 percent superphosphate; and the potash from high-grade sulphate.

The approximate nitrogen content of the materials used in these experiments is shown in table 1.³

TABLE 1.—Approximate content of principal constituents of fertilizer materials used in plot tests

Fertilizer material	Composition (percent)			Fertilizer material	Composition (percent)		
	Nitro- gen	Phos- phoric acid (P ₂ O ₅)	Potash (K ₂ O)		Nitro- gen	Phos- phoric acid (P ₂ O ₅)	Potash (K ₂ O)
Ammonium nitrate.....	34.8			Cyanamide.....	20.5		
Ammonium sulphate.....	20.6			Urea.....	48.0		
Ammonium chloride.....	25.5			Urea-Phos.....	17.7	45.0	
Monoammonium phos- phate.....	11.0	62.4		Cottonseed meal.....	6.0	3.6	2.0
Sodium nitrate.....	15.5			Superphosphate.....		16.0	
Potassium nitrate.....	13.7		46.3	Precipitated bone (dical- cic phosphate).....		40.0	
Ammo-Phos.....	11.5	47.0		Potassium sulphate.....			50.0

The treatment of the fertilized plots and those used for controls in the original major series of tests are shown in table 5, and in the supplementary series in table 7, and the arrangement of the plots is shown in figure 1.

SOIL OF THE EXPERIMENTAL PLOTS

In order to obtain as full information as practicable concerning the general characteristics of the soil used in the tests, the Bureau of Chemistry and Soils was requested to make a survey of the plots. This was done, and the results with respect to the original, principal series of plots are indicated in figure 1. The report on the soils of the farm on which the nitrogen plots are located as prepared by S. W. Phillips, Bureau of Chemistry and Soils, has been published in full elsewhere (9) in connection with certain other studies made on the farm. As indicated in figure 1, the two soil types found on the principal set of nitrogen plots are the Collington fine sandy loam and the Collington gravelly sandy loam. The supplementary set of plots is composed of the Collington fine sandy loam and Collington loamy sand types.

Prior to the initiation of these experiments the soil had not been cropped for several years. The initial reaction of the soil and subsoil, determined colorimetrically, was found to correspond to a pH value of 6.8. Readings on plots 7, 11, 13, 18, 19, and 20 (table 5) were taken in the fall of 1925 and again in 1926 and showed a pH value of about 7.1 for both soil and subsoil on all plots except 19 and 20, which received 80 pounds of nitrogen per acre as chloride and sulphate, respectively; and showed a pH of 6.8.

³ Most of the nitrogenous materials used and the data on their chemical composition were supplied by the Division of Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils.

WEATHER CONDITIONS

In interpretation of the results obtained, the amount and distribution of rainfall is of special importance as a factor because of its effect in either increasing or decreasing the available nitrogen in the

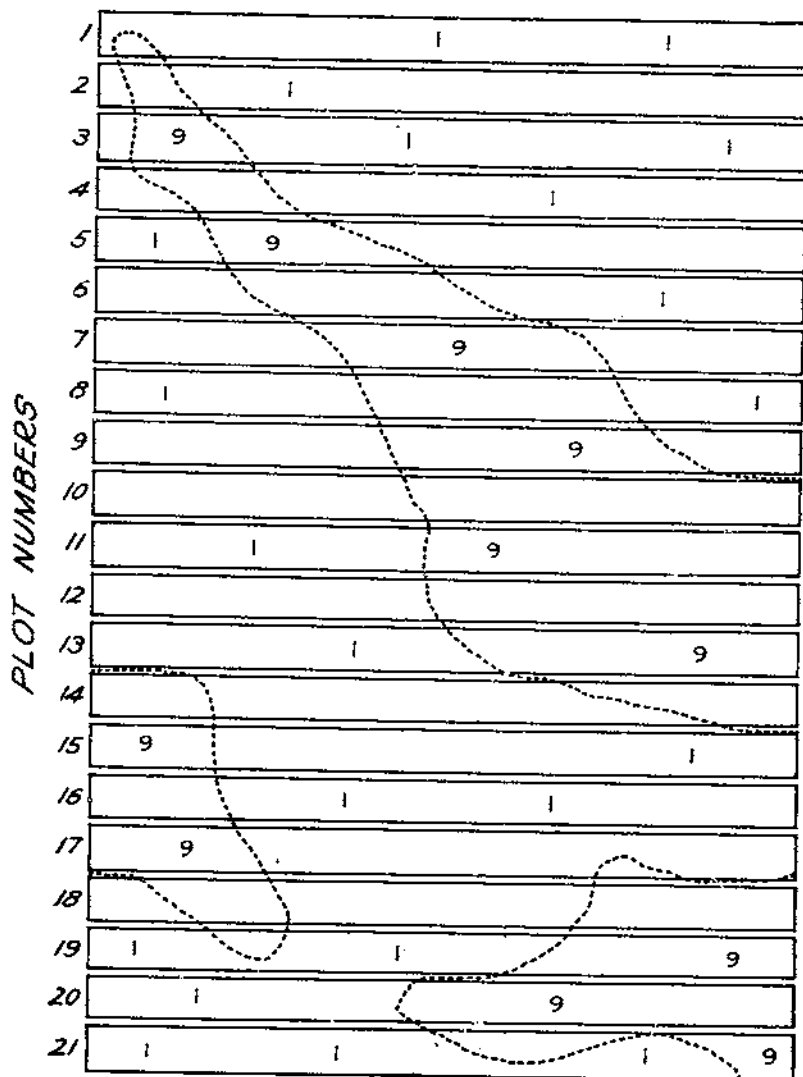


FIGURE 1.—Soil map of principal series of nitrogen test plots, Upper Marlboro, Md. Soil legend: (1) Collington fine sandy loam; (9) Collington gravelly sandy loam. Surveyed November 1921 by S. W. Phillips, Bureau of Chemistry and Soils, United States Department of Agriculture.

soil as well as the direct effect of the water supply on the growth of the plant. It is rather difficult satisfactorily to summarize the complete records of rainfall which were taken during the growing season, but the data for 10-day periods, as shown in table 2, will serve to indicate fairly well for each year the general distribution of the

rainfall through the season. In this connection the dates of transplanting to the field and of harvesting the crop each year are shown in table 3.

The temperature conditions doubtless were not greatly different from those at Washington, D. C., about 15 miles distant, where records reported by the United States Weather Bureau are available to any who may be interested in this feature of the weather conditions.

TABLE 2.—Rainfall at Upper Marlboro, Md. (in inches), for 10-day periods during the growing season, 1919-29

Month and 10-day interval	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929
April:											
1-10	2.29	2.24	0.65	0.10	0.11	2.13	0.20	1.02	1.83	0	0.70
11-20	1.03	1.77	.66	.97	1.37	.26	.66	.28	.07	.83	4.15
21-30	.69	.80	2.07	.63	1.76	.08	.80	.33	4.09	3.99	2.34
Total	3.91	4.81	2.78	1.10	3.23	2.47	1.66	1.61	5.99	4.92	7.19
May:											
1-10	1.85	.80	1.98	1.13	.90	1.40	.20	.62	1.92	.20	1.19
11-20	3.05	.08	2.33	2.81	0.88	2.66	.77	.82	.54	.46	.39
21-31	1.79	.18	.76	.00	0	2.10	1.13	.09	.57	1.02	2.56
Total	6.69	1.06	5.07	4.60	1.78	6.25	2.10	.93	3.03	1.77	4.14
June:											
1-10	1.10	2.08	0	3.69	.93	2.71	0	1.04	0	.42	.68
11-20	.33	2.40	.31	1.86	1.68	1.11	.04	.52	3.25	1.13	3.02
21-30	.57	1.24	.46	2.80	.48	1.38	1.73	.89	.34	2.25	2.61
Total	2.29	5.72	.77	8.20	2.90	5.20	1.77	2.45	3.59	3.83	6.20
July:											
1-10	1.63	2.35	1.34	2.30	.07	1.08	2.83	1.50	.46	.07	1.99
11-20	2.58	1.73	1.46	3.05	1.02	.03	.32	2.56	.60	2.35	.74
21-31	2.43	.55	.37	.73	1.13	.68	3.55	.65	.33	0	.14
Total	6.64	4.63	3.14	6.74	3.72	1.79	6.70	4.77	1.41	2.42	2.87
August:											
1-10	.83	.86	.73	.41	.70	.89	1.12	.54	.07	.89	.90
11-20	3.07	1.40	1.45	2.73	1.71	2.15	.85	3.79	2.43	11.72	0
21-31	.40	2.15	.10	.68	1.45	1.72	.65	2.37	3.06	1.15	.45
Total	4.30	4.30	2.28	3.82	2.86	4.73	2.62	6.64	5.56	13.70	1.35
September:											
1-10	.19	1.24	.36	.63	1.52	.54	.62	2.48	1.85	2.19	1.33
11-20	.77	.03	.02	0	.39	.78	.92	0	.44	1.47	1.28
21-30	.73	2.05	1.41	0	.62	4.26	.23	1.61	0	.74	1.10
Total	1.69	3.32	1.79	.63	2.53	5.50	1.77	4.09	2.20	4.31	3.71
Total for 6 months	25.40	23.84	15.83	25.15	17.11	26.03	16.68	20.40	21.87	31.01	25.65

TABLE 3.—Dates of transplanting and harvesting the tobacco of the experimental plots each year, 1919-29

Year	Date of trans-planting	Date of harvest- ing	Year	Date of trans-planting	Date of harvest- ing	Year	Date of trans-planting	Date of harvest- ing
1919	June 20	Oct. 10	1923	June 27	Sept. 28	1927	June 15	Sept. 9
1920	June 25	Sept. 17	1924	June 26	Oct. 10	1928	June 19	Sept. 14
1921	July 1	Oct. 5	1925	June 10	Sept. 4	1929	June 28	Oct. 8
1922	June 14	Sept. 22	1926	June 15	Sept. 17			

EFFECTS OF SUPPLY OF FERTILIZER NITROGEN ON CROP YIELDS

In the primary or major series of plot tests the average yields of cured leaf on the duplicate sections of the control plots (table 4) indicate only a fair degree of uniformity in the productivity of the soil as a whole, the yields of sections A and B of plot 1 and section A of plots 5 and 17 pointing to a somewhat greater natural productivity of the adjoining soil than that of the remainder of the field. When plot 1 is excluded the average yield of the remaining controls is 614 pounds, while including this plot gives an average yield of 634 pounds. It is recognized that there are differences of opinion as to the most satisfactory manner of applying the results from control plots in interpreting experimental data, but in the present instance the results on plot 1 will be excluded, and the average value of 614 pounds for the remaining controls applied uniformly in all cases in arriving at the effects of the nitrogen treatments.

In the supplementary series of plots the average yields of the three controls (table 7) are 464, 534, and 524 pounds, respectively. The general average for the three controls is 507 pounds. It appears, therefore, that the soil of this field is somewhat less productive than that of the primary series of tests.

TABLE 4.—Average yields per acre of duplicate sections of control plots in the primary series of nitrogen tests, 1919-29

Plot section	Plot 1	Plot 5	Plot 9	Plot 13	Plot 17	Plot 21
	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds
A.....	771	682	545	581	670	593
B.....	692	601	607	630	607	614
Average.....	732	642	576	600	639	604

The effectiveness of nitrogen as a fertilizer in promoting growth on the several plots in both the early and later stages of development of the plants is indicated in figures 2 and 3.

EFFECTS OF INCREASING QUANTITIES OF NITROGEN

The average annual yields of the duplicate plot sections in leaf and stalk, the 10-year average yield of leaf on each of the duplicate sections of each plot, and the 10-year combined average yield of leaf and of stalk on the two sections of each plot in the primary series are shown in table 5. This table also indicates the arrangement of the plots. The yearly yields on the duplicate sections of each plot were reasonably consistent and are not given in detail in the table. The average increase in yield over the controls for each treatment covering the entire period of the experiment, except the year 1924, is shown in table 6.

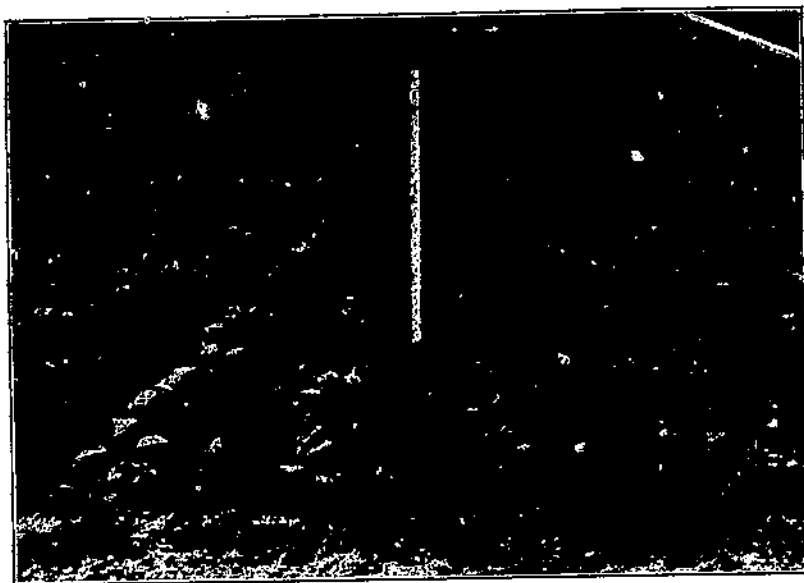


FIGURE 2.—Primary series of plots, season of 1920, showing the effectiveness of fertilizer nitrogen in the early stages of growth. The plot in the center received no nitrogen, while the plants at left received 20 pounds per acre in the form of ammonium chloride, and those at right received a like quantity in the form of ammonium sulphate.



FIGURE 3.—Supplementary series of plots, season of 1926, showing the effectiveness of nitrogen as a fertilizer in the late stages of growth. Plot at right of measuring rod received no nitrogen, while plot at left received 40 pounds of nitrogen per acre in the form of ammonium sulphate. The plants receiving no nitrogen in the fertilizer show a pale yellowish-green color, a characteristic symptom of nitrogen hunger.

TABLE 5.—Yields of leaf and stalk each year, 1919-29 (average of duplicate sections, A and B, of each plot), 10-year average yield of leaf on each of the two plot sections, and the 10-year combined average yield of leaf and of stalk on the two sections of each plot, in the primary series of tests with nitrogen applied at different rates and derived from different sources, 1919-29

[Results for 1924 are not included in the 10-year averages for reasons stated on page 4]

Plot no.	Quantity per acre	Source	Acre yields of leaf and stalk (pounds)													
			1919		1920		1921		1922		1923		1924		1925	
			Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk
1.	0		674	435	606	325	639	290	519	170	785	735	075	225	800	460
2.	20	Cyanamide	835	690	769	555	709	335	543	230	830	805	1,063	555	965	610
3.	20	Ammonium nitrate	825	705	816	660	785	435	610	285	965	865	713	290	1,035	705
4.	20	Ammonium sulphate	815	685	945	715	670	380	665	345	840	725	783	415	1,045	685
5.	0		552	360	450	240	580	250	370	135	775	590	603	225	740	340
6.	20	Ammonium chloride	895	740	950	700	660	300	740	335	930	720	690	555	1,055	625
7.	20	Nitrate of soda	605	370	800	640	770	420	675	300	940	720	873	485	1,125	740
8.	10	do	605	370	745	390	600	305	553	175	918	650	740	320	870	520
9.	0		625	375	450	230	555	290	325	65	715	510	483	145	745	375
10.	30	Nitrate of soda	780	705	970	670	845	430	580	255	1,165	840	895	590	1,140	755
11.	40	do	780	705	970	670	845	430	580	255	1,165	840	895	590	1,140	755
12.	40	Ammonium sulphate	577	380	645	330	735	350	700	355	1,008	755	530	765	1,085	790
13.	0		579	410	380	105	520	200	216	80	748	520	565	165	755	440
14.	40	Ammonium chloride	1,104	910	1,195	905	815	350	785	300	1,070	895	1,053	635	1,160	710
15.	40	Ammonium nitrate	1,038	825	1,175	885	680	546	736	350	1,310	985	1,000	740	1,190	910
16.	40	Cyanamide	879	650	960	610	555	290	315	70	795	600	625	185	835	460
17.	0		598	365	500	235	715	360	315	70	795	600	625	185	835	460
18.	80	Nitrate of soda	908	675	1,015	735	665	475	418	125	1,285	890	1,115	760	1,260	890
19.	80	Ammonium sulphate	1,133	895	1,235	775	795	420	408	100	1,120	790	1,115	140	1,230	880
20.	80	Ammonium chloride	1,205	975	1,210	850	625	205	490	145	845	475	900	446	1,200	790
21.	0		602	425	680	290	640	325	365	95	600	460	340	85	780	450
Average			815	645	844	580	704	354	534	210	940	607	767	383	1,015	640

Plot no.	Quantity per acre	Source	Acre yields of leaf and stalk (pounds)										10-year average, sections A and B	
			1926		1927		1928		1929		10-year average, section A—leaf	10-year average, section B—leaf	Leaf	Stalk
			Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk				
1.	0		635	655	985	490	395	225	1,015	600	771	002	732	425
2.	20	Cyanamide	935	840	1,160	720	510	350	1,125	505	860	827	847	504
3.	20	Ammonium nitrate	960	890	1,245	780	580	400	1,145	610	949	844	897	642
4.	20	Ammonium sulphate	885	775	1,140	700	565	485	1,115	670	882	855	860	817
5.	0		839	535	935	330	320	200	890	410	682	801	699	340
6.	20	Ammonium chloride	965	790	1,316	800	615	440	1,145	645	975	884	832	610
7.	20	Nitrate of soda	805	780	1,050	710	550	480	1,320	710	868	878	832	603
8.	10	do	770	620	905	465	455	330	995	525	746	743	745	440
9.	0		635	450	730	285	290	185	790	305	545	607	676	303
10.	30	Nitrate of soda	903	810	1,240	745	549	455	1,320	705	903	897	890	631
11.	40	do	1,005	800	1,355	855	545	550	1,240	740	937	1,007	972	670
12.	40	Ammonium sulphate	1,010	923	1,335	1,000	600	630	1,225	710	963	961	962	723
13.	0		890	615	780	310	290	180	800	330	581	630	606	334
14.	40	Ammonium chloride	1,010	705	1,325	645	610	570	1,225	660	1,030	1,024	1,034	675
15.	40	Ammonium nitrate	1,035	830	1,175	790	655	505	1,250	780	1,070	1,000	1,040	757
16.	40	Cyanamide	1,090	785	1,090	565	395	245	1,035	470	885	836	851	511
17.	0		850	460	705	255	285	170	785	280	670	607	840	326
18.	80	Nitrate of soda	1,205	895	1,525	1,110	615	415	1,400	800	1,094	1,034	1,064	609
19.	80	Ammonium sulphate	1,315	970	1,370	1,050	830	755	1,220	685	1,056	1,057	1,057	735
20.	80	Ammonium chloride	1,370	850	1,605	975	785	630	1,340	635	1,100	1,033	1,067	453
21.	0		760	405	655	270	260	155	780	295	593	614	604	316
Average			963	730	1,122	679	505	404	1,100	570	865	845	855	552

TABLE 6.—Increase in yield of tobacco resulting from use of nitrogen in different quantities and from different sources in the primary series (1919-23 and 1925-29) and the supplementary series (1921-23 and 1925-29) of field-plot tests

[The last plot in the supplementary series received a double ration of phosphoric acid]

Series of test and source of nitrogen	Acra increase in yield from indicated quantities of nitrogen				
	10 pounds	20 pounds	30 pounds	40 pounds	80 pounds
Primary series:	Pounds	Pounds	Pounds	Pounds	Pounds
Nitrate of soda.....	131	258	336	353	450
Ammonium nitrate.....	283	426
Ammonium chloride.....	318	416	453
Ammonium sulphate.....	255	348	443
Cyanamide.....	233	237
Supplementary series:
Urea.....	266	401
Ammo-Phos.....	235	381
Ammonium sulphate.....	254	423
Do.....	465

The nitrate of soda tests of the primary series show increases in yield for each increment of nitrogen but, as is to be expected, the

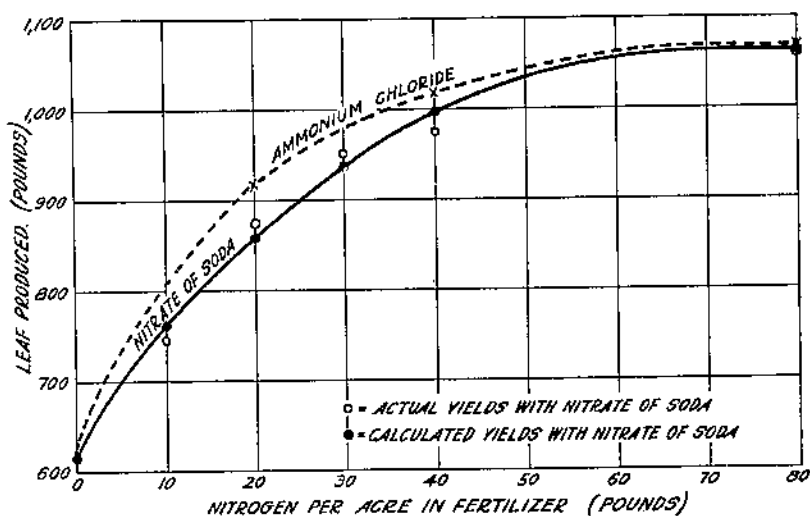


FIGURE 4.—Second-degree parabola, showing the theoretical 10-year average yields of leaf tobacco with 10-pound increments of fertilizer nitrogen derived from nitrate of soda, as compared with the actual average yields; also, comparative actual yields of leaf from equal quantities of fertilizer nitrogen derived from nitrate of soda and ammonium chloride. Note the stimulating action of the chloride ion on leaf yield.

rates of gain for the higher rates of fertilization are less than those for the lower rates (fig. 4). However, there is uncertainty as to what extent the result obtained with 80 pounds of nitrogen as compared with the lower rates is to be attributed merely to the quantity of nitrogen used. In the first 5 years of the test all the nitrogen in the 80-pound rate was applied prior to transplanting, as was done with the lower rates of fertilizing. Under these conditions the concentration of the soil solution seemed to be excessive, the plants were retarded in growth in the early stages, and the final yield from the 80-pound rate averaged only slightly better than that from the 40-

pound rate. In 1925, and thereafter, one half of the nitrogen of the 80-pound rate was applied about 30 days after transplanting. This plan of splitting the nitrogen application prevented the early retarding action on growth of the plants and materially increased the yield. For the period in which all of the nitrogen was applied prior to transplanting, the average increase from the 80-pound rate of fertilizing above the 40-pound rate was only 39 pounds or 4.5 percent, while splitting the 80-pound ration of nitrogen in later years gave an increase of 145 pounds or 14 percent. It appears, therefore, that the method of application became a factor of importance when the rate of nitrogen fertilization was increased to 80 pounds per acre. Nothing can be said as to whether fractionation of the applications in the lower rates of nitrogen fertilization would have materially affected the results.

Under the conditions of the experiment, with an average yield of 614 pounds of leaf on the control plots, for the entire 10-year period the net increases in yield per acre for each successive 10-pound increment of nitrogen in the fertilizer in the nitrate of soda series were 131, 127, 78, and 22 pounds, respectively, for the 10-, 20-, 30-, and 40-pound rates of fertilizing. The 80-pound rate of fertilization gave an average increase in yield over the 40-pound rate amounting to 92 pounds per acre. Additional information on the effects of increasing the nitrogen supply on yield of leaf is furnished by the combined average results for the 10-year period obtained with nitrate of soda, ammonium chloride, and ammonium sulphate at the rates used, namely, those supplying 20, 40, and 80 pounds of nitrogen per acre. The first 20-pound increment of nitrogen gave an increase in yield of 277 pounds of leaf and the second 20-pound increment produced a further increase of 97 pounds, as compared with 258 pounds and 100 pounds, respectively, obtained with the nitrate of soda alone. The 80-pound rate of fertilization gave an increase over the 40-pound rate amounting to 75 pounds per acre.

The results of the supplementary tests (table 7) are not strictly comparable with those of the primary series, the average yield of the controls for the period of the test of the former being 507 pounds against 631 pounds for the controls of the latter series for the same period. On this soil the increase in yield of leaf tobacco for the first 20 pounds of nitrogen applied as ammonium sulphate was 254 pounds and the second 20 pounds gave a further increase of 169 pounds. Thus, on the poorer soil the fertilizer nitrogen at the higher rate has been somewhat more effective in promoting growth of the crop although the actual yields have been lower than those of the primary series. The urea and the Ammo-Phos show about the same relative efficiency at the two rates of application as does the ammonium sulphate.

TABLE 7.—Average acre yield and value and average price per pound of tobacco on duplicate plot sections in the supplementary tests with nitrogen applied at different rates and derived from different sources, 1921-29

[Plot 30 received an additional application of 80 pounds of phosphoric acid per acre. Results for 1924 are not included in the 8-year averages]

Plot no.	Nitrogen applied		Yield									
	Quantity per acre	Source	1921	1922	1923	1924	1925	1926	1927	1928	1929	8-year average
			Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.
22	0	-----	525	162	520	535	600	505	550	475	675	484
23	20	Urea	855	365	775	855	1,025	915	1,110	140	960	806
24	20	Ammonium sulphate	780	350	795	477	855	875	1,000	460	965	761
25	20	Ammo-Phos	570	432	660	895	875	935	975	480	1,010	742
26	0	-----	505	245	615	567	725	690	505	250	740	534
27	40	Urea	775	730	980	1,227	1,145	1,190	1,230	540	1,395	998
28	40	Ammonium sulphate	760	522	820	582	1,120	1,095	1,265	540	1,320	930
29	40	Ammo-Phos	470	457	865	1,155	1,040	1,080	1,300	610	1,265	888
30	40	Ammonium sulphate	850	622	975	562	1,130	1,090	1,210	640	1,255	972
31	0	-----	380	217	575	692	725	835	660	245	655	524
Average			648	410	758	756	924	921	971	438	1,024	762

Plot no.	Nitrogen applied		Value										8-year average price per pound
	Quantity per acre	Source	1921	1922	1923	1924	1925	1926	1927	1928	1929	8-year average	
			Dol.	Dol.	Dol.	Dol.	Dol.	Dol.	Dol.	Dol.	Dol.	Dol.	
22	0	-----	98	17	87	70	65	65	34	16	38	52	
23	20	Urea	201	75	154	138	163	194	220	91	174	159	
24	20	Ammonium sulphate	203	65	216	79	171	178	180	94	158	158	
25	20	Ammo-Phos	130	75	150	149	172	187	165	100	171	144	
26	0	-----	94	37	113	66	80	97	38	36	87	73	
27	40	Urea	197	157	295	249	295	234	246	126	184	213	
28	40	Ammonium sulphate	168	99	214	66	246	240	260	109	229	196	
29	40	Ammo-Phos	107	79	260	183	257	215	267	134	207	190	
30	40	Ammonium sulphate	198	101	278	60	291	237	260	143	219	216	
31	0	-----	66	18	114	88	68	98	30	31	72	62	
Average			146	72	187	115	178	175	170	88	154	146	

Tables 5 and 7 show clearly that weather conditions have profoundly affected the results. Taking the annual averages of all treatments as an index, in the primary series the yield in 1922 and 1928 was only about 500 pounds, while in 1927 and 1929 the yield was about 1,100 pounds. In the supplementary series the corresponding yields were approximately 400 and 1,000 pounds. These wide variations in yield from year to year apply to the controls and to all treatments. However, the widest fluctuations in yield are seen in the heaviest rates of fertilizing. It is interesting to note, also, that the changes in yield in the control plots from year to year are not always in the same direction as the changes in yield in the plots receiving nitrogen. For the most part, however, the same general direction of change in yield is seen in the plots receiving different quantities of nitrogen. Considering both the bad and the good years, it appears that 20 pounds of nitrogen per acre has given more consistent and relatively larger increases in yield than the 40-

and 80-pound rates of fertilizing. The generally poor results of 1928 were due to the extraordinarily heavy rainfall in August, and the very low yields of 1922 probably were caused also by the heavy rainfall of June and July. Taking the results as a whole, nitrogen was especially effective as a fertilizer in 1920, 1925, 1927, and 1929. While there is no close correlation with rainfall, it appears that the good results obtained in these years are associated with a moderate to a fairly liberal rainfall during June, July, and August. The results were less satisfactory in the relatively dry season of 1921. In 1926, the only year in which the first 20 pounds of fertilizer nitrogen was markedly ineffective, the rainfall for July and August was rather heavy.

The duration of the tests was too short, of course, to develop definite trends in the results which would be independent of seasonal effects. However, it is evident that there has been no sustained or progressive decline in yield through the period of the tests even on the control plots which received no nitrogen during the 11 years of the experiment. It seems that a low balance in the nitrogen supply of the soil is established and maintained through additions from outside sources, such as fixation of nitrogen by micro-organisms of the soil.

EFFECTS OF FORM OF NITROGEN APPLIED

The data in table 6 show the comparative effects of the different forms of nitrogen used in the experiments on the average yield of tobacco over a period of 10 years for the primary series and 8 years for the supplementary series. All forms of nitrogen tested were used at the rates of 20 and 40 pounds per acre and 3 forms also were tested at the rate of 80 pounds per acre. At the 20-pound rate, ammonium chloride stands out as producing the highest yield in the primary series and ammonium nitrate ranks second, while at the 40-pound rate these 2 forms of nitrogen jointly head the list, with no material difference between them. In these tests nitrate of soda and ammonium sulphate gave somewhat lower yields, and at both the 20- and 40-pound rates these 2 sources of nitrogen produced results agreeing rather closely. At the 80-pound rate of application ammonium chloride, ammonium sulphate, and nitrate of soda gave practically identical results.

When used to supply 20 pounds of nitrogen per acre cyanamide gave fairly satisfactory results, the yield falling somewhat below that of nitrate of soda or ammonium sulphate. At the 40-pound rate the results with cyanamide were unsatisfactory. The yield was the same as with the 20-pound rate and the lower leaves of the plant developed a characteristic spotting although in some years the leaves eventually recovered from these toxic effects. The spots produced on the leaves of tobacco by cyanamide resemble somewhat those caused by magnesium deficiency but do not so definitely originate at the tip or margin of the leaf.

In the supplementary series of tests urea gave appreciably better yields than ammonium sulphate at both the 20- and 40-pound rates of fertilization. Ammo-Phos fell somewhat below the ammonium sulphate in efficiency at the two rates of application because of poor results prior to application of magnesian limestone to the soil. The yield from ammonium sulphate was increased slightly by an increased supply of precipitated dicalcic phosphate. In the plot tests with a

urea phosphate which were added to the primary series in 1923 the average increase in yield for the 8-year period with 20 pounds of nitrogen per acre from this source was 187 pounds and with 40 pounds of nitrogen per acre the increase in yield was 407 pounds. The average yield of the control (control plot 1) for the same period was 817 pounds. As previously pointed out (p. 9), the yield on plot 1 has materially exceeded that of the remaining controls, indicating that the soil of the urea-phosphate plot was somewhat more productive than that of the other plots in the series. The indications are that urea phosphate will compare favorably with the other sources of nitrogen used in the experiments.

The relative efficiency of the different sources of nitrogen varied somewhat from year to year. On a comparative basis, nitrate of soda gave poorest results in 1919 and 1926 and best results in 1925; ammonium sulphate ranked low in 1921 and high in 1920; ammonium nitrate was not notably low in efficiency in any year but was particularly effective in 1921 and 1923; ammonium chloride made its poorest showing in 1921 and its best showing in 1919 and 1927. These results, however, do not seem to be closely correlated with differences in rainfall. In the supplementary series there were no striking variations from year to year in relative efficiency of urea, Ammo-Phos and ammonium sulphate.

RESULTS WITH MONOAMMONIUM PHOSPHATE AND NITRATE OF POTASH

In a series of tests with monoammonium phosphate and nitrate of potash the treatments were run in duplicate, and, as in the other tests, each plot was divided at harvest time into two equal sections, A and B. The tests have been made each year beginning with 1925, but the crop was so badly damaged by a severe rain and wind storm in August 1928 that reliable yield data could not be obtained for that year. The control plots received no fertilizer at all. The detailed results for the years 1925-27, and 1929 are shown in table 8.

TABLE 8.—Average yield of tobacco on duplicate sections, A and B, of plots run in duplicate in the years 1925-27 and 1929, showing the rapid decline in yield obtained with a fertilizer composed of ammonium phosphate and nitrate of potash as a result of rapid depletion of the soil supply of calcium and magnesium

Plot no.	Sources of nitrogen	Acre yield of tobacco											
		1925			1926			1927			1929		
		A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
1.....	Control.....	Lb. 490	Lb. 520	Lb. 565	Lb. 220	Lb. 360	Lb. 200	Lb. 110	Lb. 300	Lb. 205	Lb. 0	Lb. 0	Lb. 0
2.....	Cottonseed meal and nitrate of soda.....	910	890	900	1,000	820	910	860	1,020	940	800	950	875
3.....	Ammonium phosphate and nitrate of potash.....	850	770	810	540	810	675	290	870	450	190	640	370
4.....	Control.....	320	320	420	310	200	300	120	220	170	0	0	0
5.....	Cottonseed meal and nitrate of soda.....	880	720	800	960	840	900	940	970	955	950	770	800
6.....	Ammonium phosphate and nitrate of potash.....	800	790	795	610	750	780	500	640	570	200	300	250

The two outstanding features of the results are: (1) The rapid decline in average yield from year to year obtained with the mixture of ammonium phosphate and nitrate of potash, and (2) the marked differences in rate of this decline in yield on different portions of the experimental area, as compared with the relatively uniform yields produced by a combination of nitrate of soda, cottonseed meal, superphosphate, and sulphate of potash. In the first year of the test the highly concentrated fertilizer supplying only nitrogen, phosphorus, and potassium gave rather satisfactory results, but beginning with the second year the yield on section A of plot 3 rapidly declined to the point of almost complete failure. The decline on sec-



FIGURE 5.—Tobacco plants showing poor results obtained with a fertilizer consisting of nitrate of potash and monoammonium phosphate when no calcium or magnesium is added to the soil. Plot at left of measuring rod was fertilized with cottonseed meal, nitrate of soda, superphosphate, and sulphate of potash. Plot at right (four rows) received the same quantities of nitrogen, phosphoric acid, and potash, derived from nitrate of potash and monoammonium phosphate, the two rows at extreme right receiving in addition 500 pounds per acre of magnesian limestone.

tion B of this plot was definite but much less pronounced. Rapid decline in yield first began in 1927 on section A of plot 6, closely followed by similar results on section B of this plot.

In 1928 limestone containing 20 percent of magnesia was applied in the drill to two of the four rows in plots 3 and 6 at the rate of 500 pounds per acre, and this treatment was repeated in 1929. That the magnesian limestone was effective as an adjunct to the ammonium phosphate and nitrate of potash combination as a fertilizer is shown in figure 5.

These results with ammonium phosphate and nitrate of potash furnish a logical explanation of the comparatively unfavorable results obtained with Ammo-Phos and the increased yield with ammonium sulphate when additional phosphate was used in the supplementary

series discussed above. The poor results with Ammo-Phos were obtained in the early years of the experiment before the necessary quantities of calcium and magnesium were supplied in the form of magnesian limestone. Extensive observation indicates that the normal application of phosphoric acid used on the ammonium-sulphate plots was ample for crop needs, and it is believed that the beneficial effect of the additional phosphate was due to the increased supply of calcium which was thus provided.

APPLICATION OF THE MITSCHERLICH FORMULA

Mitscherlich (18) in 1909 and in later years developed the theory that the relationship existing between successive increments of a growth factor and the corresponding increments of yield can be expressed mathematically by the well-known equation—

$$\frac{dy}{dx} = (A - y) \cdot c \quad (1)$$

in which y is the increment of yield resulting from an increment x of a growth factor, such as one of the essential chemical elements, A is the limit toward which the yield approaches as x increases indefinitely, and c is a constant which is characteristic of the growth factor. According to this "effect law of growth factors" the increases in yield resulting from successive equal increments of a growth factor are proportional to the differences between the obtained yields and the maximum yield attainable. In other words, the successive increments of yield form a decreasing geometric series having a constant ratio. Thus, if the first unit of a growth factor produces 50 percent of the maximum yield obtainable the second unit will produce a further increase amounting to 50 percent of the difference between the yield from the first unit and the maximum or 25 percent, so that the total yield from the two units is 75 percent of the maximum. In the same way the third unit will produce a further increase of half the remainder of the maximum or 12.5 percent, the total yield being 87.5 percent of the maximum, and so on.

From the above equation the author derives by integration and transformation the equation—

$$\log (A - y) = \log A - c \cdot x \quad (2)$$

by means of which the expected yield y from a quantity x of a growth factor may be computed for given values of A and c . Where the yield produced by a soil without addition of a particular plant nutrient or fertilizer has been determined by field or pot tests under a given set of conditions, and the yield y produced by a quantity x of this nutrient likewise has been determined, the value of A may be computed according to Mitscherlich, by a modification of equation (2)—

$$A = \frac{ky - y_0}{k - 1} \quad (3)$$

in which $k = 10^{c \cdot x}$ and y_0 is the yield obtained when none of the plant nutrient involved is added to the soil. This method is commonly

employed by him for determining A in the case of the fertilizer element nitrogen, and it presupposes of course that the numerical value of c is known.

In fertilizer experiments with agricultural soils, in addition to the quantity of fertilizer x added to the soil, account must be taken of the quantity of the particular element involved, which is already present in the soil. This the author does by introducing the value for the soil content of this element, designated as b , into equation (2):

$$\log (A-y) = \log A - c \cdot (x+b) \quad (4)$$

When none of the fertilizer element is added to the soil x , of course, becomes zero so that for determining b , Mitscherlich employs the equation—

$$b = \frac{\log A - \log (A-y_0)}{c} \quad (5)$$

After finding the values of A and b it is a simple matter to ascertain by means of equation (4) the expected yield for any value of x .

Mitscherlich's law requires that for any particular growth factor the value of c be constant under all conditions, and the author himself has stated that on the soundness of this conception the whole law must stand or fall.⁴ For example, the element nitrogen is said to have a constant effect value, c , which is independent of weather, the plant food supply of the soil, or even the character of the plant. From a very large number of pot tests Mitscherlich has arrived at the numerical value of 0.122 for c in the case of the fertilizer element nitrogen. However, the author points out that c can have a constant value only so long as the value for x is expressed in terms of a fixed unit, and the above numerical value for c in the case of nitrogen applies when x represents the number of quintals of fertilizer nitrogen per hectare of land, which corresponds to approximately 90 pounds per acre.

In applying this law to the yield data for leaf and stalk and the combined yields of the two plant parts which were obtained with nitrate of soda, only the average values for a period of 10 years will be considered. These data cover the period 1919-29 except that the results for 1922 are omitted because of the very unsatisfactory yields obtained in that year. Taking 90 pounds per acre as the unit of fertilizer nitrogen, the rates of nitrogen fertilization employed, namely 10, 20, 30, 40, and 80 pounds per acre, correspond to 0.11, 0.22, 0.33, 0.44, and 0.88 units, respectively. The actual yields of leaf, stalk, and leaf and stalk combined are contained in table 9 and are shown graphically in figure 6. When the maximum possible yields, A , are computed by means of equation (3) with c having the numerical value 0.122 the respective values obtained for leaf, stalk, and leaf and stalk combined are 5,191, 3,936, and 9,127 pounds per acre. By means of equation (5) the indicated quantity of reserve nitrogen in the soil, b , is found to be 41.4 pounds per acre when leaf-yield data are considered and 30.3 pounds when yields of stalk are

⁴ Since this bulletin was prepared for publication there has appeared an article by the late W. J. Spillman (U. S. Dept. Agr. Tech. Bull. 348. Use of the Exponential Yield Curve in Fertilizer Experiments, 1933) in which the author expresses the view that Mitscherlich's values for c are not always applicable to the results of field experiments in the United States and new values must be obtained for each new set of conditions.

considered, the mean value being about 36 pounds. The expected yields for the several rates of fertilization are determined by introducing into equation (4) the values for *A* and *b* (table 9 and fig. 6).

TABLE 9.—Actual average yields of leaf and of stalk and the two combined for a period of 10 years and the corresponding theoretical yields computed by the Mitscherlich equation, using as base values the actual yields at three rates of fertilization and two numerical values for the constant *c*; including in each case the computed maximum possible yield *A*, and the quantity of the soil's reserve supply of nitrogen *b*

YIELDS OF LEAF PER ACRE

Quantity of fertilizer nitrogen used per acre		Actual yield per acre	Computed yields, using as base values the actual yields with—													
			10 pounds of nitrogen per acre				40 pounds of nitrogen per acre				80 pounds of nitrogen per acre					
			c=0.122		c=1.2		c=0.122		c=1.2		c=0.122		c=1.2			
			Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)		
Pounds	Units	Lb.	Lb. (629)	Lb.	Lb. (768)	Lb.	Lb. (629)	Lb.	Lb. (629)	Lb.	Lb. (629)	Lb.	Lb. (629)	Lb.	Lb. (629)	
0	0															
10	.11	768	(768)			727	-41	769	+1	690	-79	774	+6			
20	.22	803	903	+10	871	-22	822	-71	872	-21	767	-124	881	-12		
30	.33	986	1,033	+47	947	-39	914	-72	948	-38	833	-153	960	-26		
40	.44	1,064	1,159	+95	1,003	-61	(1,004)		(1,004)		897	-107	1,018	+14		
80	.88	1,134	1,629	+495	1,113	-21	1,335	+201	1,115	-19	(1,134)		(1,134)			
<i>A</i>			5,191		1,160		3,853		1,163		2,034		1,182			
<i>b</i>			41.4		25.5		57.0		25.4		77.3		24.8			

YIELDS OF STALK PER ACRE

		Actual yield per acre	Computed yields, using as base values the actual yields with—												
			10 pounds of nitrogen per acre				40 pounds of nitrogen per acre				80 pounds of nitrogen per acre				
			c=0.122		c=1.2		c=0.122		c=1.2		c=0.122		c=1.2		
			Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	
Pounds	Units	Lb.	Lb. (355)	Lb.	Lb. (464)	Lb.	Lb. (355)	Lb.	Lb. (355)	Lb.	Lb. (355)	Lb.	Lb. (355)	Lb.	Lb. (355)
0	0	355	(355)			440	-85	485	+21	411	-53	472	+8		
10	.11	464	(464)			534	-88	581	-41	496	-156	557	-65		
20	.22	622	570	-52	654	-78	619	-46	651	-14	519	-140	621	-44	
30	.33	685	672	-13	664	-61	619	-46	651	-14	519	-140	621	-44	
40	.44	763	772	+9	648	-55	(703)		(703)		571	-132	608	-35	
50	.55	761	1,140	+379	734	-27	1,000	+248	867	+46	(761)		(761)		
<i>A</i>			3,036		771		3,351		850		2,208		800		
<i>b</i>			30.3		20.0		30.0		17.0		50.2		19.1		

YIELDS OF LEAF AND STALK COMBINED PER ACRE

		Actual yield per acre	Computed yields, using as base values the actual yields with—												
			10 pounds of nitrogen per acre				40 pounds of nitrogen per acre				80 pounds of nitrogen per acre				
			c=0.122		c=1.2		c=0.122		c=1.2		c=0.122		c=1.2		
			Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	Value obtained	Excess (+) or deficit (-)	
Pounds	Units	Lb.	Lb. (984)	Lb.	Lb. (1,232)	Lb.	Lb. (984)	Lb.	Lb. (984)	Lb.	Lb. (984)	Lb.	Lb. (984)	Lb.	Lb. (984)
0	0	984	(984)			1,174	-188	1,253	+21	1,111	-121	1,246	+14		
10	.11	1,232	(1,232)			1,357	-125	1,451	-64	1,233	-282	1,439	-76		
20	.22	1,515	1,472	-43	1,416	-99	1,535	-116	1,598	-53	1,352	-201	1,581	-70	
30	.33	1,651	1,705	+54	1,551	-100	1,535	-116	1,598	-53	1,352	-201	1,581	-70	
40	.44	1,707	1,931	+224	1,650	-57	(1,707)		(1,707)		1,468	-239	1,687	-20	
80	.88	1,805	2,768	+963	1,848	-17	2,346	+499	1,921	+26	1,895		1,895		
<i>A</i>			9,127		1,931		7,204		2,011		5,143		1,983		
<i>b</i>			36.5		23.2		47.0		21.9		68.0		32.3		

In the above computations the increases in yield resulting from the first 10 pounds of fertilizer nitrogen are used as base values in determining *A* and *b*. When the increases in yield obtained with higher rates of fertilization are employed for the computations progressively lower values for *A* and correspondingly higher values for *b* are

obtained. As regards the data for the leaf, this discrepancy can be removed only by greatly increasing the numerical value of c , as, for

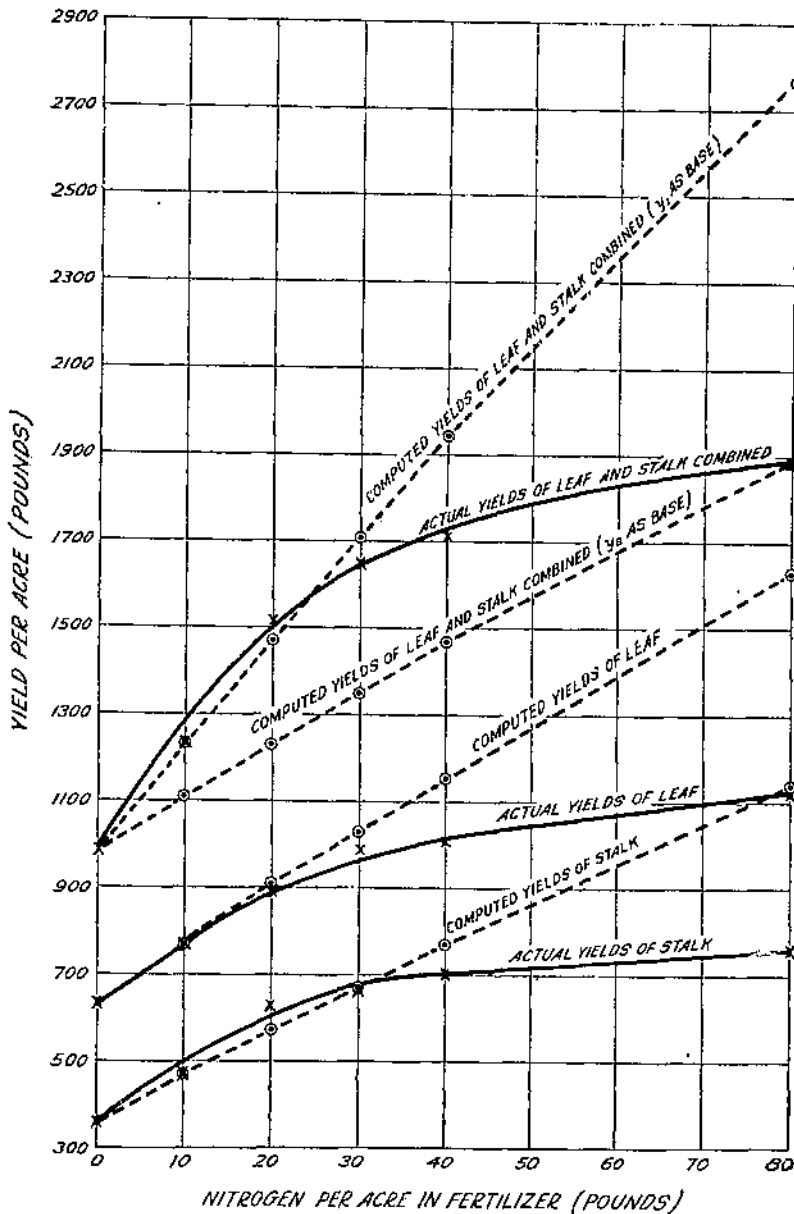


FIGURE 6.—Average actual yields of leaf and stalk and the two combined at various rates of fertilization with nitrogen for a period of 10 years, and the corresponding theoretical yields computed by means of the Mitscherlich formula, using 6,122 as the numerical value of the proportionality factor, c , of the Mitscherlich equation. The increases in yield obtained from the first 10 pounds of fertilizer nitrogen per acre (y_1) were used as base values in the computations, as was also the increase from 80 pounds of nitrogen (y_80) in case of the leaf and stalk combined.

example, by raising it to 1.2, which is 10 times the value assigned to it by Mitscherlich. In this case the maximum yield obtainable, A , is

reduced to slightly less than 1,200 pounds, and the value for the effective supply of nitrogen in the soil, *b*, is lowered to about 25 pounds. With this value for *c* the computed yields for the various rates of fertilization also agree fairly well with the actual yields, although the theoretical yields for the intermediate rates of fertilization are somewhat too low (table 9).

As to the data for the stalk, no value for *c* has been found which will give consistent figures for *A* and *b* when the yields at different rates of fertilization are used in the calculations. On the whole, best results are obtained when *c* is assigned a numerical value approximating unity. However, this value of *c* does not give satisfactory agreement between the computed and the actual yields for the several rates of nitrogen fertilization. For leaf and stalk combined there is only a fair degree of consistency in the values of *A* and *b* obtained by using the yields produced by different rates of fertilization in making the computations and assigning to *c* a value of 1.2. The differences between the computed and the actual yields for the several rates of fertilization in some instances are rather large.

INFLUENCE OF NITROGEN SUPPLY ON MARKET VALUE OF LEAF

In order to obtain reliable information on the comparative commercial value of the crops produced with the different quantities and forms of nitrogen, representative samples were laid before experienced buyers of Maryland tobacco each year for their valuation of the product. The value per acre and the average price per pound of the crop for each treatment as thus determined are shown in tables 7 and 10.

TABLE 10.—*Acre value and average price per pound of tobacco in primary series of tests with nitrogen applied at different rates and derived from different sources, 1919-29*

Plot no.	Nitrogen applied		Acre value (dollars)										10-year average price per pound		
	Quantity per acre	Source	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928		1929	Average
	Lb.														Cents
1	0	182	133	05	61	189	104	115	145	93	07	125	119	17
2	20	Cyanamide.....	240	227	109	101	208	240	195	108	143	113	164	173	21
3	20	Ammonium nitrate.....	269	259	147	103	203	170	109	184	257	137	161	100	23
4	20	Ammonium sulphate.....	250	288	133	129	209	174	215	163	219	129	148	104	22
5	0	134	108	86	25	193	90	83	107	80	55	91	97	16
6	20	Ammonium chloride.....	198	185	63	170	250	236	209	197	76	133	135	161	18
7	20	Nitrate of soda.....	201	227	163	121	237	180	228	142	205	119	145	180	21
8	10	do.....	207	213	104	40	167	149	152	101	112	101	135	134	18
9	10	123	102	81	20	159	61	98	91	67	42	69	84	15
10	30	Nitrate of soda.....	249	277	309	61	360	202	218	187	208	119	150	206	22
11	40	do.....	227	340	222	103	281	188	252	167	170	121	134	201	21
12	40	Ammonium sulphate.....	292	353	221	161	270	103	210	200	177	141	150	214	23
13	0	133	87	88	20	177	57	84	101	76	45	78	80	15
14	40	Ammonium chloride.....	231	242	191	108	199	283	237	192	137	142	96	177	18
15	40	Ammonium nitrate.....	327	401	272	112	290	210	240	222	149	126	144	228	22
16	40	Cyanamide.....	254	289	117	80	157	185	184	200	155	78	115	104	19
17	0	134	118	117	19	176	60	98	67	32	39	59	89	16
18	80	Nitrate of soda.....	230	301	202	35	302	167	175	144	214	123	163	181	17
19	80	Ammonium sulphate.....	332	345	138	28	146	86	232	165	139	95	185	17	
20	80	Ammonium chloride.....	267	249	53	27	87	124	74	112	96	131	90	123	11
21	0	143	138	80	21	130	40	85	84	31	39	54	82	14
	Average.....	219	231	188	72	218	150	170	155	136	102	126	150

* Results for 1924 are not included in the 10-year average.

All rates of application of nitrogen increased the gross value of the crop over that of the control plots. Considering the data with nitrate of soda, the first 10 pounds of nitrogen per acre gave an increase in value of \$50 per acre, the second 10 pounds a further increase of \$46, while the third 10 pounds gave the maximum value, representing an additional gain of \$26. There was no significant difference in value of crop between the 30- and the 40-pound rates of fertilizing, but there was a material decrease in crop value with the use of 80 pounds of nitrogen per acre. Substantially the same relationships obtain with respect to price per pound of the tobacco. All sources of nitrogen except cyanamide gave somewhat higher gross values per acre at the 40-pound rate than at the 20-pound rate, but differences in price per pound of the leaf were hardly large enough to be significant. The effects of the 80-pound rate were the same with ammonium sulphate as with nitrate of soda, but with ammonium chloride the decreases in value per acre and price per pound of leaf were much more marked.

The effects of the different sources of nitrogen on the price per pound of the tobacco leaf and their action in increasing the gross value of the crop per acre are summarized in table-11. The average price per pound of the crop on the control plots receiving no nitrogen was 15.5 cents in the primary series and 12.5 cents in the supplementary series. The corresponding gross crop values per acre were \$93 and \$62, respectively. In the primary series, ammonium nitrate and ammonium sulphate gave the best quality of tobacco at both the 20- and the 40-pound rates of fertilizing, and the ammonium nitrate gave slightly higher crop values per acre than the sulphate. Nitrate of soda ranked next in value per pound and gross value per acre of the crop produced. Cyanamide produced a fairly good quality of leaf at the 20-pound rate while with ammonium chloride a comparatively poor quality was produced at all rates of fertilizing. Until rather recently it has not been the practice of buyers in southern Maryland to determine the burning qualities of tobacco in estimating its market value, the assumption being that all tobacco having the desired color and texture will burn satisfactorily. Consequently, the values shown in table 11 were arrived at independently of the factor of combustibility. But for this circumstance the market value of the tobacco grown with ammonium chloride would have been placed considerably lower.

In the supplementary series urea and ammonium sulphate at either rate of fertilizing did not differ materially in quality of the tobacco produced, but at the higher rate of fertilizing with ammonium sulphate an increased rate of application of calcium phosphate was necessary to obtain best results as to value of crop per acre. The reason for this situation has already been given. The somewhat poorer showing of Ammo-Phos is due primarily to the reduced growth obtained in the early years of the test before dolomitic limestone had been applied. After calcium and magnesium had thus been added to the soil Ammo-Phos gave good results.

TABLE 11.—Increase in value per acre and in the estimated value per pound of tobacco leaf in the primary and supplementary series of tests obtained with nitrogen derived from different forms and applied at different rates

[The last plot in the supplementary series received a double ration of phosphoric acid]

Series of test and source of nitrogen	Increase in acre value of crop			Average value per pound of leaf		
	20 pounds nitrogen	40 pounds nitrogen	80 pounds nitrogen	20 pounds nitrogen	40 pounds nitrogen	80 pounds nitrogen
Primary series:	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Cents</i>	<i>Cents</i>	<i>Cents</i>
Cyanamide.....	80	71	21	19
Ammonium nitrate.....	106	135	23	22
Ammonium sulphate.....	101	121	92	23	23	17
Ammonium chloride.....	71	84	30	18	18	11
Nitrate of soda.....	97	108	88	21	21	17
Supplementary series:						
Urea.....	97	151	20	21
Ammonium sulphate.....	96	134	21	21
Ammo-Phos.....	82	125	10	21
Ammonium sulphate.....	154	22

RELATION OF NITROGEN SUPPLY TO RATE AND TYPE OF GROWTH LEAF, STALK, AND ROOT GROWTH

In 1927, data on air-dried weights of leaf, stalk, and root were obtained on 20 plants in a control treatment and in the plots receiving nitrogen in the form of nitrate of soda at each rate included in the primary series. The mature plants were harvested by cutting the stalk at the ground level, and the weights of leaf and stalk were obtained after the curing process was completed. After harvest the roots were carefully dug, washed with water, and allowed to dry in the air under shelter. It was not possible to make complete recovery of the roots, and the data for this part of the plant are only approximately correct. The percentages of leaf, stalk, and root thus obtained are shown in table 12.

TABLE 12.—Percentages of air-dried leaf, stalk, and root in plants receiving different quantities of nitrogen, crop of 1927

Plot no.	Nitrogen applied per acre	Percentages of plant parts			Plot no.	Nitrogen applied per acre	Percentages of plant parts		
		Leaf	Stalk	Root			Leaf	Stalk	Root
	<i>Pounds</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>		<i>Pounds</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
9.....	0	68.5	16.5	15.0	10.....	30	68.1	22.0	14.9
8.....	10	64.3	19.4	16.3	11.....	40	61.9	23.1	15.0
7.....	20	65.4	20.9	13.7	12.....	80	66.3	20.5	13.2

The range in percentage of root under the different treatments is rather narrow and does not indicate any clearly defined effect of increasing quantities of nitrogen in the fertilizer. The difference in proportions of leaf and stalk are more definite and are pretty well in line with the much more comprehensive data contained in table 5. The proportions of leaf obtained with increasing quantities of nitrogen derived from nitrate of soda, ammonium sulphate, and ammonium chloride, expressed as percentages of the combined weight of cured leaf and stalk, are shown graphically in figure 7. The source of the

nitrogen affects somewhat the proportions of leaf and stalk. At all rates used, ammonium sulphate gave the lowest percentage of leaf. Reference has already been made to the stimulating action of the chloride ion on leaf development, and it will be seen that ammonium chloride has given the highest percentage of leaf of all the forms of nitrogen tested. The sharp rise in percentage of leaf with the 80-pound rate of fertilizing is especially noticeable.

The annual average yields of leaf and stalk for all treatments, as shown in table 5, demonstrate that weather conditions also may have a marked effect on the proportions of leaf and stalk. In general, in years of low crop yield there is a tendency toward a high percentage of leaf, and in years of high crop yield the tendency is toward a high percentage of stalk. This is equally true for the control plots receiving no nitrogen and those well supplied with this element. It appears

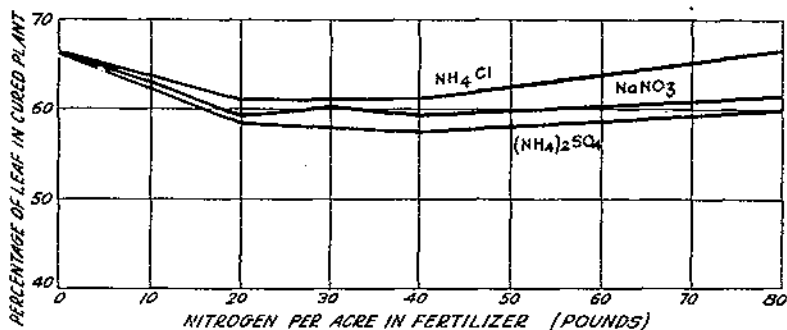


FIGURE 7.—Effects of quantity and source of the fertilizer nitrogen on the proportions of leaf and stalk in the cured plant. Nitrogen deficiency and a high nitrogen supply alike produce a small stalk as compared with a moderate supply of nitrogen. A stimulating action of the chloride ion on leaf development is shown by the results with ammonium chloride, while ammonium sulphate apparently tends to produce a relatively large stalk.

that environmental conditions exert a somewhat greater effect on the yield of stalk than on the yield of leaf.

To obtain information as to the effect of the supply of nitrogen on the proportions of midrib and lamina or web in the leaf, observations were made on samples of the cured crop of 1929 from the nitrate of soda plots receiving nitrogen at the rates of 20, 40, and 80 pounds per acre and the control plots which received no nitrogen. In the leaf from the latter plots the percentage of midrib was 27.1 and from the plots receiving 20, 40, and 80 pounds of nitrogen per acre the respective values were 29.3, 31.1, and 27.8. These results were confirmed in additional tests. Thus, the maximum percentage of midrib is obtained with a moderate supply of nitrogen, and either a very low or a high supply tends to reduce the relative weight of the midrib. In these experiments it was observed that the percentage of midrib is decidedly lower in the lower leaves of the plant than in the middle and upper leaves.

THE GROWTH RATE

In 1920 and again in 1926 observations were made on the rate of increase in height of selected plants in the primary series of plots. Since the results in the 2 years were about the same, only the 1926 data need be considered here. In a control plot and the plots receiving 20, 40, and 80 pounds of nitrogen from nitrate of soda and 40 pounds

from ammonium sulphate, ammonium nitrate, and ammonium chloride, respectively, two groups of plants were selected for study. One group representing each treatment consisted of 25 larger plants which had obtained an early start, while the other group contained 14 smaller plants, and measurements of height were made at intervals of 9 to 15 days. The results are shown in table 13. At the time of last measurement of total height none of the plants in either group receiving no nitrogen had flowered, but in all other instances many of the plants were in bloom.

TABLE 13.—Effects of nitrogen derived from different sources and applied at different rates on the average rate of increase in height, the number of leaves, and the length of internodes of 25 larger and 14 smaller tobacco plants, season of 1926

Number and size of plants	Nitrogen applied		Average total height on—					Average final height to first leafless sucker	Average number of leaves	Average length of internodes
	Quantity per acre	Source	July 23	Aug. 2	Aug. 11	Aug. 26	Sept. 4			
	Pounds		Inches	Inches	Inches	Inches	Inches	Inches		Inches
25 larger	10		5.7	9.8	14.5	27.7		49.2	34.6	1.16
	20	Nitrate of soda	6.0	18.0	28.8	55.1		49.8	27.2	1.48
	40	do.	5.7	14.6	20.1	53.2		42.6	27.2	1.55
	40	Ammonium sulphate	5.0	10.4	28.8	54.8		42.4	27.2	1.57
	40	Ammonium chloride	5.8	14.9	27.1	52.7		41.8	26.2	1.60
	40	Ammonium nitrate	5.9	15.5	29.2	54.5		40.6	27.3	1.40
14 smaller	80	Nitrate of soda	5.1	12.9	21.2	50.9		43.1	28.0	1.54
	0		2.9	5.0	8.5	18.0	21.0	32.8	34.9	.94
	20	Nitrate of soda	2.5	7.8	15.6	33.9	43.9	41.0	28.3	1.45
	40	do.	2.8	8.6	16.9	38.1	41.4	43.0	27.5	1.67
	40	Ammonium sulphate	3.0	9.0	17.4	36.1	47.3	42.5	28.4	1.48
	40	Ammonium chloride	2.8	8.0	18.0	40.6	52.2	44.4	27.4	1.60
	80	Ammonium nitrate	3.0	10.3	19.1	43.1	55.6	42.6	27.9	1.53
		Nitrate of soda	2.3	7.4	13.0	32.9	44.7	44.6	31.1	1.43

With no nitrogen supplied in the fertilizer the increase in height was at a slow rate while, on the whole, the most rapid rate was obtained with 40 pounds of nitrogen per acre in case of the smaller plants (fig. 8) and with 20 pounds of nitrogen per acre in the case of the larger plants. In both instances the rate of growth with 80 pounds of nitrogen was slightly less than with the lower rates of nitrogen fertilization, particularly in the early stages. The effects on the growth rate produced by differences in the form of nitrogen used were comparatively small. The final height of the stalk measured up to the position of the lowermost leafless branch increased somewhat with increase in the nitrogen supply. The data on increase in size of the leaf in the 1929 crop indicate that according to this method of measurement the intermediate and the highest rates of nitrogen fertilization produced about the same rate of growth in the later stages of leaf development. (See table 20.) It is apparent, however, that the maximum application of nitrogen produced the maximum rate of increase in size of leaf in the earlier stages of growth.

NUMBER, SIZE, AND SHAPE OF LEAVES

Counts were made of the total number of leaves produced per plant in the two groups of individuals used in the study of growth rate. The first counts were made in the early stages of growth so as to

avoid overlooking lower leaves which would disappear before the plants had reached maturity. All leaves up to and including the position of the lowermost leafless sucker or lateral branch were counted. The results are shown in table 13.

It will be seen that the delayed growth and flowering in the no-nitrogen treatments are associated with a definite increase in the number of leaves per plant, accompanied by a decrease in length of the internodes. Also, the 80-pound rate of nitrogen fertilization in comparison with lower rates produced a slight increase in number of leaves per plant in association with delayed growth and flowering.

In 1919, 1920, and 1923, measurements of length and width of leaves located at the center of the plant were made on 25 selected individuals in each of the plots receiving 20, 40, and 80 pounds of nitrogen as nitrate of soda, respectively, and in the controls. Measurements were made at time of topping on one series of leaves, about 2 weeks after topping on a second series of leaves immediately above the first series, and about 4 weeks after topping on a third series directly above the second series on the plant. Similar observations had been made in 1918 in another nitrogen test conducted on the same general plan and

located on the same soil type. It is known that the area of a tobacco leaf is approximately two thirds the product of the length and the width, and this formula was used to compute the leaf areas for comparison. To arrive at the effect of the nitrogen supply on the general shape of the leaf the ratio between the length and width was used. The averaged results for size and shape of leaf under the different treatments for the 4 years mentioned are shown in table 14. This table also contains data on observations made in 1929 on the effects of the nitrogen supply on the area of upper, middle, and lower leaves of the plant. The leaves used for the purpose were those employed for determining the relative weight per square foot as affected by the nitrogen supply (p. 29).

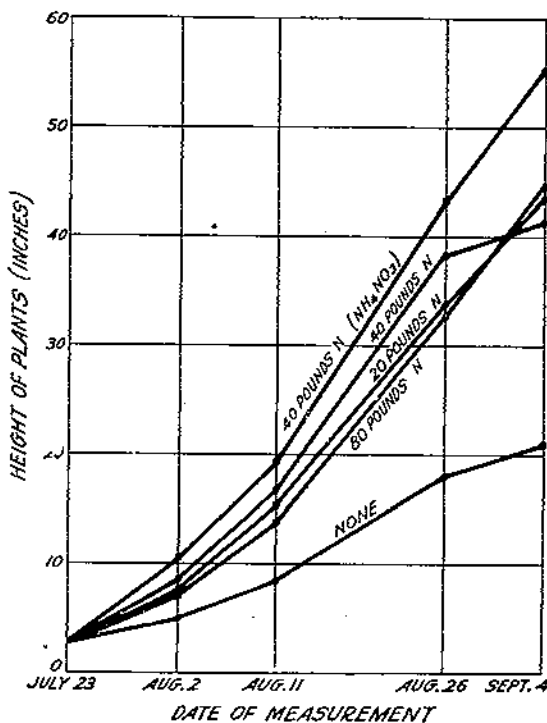


FIGURE 8.—Effect of the supply of fertilizer nitrogen on the growth rate as measured by increase in height. For the period covered, the growth rate of only the plants receiving no nitrogen and those receiving 40 pounds per acre from nitrate of soda show the typical sigmoid form of curve. Ammonium nitrate, supplying both ammonia and nitrate nitrogen, produced the most rapid rate of growth, while 40 pounds of nitrogen from nitrate of soda caused more rapid growth than 80 pounds.

TABLE 14.—*Effects of nitrogen applied at different rates as a fertilizer on the length, width, and area, and the ratio of length to width of green tobacco leaves in different years*

Quantity of nitrogen applied per acre (pounds)	Average shape and size of leaf, 1918-20, 1923				Area of leaves at different positions on stalk, 1920			
	Length	Width	Ratio of length to width	Area	Upper leaf	Middle leaf	Lower leaf	Average
	Inches	Inches		Sq. in.	Sq. in.	Sq. in.	Sq. in.	Sq. in.
0.....	17.65	8.17	2.17	90.7	71.4	99.9	104.8	92.0
20.....	21.11	10.35	2.05	146.9	68.5	145.2	146.1	119.9
40.....	21.17	10.70	1.98	152.4	87.4	171.1	156.0	138.2
80.....	23.45	12.50	1.88	197.0				

Increase in the nitrogen supply has a marked effect in increasing the size of the leaf, the area per leaf produced by the 80-pound rate being double that of the controls. The increase produced by the 40-pound rate over that of the 20-pound rate, however, is relatively small except in the middle and upper leaves of 1929. As the nitrogen supply is increased the leaf becomes progressively broader in proportion to length. To compare the effects of nitrate and ammonia nitrogen, observations were made in 1919, 1920, and 1923 on leaf dimensions in the plots receiving 40 pounds of nitrogen as ammonium sulphate. For the 3 years the average area per leaf obtained with the ammonia nitrogen was 141.7 square inches as compared with 143.1 square inches obtained with 40 pounds of nitrogen from nitrate of soda. The corresponding values for ratio of length to width of leaf were 2.06 and 2.09, respectively. It appears, therefore, that there was no difference of importance in the effects of the two forms of nitrogen on the dimensions of the leaf produced.

WEIGHT AND THICKNESS OF LEAF AND SPECIFIC GRAVITY OF LEAF MATERIAL

In 1929, groups of 20 plants each were selected on the plots receiving 20 and 40 pounds of nitrogen per acre in the fertilizer and on a control plot in order to make observations on effect of the nitrogen supply on the weight of leaf per unit of area after completion of the curing. The plants were topped in the usual manner, and just before harvest the length and width of an upper, middle, and lower leaf on each of the selected plants were measured and the area computed. The upper leaf in each instance was located approximately in the center of the upper third portion of the leaves of the plant, and the lower leaf likewise was located at or near the center of the lower third portion of the leaves. The plants were harvested and cured without removing the leaves from the stalks. After ascertaining the dry weight of the cured leaves the average weight per square foot of each lot was calculated. The results are shown in table 15.

TABLE 15.—Effects of nitrogen applied as a fertilizer at different rates on the weight per square foot, the thickness and the specific gravity of cured tobacco leaves, crop of 1929

Quantity of nitrogen applied per acre (pounds)	Weight of cured leaf per square foot				Average thickness of middle leaves in hundredths of a millimeter, measured with—		Specific gravity of the powdered leaf material		
	Upper leaf	Middle leaf	Lower leaf	Average	Large spindle head	Small spindle head	Upper leaf	Middle leaf	Lower leaf
	Grams	Grams	Grams	Grams					
0.....	5.458	5.490	6.405	5.817	50	26	1.4706	1.4966	1.5056
20.....	5.602	4.795	5.648	5.415	39	27	1.4540	1.4833	1.5175
40.....	4.979	4.356	5.744	5.026	36	22	1.4607	1.4839	1.5350
80.....					28	20			

The data in table 15 show that increase in the supply of fertilizer nitrogen results in a decrease in weight of a unit area of the leaf. This effect is produced alike in the lower, middle, and upper leaves of the plant. Confirmatory data are contained in the section of this bulletin dealing with processes of nutrition and metabolism. (See tables 20 and 32.)

It is a matter of some importance to know whether decrease in thickness of the leaf lamina is entirely responsible for the decided decrease in weight per unit area brought about by the high nitrogen supply but, unfortunately, no entirely satisfactory method of measuring the thickness of the cured leaf has been found. The leaf, after curing, presents an extremely uneven surface, and it is difficult to obtain consistent results in measurements made with a micrometer caliper. The readings obtained are greatly influenced by the moisture content of the leaf and by the size of the head of the measuring spindle of the instrument. Most work on the subject has been done with moist material, but in this state the tissues are easily compressed so that even with the lowest pressures that can be employed the readings are decidedly lower than when the material is measured in a dry condition. In the experience of the writers, best results are obtained with dry material. The apparent thickness is, of course, that of the fine network of veins in the leaf lamina, and naturally, when the head of the measuring spindle is small and precautions are taken to avoid the veins which are readily visible, lower readings will be obtained than when a measuring spindle with relatively large head is used. A large number of measurements were made with air-dried cured-leaf samples of the 1929 crop from the plots employed in the study of the weight of leaf per square foot as affected by the nitrogen supply. The leaves were taken from the middle portion of the plants. For the measurements, a micrometer dial gage reading to one hundredth of a millimeter, such as is used in measuring thickness of paper, was employed. Comparative measurements were made with spindle heads of approximately 13 and 6 millimeters diameter. It is well known that different portions of an individual leaf vary materially in thickness, the apical and marginal portions being decidedly thicker than the basal and interior parts (14). In the present case, readings were taken on small disks cut from various parts of the leaf and the results were averaged for the entire leaf. The final data are given in table 15

While the results are not entirely consistent, as a whole, they indicate that the reduced weight per unit area obtained with an increased supply of nitrogen is due at least in part to decrease in thickness of the leaf. The apparent decrease in the differences in thickness of leaf from the various treatments obtained with the smaller spindle head of the measuring instrument suggests that the vascular tissues are more prominent in the leaf grown with a low nitrogen supply. Doubtless the fine network of vascular tissue in the leaf plays a prominent part in the judgment formed by the expert tobacco buyer as to the so-called body and texture of the cured product. In any case, it is hardly possible to avoid the effects of the smaller veins in making direct measurements of leaf thickness by such methods as here employed, but the results have value from a practical standpoint, at least for comparative purposes. Since the material was measured in an air-dry condition the values obtained are higher than those reported by most other investigators for leaf thickness.

A limited number of observations were made on the apparent specific gravity of the ground-cured leaf material, using the samples from the 1929 crop that had been employed in the study of weight per unit area of the leaf. The determination was made simply by measuring the volume of toluol displaced by a known weight of the leaf material at 25° C., employing for the purpose the usual type of pycnometer. Toluol was used because of its slight solvent action on the constituents of tobacco. To facilitate removal of air from within and about the leaf particles, the apparatus was placed under a partial vacuum before completing the filling with toluol. A correction was applied for moisture content and adherent earthy matter of the air-dried sample. The results expressed as weight per cubic centimeter of the tobacco are contained in table 15. As compared with differences in weight per unit of area, the apparent differences in specific gravity or density of the solids of the leaf are very small. Assuming that the toluol effectively penetrated the leaf tissues, these values, of course, throw no light on the relative development of intercellular air spaces in the tissues of the leaf under the different nitrogen treatments.

TIME OF FLOWERING AND RIPENING OF LEAF

The terminal flower bud is normally the first to open on the tobacco plant, and its opening marks a definite stage of development which is easily observed. No personal equation is involved in determining the date of its opening, and for comparative purposes the appearance of this blossom furnishes a convenient point from which to reckon relative stages of development in the plant. It is recognized, however, that the opening of the terminal flower bud does not necessarily indicate a fixed stage of development of any particular leaf on the plant. Its significance attaches rather to developmental stage of the plant as a whole. Throughout this bulletin data on time of flowering and, with certain exceptions, the time of topping are based on the time of opening of the terminal blossom unless otherwise stated.

In the 4 years, 1919-22, observations were made on date of flowering of all individuals in each treatment of the primary series of plots. The counts were made at intervals of 3 days, and the plants were topped as they flowered. When 80 to 85 percent of all plants in the series had flowered, the remaining ones were topped without regard to stage of development. The data on flowering for the 4 years were

assembled in three groups: (1) Number of plants which had flowered up to 20 days before the final topping; (2) number which flowered in the 20 days immediately preceding the final topping; (3) number which had failed to flower at final topping. The results expressed in percentages of the total number of plants in each treatment are shown in table 16.

TABLE 16.—Average effect of varying quantities and forms of nitrogen applied in the fertilizer on time of flowering of tobacco, 1919-22

Plot no.	Nitrogen applied		Plants flowering—		Plants not flowering prior to final topping
	Quantity per acre	Source	20 to 40 days prior to final topping	During 20 days immediately preceding final topping	
	Pounds		Percent	Percent	Percent
1, 5, 9, 13, 17, 21.....	None		8.2	29.8	62.0
8.....	10	Nitrate of soda.....	17.4	47.6	35.0
7.....	20	do.....	28.1	54.6	17.3
10.....	30	do.....	36.4	43.2	10.4
11.....	40	do.....	37.8	46.9	15.3
18.....	80	do.....	31.3	50.6	18.1
4.....	20	Ammonium sulphate.....	55.1	34.4	10.5
12.....	40	do.....	67.1	24.0	8.9
19.....	80	do.....	49.4	42.1	8.5
6.....	20	Ammonium chloride.....	43.7	41.2	15.1
14.....	40	do.....	61.9	32.4	5.7
20.....	80	do.....	33.1	45.3	21.6
3.....	20	Ammonium nitrate.....	43.0	39.9	17.1
15.....	40	do.....	71.5	21.7	6.8
2.....	20	Cyanamide.....	26.0	33.3	40.7
16.....	40	do.....	25.4	42.2	32.4

It is evident that a very low nitrogen supply as represented by the control plots had a marked effect in delaying flowering. Addition of 10 pounds of nitrogen per acre had a decided effect in overcoming this delay, and an additional 10 pounds further hastened the reproductive process. Further increase in the nitrogen supply produced less significant results. On the whole, however, it appears that optimum conditions for flowering were supplied by 30 to 40 pounds of nitrogen per acre. The maximum rate of 80 pounds per acre seems to have slightly delayed flowering as compared with the 40-pound rate, at least in some cases.

As to the form of the nitrogen, cyanamide had a definite retarding action. When compared with the remaining sources nitrate of soda also tended to delay flowering.

Effects of the nitrogen supply on rate of development and subsequent decline of the leaf, more particularly as regards the lower leaves on the plant, were not entirely the same as those on time of flowering. Gain in weight per unit of area, that is, the accumulation of total dry matter which usually takes place during the ripening of the leaf, and the effects on these changes of the operation of topping or disbudding the plant, can be more satisfactorily dealt with in the section devoted to biochemical studies. Only changes in the general appearance of the leaves are considered here.

In general, the lower leaves reached full maturity earlier with the 20-pound rate of nitrogen fertilization than with the other treatments, and after a short time these leaves began to deteriorate and dry, a process popularly known as firing. Thus, with a relatively low content of nitrogen in the fertilizer there is a tendency for the lower leaves to

perish before the remaining leaves on the plant have attained full maturity. This tendency is less evident, however, where all nitrogen is omitted from the fertilizer. With the heavier rates of nitrogen fertilization there was little or no tendency toward firing in the lower leaves.

COLOR OF LEAF

Where all nitrogen was omitted from the fertilizer the leaves of the plants showed a pale yellowish-green color throughout the season, which is perhaps the most distinctive symptom of nitrogen hunger. As the nitrogen supply was increased the leaves showed a progressively darker green color. Also, during the ripening period of the leaves, following topping, there was less tendency with heavy nitrogen fertilization for the leaves to gradually lose their dark-green color, as usually happens in normal ripening of the leaf. In the cured leaf, the color was comparatively dark when nitrogen was omitted from the fertilizer and there was a peculiar dullness or lack of luster. As a rule the lighter and brighter colors were obtained with 20 to 40 pounds of nitrogen per acre. With the 80-pound rate dark colors with a decided greenish cast were often obtained, especially in the earlier years of the tests, although in the later years this was not always the case.

As to the effects of the form of nitrogen on color of the green and cured leaf, no clearly defined differences were observed except in the case of ammonium chloride. At the lower rates of application of this material no decided abnormality in color was seen. With the 80-pound rate, however, the green leaf in the field showed a characteristic yellowish-green color having an oily appearance, and the cured leaf was almost black.

BURNING QUALITIES OF LEAF

Samples of cured leaf of the 1925, 1927, and 1929 crops from the control plots and the nitrate of soda treatments, and from the 1925 and 1929 crop from the ammonium sulphate and ammonium chloride treatments were used for making the usual laboratory tests of fire-holding capacity of the leaf. As a hot point for igniting the leaf an electric cigar lighter was employed. On one half of each leaf the test was applied at four points near the margin and ranged from the tip to the base. The 1925 and 1927 samples were tested under ordinary laboratory conditions during the winter, that is, at a temperature of 70° to 75° F. and with a very low relative humidity. The 1929 samples were tested under accurately controlled conditions of temperature and humidity, namely, 77° and 54 percent relative humidity. The results are summarized in table 17.

TABLE 17.—Effects of form and quantity of the nitrogen supplied in the fertilizer on the fire-holding capacity of the cured leaf in the years 1925, 1927, and 1929

Nitrogen applied in fertilizer		Duration of glow		
Quantity per acre (pounds)	Source	1925	1927	1929
		Seconds	Seconds	Seconds
None.....		23	39.5	12.1
20.....	Nitrate of soda.....	9.9	14.6	3.1
40.....	do.....	11.8	8.1	3.2
80.....	do.....	10.6	4.6	2.7
20.....	Ammonium sulphate.....	13.5		5.0
40.....	do.....			3.9
80.....	do.....	13.5		3.8
20.....	Ammonium chloride.....			1.4
40.....	do.....			1.1
80.....	do.....	2.5		.8

It is apparent that both the quantity and the form of nitrogen used in the fertilizer materially affected the fire-holding capacity or duration of glow of the leaf. It will be seen, moreover, that seasonal conditions in the different years greatly affected the results as a whole. It is well known that dry weather tends to reduce the fire-holding capacity and comparatively wet weather tends to produce the opposite effect. The best burn was obtained in 1927 when there was an abundant and fairly evenly distributed rainfall. Rather good results also were obtained in 1925 when July was wet and the August rainfall, though moderate, was very evenly distributed and adequate for rapid growth. The burn of the 1929 crop was very poor and far below the normal for Maryland tobacco, possibly as a result of light rainfall in August and the latter part of July. It is possible also that severe leaching of the soil by the very heavy rainfall of spring and early summer prior to transplanting was a factor in the poor results of 1929.

As regards the influence of the nitrogen supply, it is obvious that the first 20 pounds of nitrogen supplied in the fertilizer had a marked effect in reducing the combustibility of the tobacco. Additional quantities of nitrogen further lowered the capacity of the leaf to hold fire. It appears that the depressing effects of increasing quantities of nitrogen on combustibility were less consistently maintained in 1925 than in the other 2 years, but the reason for this is not known. As to sources of nitrogen, it seems that under the conditions of the fertilizer test ammonium sulphate produced a slightly better burn than nitrate of soda. It is likely that this result is correlated with the effect of ammonium sulphate in promoting rapid growth and development as indicated by relative time of flowering (table 16). As subsequently stated (p. 64), it is believed that the soil on which these tests were conducted is deficient in sulphur and this deficiency tends to delay the growth of the plant. Ammonium chloride as a source of nitrogen greatly impairs the burning qualities of the leaf. This effect is to be expected, perhaps, since it is well known that chlorine in the quantities here supplied in the ammonium chloride tends to seriously lower the fire-holding capacity of tobacco.

INFLUENCE OF NITROGEN SUPPLY ON NUTRITION AND METABOLISM

METHODS OF SAMPLING AND ANALYSIS

The chemical and physical characteristics of the individual leaf of the tobacco plant are profoundly affected by its relative position on the stalk and by its age. Hence, to obtain satisfactory comparison of the effects of different treatments it is essential that all leaf samples be chosen from the same position on the plant unless all of the leaves are taken. In the present case those from the lower half of the plant were generally used. The factor of age was controlled by making observations on a series of samples taken at intervals extending over a considerable period of development of the plant. In fixing the dates for collecting successive samples, account was usually taken of the date on which the first blossom appeared.

In the commercial culture of nearly all domestic types of tobacco it is the general practice to top the plants, that is, to break out the terminal portion of the stem, thus removing the flower bud together with some of the top leaves. However, the number of leaves removed in this way varies widely in the production of the different types of

tobacco, and there is also wide variation as to the stage of development at which the topping is done. In the experiments reported in the following paragraphs, except as otherwise stated, the plants were topped to the lowermost leafless sucker or lateral branch, and the topping was done when the first blossom opened. This sort of high and late topping approximates common practice in southern Maryland and in some of the other tobacco districts.

In sampling, as many plants as conditions would permit were utilized and, ordinarily, for each sample a single leaf or only one half of a leaf was taken from each plant. In studies on the cured product, with certain exceptions all the leaves on the plant were used. The fresh leaves were weighed as quickly as possible after harvest, and usually the outline of each leaf was traced on paper as a means of ascertaining the area. For some purposes the leaf material was at once frozen, macerated, ground, or otherwise prepared for immediate use. Where it was desired to preserve the leaves for later study they usually were plunged into boiling alcohol or exposed to chloroform vapor and then dried at about 60° C. or by a blast of dry air at room temperature. In certain cases, special methods of sampling and preparing the material were necessary, and these are mentioned in connection with the individual experiments. In some instances the whole leaf was used for study, while in others the midrib was removed and rejected.

It is desirable at this point to refer to the surprisingly large quantities of sand and fine earth that may adhere to the leaves of the tobacco plant, especially the lower leaves. It was found that in some cases this material accounted for a third of the total dry weight of the leaf. The largest relative weight of soil material adhering to the leaf was on the no-nitrogen plots where the plants were abnormally small. It was found desirable to apply the proper correction for the content of this soil material to many of the analytical data.

In preparation for analysis the air-dried leaf material was ground to pass a 40-mesh sieve and preserved in tightly stoppered jars. The stalks were first passed through a high-speed hammer mill of the type used in grinding cattle feeds, in preparation for the final grinding in a Wiley mill.

In the analytical work the following methods were employed unless otherwise stated:

Moisture.—The procedure, used chiefly, was to dry for 4 hours at 100° C. An alternative method consisted in drying to constant weight over concentrated sulphuric acid.

Total nitrogen.—The official Gunning method modified to include nitrates as applied to fertilizers (3, p. 21), but with addition of 0.7 g of mercuric oxide, was employed. Thorough digestion of the acid mix is necessary to insure complete decomposition of nicotine and related bodies.

Protein nitrogen.—The tobacco powder (2 g) was boiled 5 minutes with 0.5 percent acetic acid and the mixture was filtered when cold. The residue was washed with hot 0.5 percent acetic acid until the filtrate was colorless. The nitrogen in the residue was determined by the official Kjeldahl-Gunning-Arnold method (3, p. 21). In some cases, however, the official (Stutzer) method for albuminoid nitrogen in grain and stock feeds (3, p. 279), which gives slightly higher figures, was followed. The nitrogen value found was multiplied by the factor 6.25 to obtain the protein figure.

Nicotine.—A modification of the Keller method developed in the Division of Tobacco and Plant Nutrition was chiefly employed. Six grams of tobacco in a ½-pint E-Z seal type of preserve jar was thoroughly mixed with 5 cc of 7.5 percent aqueous caustic soda by means of a spatula. One hundred cubic centimeters of gasoline was added from a pipette, the jar being covered with a thin block of wood

properly notched to receive the stem of the pipette. The jar was sealed with a rubber ring and glass cover, rotated to mix the contents and allowed to stand overnight. After thoroughly mixing the extract, the tobacco was caused to collect on one side by tapping the edge of the jar, which was maintained in an inclined position for an hour or longer to allow the extract to clarify. Fifty cubic centimeters of the extract was withdrawn by means of a pipette the end of which was fitted with a filter tube. This tube was a glass cylinder about 2 inches long and $1\frac{1}{2}$ inches in diameter, over one end of which was placed a filter paper held in place with a rubber ring. The open end was connected with the pipette by means of a one-hole rubber stopper. With the wooden cover in place on the jar, the extract was drawn through the filter into the pipette and placed in a dry 200-cc Erlenmeyer flask. While standing uncovered for an hour the flask was gently rotated several times to cause all ammonia to escape. Exactly 10 or 15 cc of 0.1 N sulphuric acid and 50 cc of water containing the indicator (methyl red or cochineal) were added. The contents were thoroughly mixed and allowed to stand until the two layers had sharply separated. Fifty cubic centimeters of the aqueous layer was withdrawn with a pipette and titrated with 0.1 N alkali. One cubic centimeter 0.1 N sulphuric acid is equivalent to 0.01621 g of nicotine. In certain instances the official silicotungstic-acid procedure (3, p. 55) was followed.

Ammonia.—Twelve grams of the tobacco sample were boiled 5 minutes with 150 cc of 0.5 percent acetic acid under a reflux condenser. After cooling, the extract was passed through paper pulp on a Buchner funnel, using suction. The residue was washed with hot 0.5 percent acetic acid till the filtrate was colorless. The filtrate was transferred to a 300-cc flask, sufficient 12 percent hydrochloric acid was added to form a 0.3 percent solution and sufficient 12 percent silicotungstic acid solution to precipitate all of the nicotine. After the mixture had stood overnight the flask was filled to the mark with water and the contents filtered. A 250-cc aliquot of the filtrate corresponding to 10 g of tobacco was used for determination of ammonia, amide, amino, and nitrate nitrogen. For determination of ammonia an apparatus train was used consisting of: (1) A flask containing dilute sulphuric acid; (2) a similar flask containing ammonia-free water; (3) a Kjeldahl flask containing the test solution; (4) a small guard flask; (5) a liter flask containing 30 cc of 0.2 N sulphuric acid diluted with ammonia-free water and connected with an absorption tower containing bits of small glass tubing; and (6) a guard flask leading to the suction. An open-end manometer filled with glycerol connected with the apparatus immediately beyond the absorption tower aids in maintaining a steady rate of aeration. To facilitate the scrubbing action of the air current the lower ends of the inlet tubes of flasks 1, 2, and 3 are provided with bulbs containing several small openings. The test solution was made nearly neutral with sodium hydroxide and a few drops of kerosene added to prevent frothing, after which 2 cc of saturated potassium oxalate solution and 3 cc of saturated potassium carbonate solution also were added. A current of air was drawn through the apparatus for 3 hours at the rate of 500 l per hour and the residual acid in the absorption flask and tower was titrated with 0.2 N sodium hydroxide, using methyl red as indicator.

Amide nitrogen.—The residual extract from the ammonia determination is neutralized and evaporated to 100 cc, after which sufficient sulphuric acid is added to produce a 5 percent solution. The mixture is boiled 2 hours under a reflux condenser. After cooling and diluting, a little paraffin, a few pieces of pumice, and sufficient caustic soda to give an approximately 0.1 N solution are added. One hundred cubic centimeters are distilled into an excess of 0.05 N sulphuric acid and the ammonia determined by titration.

Amino nitrogen.—The tentative official Van Slyke method for meats and meat products (3, p. 297) was used. An aliquot of the residual solution from determination of amide nitrogen corresponding to 2 g of tobacco was made slightly acid to litmus with acetic acid and evaporated to a small volume. This was made up to 20 cc and 1^o cc of the solution, corresponding to 1 g of tobacco, was used for the determination.

Nitrate nitrogen.—To an aliquot of the residual solution from determination of amide corresponding to 8 g of tobacco adjusted to 0.1 N Caustic Soda 2 g of Devarda alloy powder were added. The mixture was distilled and the distillate collected in an excess of sulphuric acid. The ammonia thus obtained represents the nitrate present. To check against possible mechanical transfer of alkali to the distillate this was redistilled into 0.1 N sulphuric acid after adding magnesia free from carbonate.

Starch.—The official method for starch in the presence of interfering polysaccharides as applied to grain and stock feeds (3, p. 282) was employed in the

analyses presented in table 26. In other cases the alcohol treatment for precipitation of pectins or gums was omitted. It is believed that any errors resulting from this omission were small and of little significance for comparative purposes.

Sugars.—The official methods for reducing sugars and sucrose in grain and stock feeds (3, p. 281) were used. For preparation of the solution 5 to 10 g of the tobacco were employed. In clarifying the extract, addition of neutral lead acetate was continued till no further precipitate was formed. In samples of uncured leaf some trouble was experienced from yellowish, flaky copper precipitates which were rather difficult to filter. The reduced copper was weighed as cupric oxide after heating the precipitate to red heat in a muffle furnace.

Fiber.—As a rough measure of cellulose content the official method for crude fiber in grain and stock feeds (3, p. 280) was employed.

Pectic constituents.—The procedures of Conrad (?) and Appleman and Conrad (2) as applied to tomatoes and based on the methods of Carré and of Carré and Haynes were used to obtain the approximate content of pectin, protopectin, and pectic acid. The tobacco (2.5 g) was washed on a filter with water till the filtrate was free of pectin. Soluble pectin if present was determined in the filtrate in accordance with the procedure of Appleman and Conrad. The tobacco residue was washed with alcohol, dried at 70° C., boiled 30 minutes with N/30 hydrochloric acid, neutralized with caustic soda, and boiled 30 minutes with sufficient ammonium citrate to give a 1 percent solution. After cooling, the solution was diluted to a volume of 250 cc and filtered. The pectic acid was determined as calcium pectate in an aliquot (20 cc), following the procedure of Appleman and Conrad. In a second aliquot of 20 cc the combined protopectin and pectic acid were determined as calcium pectate by successive treatment with sodium hydroxide, acetic acid, and calcium chloride, as outlined by Conrad. The nitrogen content of the calcium pectate precipitates was found to be too low to justify attempts to further purify these precipitates. This may be accomplished when required, however, by redissolving in boiling ammonium citrate and applying a correction for any undissolved residue.

Nonvolatile organic acids.—Malic, citric, and oxalic acids have been generally regarded as the most important nonvolatile organic acids in leaf tobacco. Unfortunately, there is no entirely satisfactory method available for the quantitative separation of these acids in tobacco. Vickery and Pucher (27) have recently pointed out that in the Kissling method (15), which has been used to a considerable extent in the analysis of tobacco leaf, there can be no assurance that the precipitates obtained represent pure salts of malic, citric, and oxalic acids. This criticism has special significance perhaps with respect to malic and citric acids. However, Piatnitzki (26) considers that the Kissling method is of value for purely comparative purposes. This investigator believes that with certain modifications the method yields accurate results for oxalic acid and fairly satisfactory results for malic acid, while the values for citric acid are distinctly low. He recommends that citric acid be extracted by the Kissling method and determined by conversion into pentabromacetone.

In obtaining the data presented in table 35 the acids were first obtained in ether extracts both by the Kissling procedure and by first extracting the tobacco with 2 N sulphuric acid and then exhausting this extract with ether, according to the method used by Vickery and Pucher in extracting and recovering the oxalic acid in their material. For the latter extraction with ether the Palkin automatic extraction apparatus was used. The citric acid was determined by conversion into pentabromacetone according to the official method for citric acid in fruits and fruit products (3, p. 273). The oxalic acid was precipitated as the calcium salt in the dilute solution moderately acidified with acetic acid. The calcium salt was dried and weighed as such, after which it was dissolved in dilute sulphuric acid and titrated with potassium permanganate. The citric and oxalic acid values obtained by the two methods of extraction showed fairly good agreement, and there was excellent agreement in the oxalic acid values obtained gravimetrically and by titration with permanganate. The data for citric and oxalic acids in the uncured leaf presented in table 24 were obtained by the Kissling method before the work of Vickery and Pucher appeared and, unfortunately, material was not available for checking these data by the revised procedure. The values for malic acid in tables 24 and 35 were obtained by the Kissling method, and it is recognized that the fractions thus designated may have contained small quantities of impurities. It has been the experience of the writers that in applying the Kissling method of extracting the acids from tobacco it is often necessary to increase by 25 percent the quantity of sulphuric acid recommended by Kissling to insure complete liberation of oxalic acid in the product.

Tobacco resins.—The empirical procedure of Degrazia (8) was applied to the cured leaf, using however only 10 g of material for extraction. It was found extremely difficult to obtain quantitative extraction of the resene fraction with ether after saponification of the extract with alkali, so that the values for resene probably are only approximately correct.

Ash constituents.—For total ash 5 to 10 g of material were ignited to a dull red in a muffle furnace till the residue was white or nearly so. The several constituents of the ash were determined in accordance with the official methods for plants (3, p. 102).

RECOVERY OF FERTILIZER NITROGEN BY THE CROP AND ITS DISTRIBUTION IN THE LEAF AND STALK

In 1919, 1925, and 1929 representative samples of the cured leaf and stalk from the plots receiving nitrogen at the rates of 20, 40, and 80 pounds per acre in the form of nitrate of soda and from the control plots were analyzed to ascertain the relative quantities of nitrogen recovered by the crop when the nitrogen supply of the soil was increased. The results are presented in table 18. In this table all analytical data are calculated to a uniform moisture content of 15 percent, which is approximately the moisture content of Maryland leaf tobacco when in proper condition for handling. The observed plot yields were used as a basis for computing the quantity of nitrogen recovered by the crop.

TABLE 18.—*Recovery by the tobacco crop of fertilizer nitrogen applied at various rates in 1919, 1925, and 1929*

PERCENTAGE OF NITROGEN IN THE PLANT

Quantity of nitrogen in fertilizer, per acre (pounds)	1919			1925			1929			3-year average		
	Leaf	Stalk	Whole plant	Leaf	Stalk	Whole plant	Leaf	Stalk	Whole plant	Leaf	Stalk	Whole plant
0.....	1.40	1.58	-----	1.03	1.55	-----	1.36	1.58	-----	1.27	1.60	-----
20.....	1.01	1.09	-----	1.36	1.62	-----	1.60	1.91	-----	1.52	1.74	-----
40.....	1.58	1.66	-----	1.50	1.84	-----	1.87	1.90	-----	1.67	1.80	-----
80.....	1.83	1.81	-----	2.07	2.35	-----	2.06	2.06	-----	1.99	2.07	-----

QUANTITY OF NITROGEN CONTAINED IN THE CROP (POUNDS PER ACRE)

0.....	7.9	6.0	13.9	8.6	6.3	14.0	11.0	5.6	16.0	9.2	6.0	15.2
20.....	11.2	10.6	21.8	15.3	12.0	27.3	21.1	13.6	34.7	15.9	12.1	28.0
40.....	12.5	11.7	24.2	19.1	14.5	33.6	23.2	14.1	37.3	18.3	13.4	31.7
80.....	15.0	12.2	26.1	26.1	20.0	47.0	30.7	16.5	47.2	24.2	19.0	40.8

Of the first 20 pounds of nitrogen applied in the fertilizer the crop recovered in round numbers 40, 60, and 90 percent, respectively, in the years 1919, 1925, and 1929, the 3-year average being 64 percent. The apparently abnormally high recovery in 1929 is coupled with an exceptionally high crop yield on the particular plot involved, the reason for which is not known. If the 1929 value is disregarded, the indicated recovery of the first 20 pounds of fertilizer nitrogen amounts to 40 to 60 percent of the total applied to the soil. The additional quantity of nitrogen recovered by the crop when 40 pounds of nitrogen was applied is small, the average for the 3 years amounting to less than 4 pounds or 20 percent of the second 20 pounds of nitrogen included in the fertilizer. The recovery was comparatively small in each of the 3 years, possibly due in part to some soil

condition on this plot which was unfavorable for absorption of the additional fertilizer nitrogen. It is worthy of mention, however, that nitrogen recovery in this treatment as compared with the 20-pound rate of fertilization was distinctly better in 1925 than in the other 2 years. Of the 40 pounds supplied in the fertilizer in this treatment, the quantity contained in the crop in excess of that absorbed by the crop in the no-nitrogen treatment averaged for the 3 years 16.5 pounds, or 41 percent of the quantity applied. The nitrogen recovery in the 80-pound rate of fertilization was approximately 18 percent in 1919, 40 percent in 1925, and 38 percent in 1929.

There is a definite though not a uniform increase in the percentage content of nitrogen in both leaf and stalk as the quantity of nitrogen in the fertilizer is increased. Usually this increase is more marked in the leaf, however, than in the stalk. The greatest increase in percentage content of nitrogen, at least in the leaf, results from the first 20 pounds of nitrogen supplied in the fertilizer, even though much the largest increase in yield of both leaf and stalk also is produced by this first 20 pounds of fertilizer nitrogen. The percentage of nitrogen in the stalk usually exceeds somewhat that of the leaf, especially when the supply of nitrogen in the soil or fertilizer is low.

The actual quantity of nitrogen contained in the leaf exceeds that in the stalk in all cases, but the amount of the excess varies considerably from year to year. The greatest difference in nitrogen content of leaf and stalk is found in the 1929 crop because of the unusually high yield of leaf in comparison with the yield of stalk. On an average, about 60 percent of the nitrogen is found in the leaf and 40 percent in the stalk with both light and heavy rates of nitrogen fertilization. A striking feature of the results for the control plots is that in 1929, after 10 years of continuous cropping without addition of any nitrogen in the fertilizer, the quantity recovered by the crop exceeds that recovered in the first year of the experiment. For the 3 years in which observations were made the average quantity of nitrogen in the crop from the control plots amounted to about one third of the quantity in the crop most heavily fertilized with nitrogen.

The leaf material obtained from selected plants in 1929 for study of weight of cured leaf per unit area (p. 28), and the corresponding stalks also were employed in observations on the distribution of nitrogen in the midrib and the lamina of upper, middle, and lower leaves, and in the stalk, as affected by increase in the supply of fertilizer nitrogen. In addition to these individual leaves from the upper, middle, and lower portions of the plant, which were used to obtain data on the nitrogen content of the lamina and the midrib, all the remaining leaves from the three parts of the plant were employed for ascertaining the nitrogen content of the entire leaves in order to insure better average values. The lowermost or ground leaves which had partially dried at time of harvest also were collected separately and analyzed. These ground leaves are often collected, cured, and marketed by growers in southern Maryland. The analytical data are summarized in table 19.

TABLE 19.—Content of nitrogen in the lamina and midrib of upper, middle, and lower leaves, in the leaves as a whole, and in the stalk, as affected by the quantity of nitrogen applied in the fertilizer, season of 1920

Quantity of nitrogen applied per acre (pounds)	Position of leaf on stalk	Percentage of nitrogen in—				Milligrams of nitrogen per square foot of leaf
		Leaf		Whole leaf	Stalk	
		Lamina	Midrib			
None	Upper.....	2.34	1.40	2.13	114.1
	Middle.....	1.94	.82	1.68	93.5
	Lower.....	1.67	.68	1.40	90.1
	Ground.....	1.39
	All leaves.....	1.47
20	Upper.....	2.65	2.23	2.45	141.2
	Middle.....	2.13	1.26	1.92	99.6
	Lower.....	1.79	.78	1.60	91.2
	Ground.....48
	All leaves.....	1.70	2.01
40	Upper.....	3.81	2.18	3.22	160.8
	Middle.....	2.67	1.68	2.46	104.1
	Lower.....	1.97	1.02	1.80	101.1
	Ground.....69
	All leaves.....	2.24	2.42

Considering the results as a whole, the percentage content of nitrogen in the midrib averages slightly more than one half that in the lamina or web of the leaf and is lower than that of the stalk. In comparison with the lamina the midrib contains relatively least nitrogen in the lower leaf and most in the upper leaf, especially when the soil supply of nitrogen is low. The actual percentage of nitrogen in both midrib and lamina is highest in the upper and lowest in the lower leaves. In the lower and middle leaves and to a lesser extent in the upper leaves the effect of an increased supply of fertilizer nitrogen is to increase the nitrogen content of the midrib, and this applies generally also to the lamina. Taking the leaf as a whole, there is a well-defined increase in nitrogen from the ground leaf to the upper leaf, the former being rather low in this element. However, there is little difference in nitrogen content per unit area between the lower and the middle leaves. The greatest effect of an increased supply of nitrogen in the fertilizer in increasing the nitrogen content of the leaf on a percentage basis or on a basis of equal area is in the upper leaves of the plant. As in table 18, the percentage of nitrogen is somewhat higher in the stalk than in the leaf. The distribution of total nitrogen between stalk and leaf and between the web and midrib of the leaves is of interest chiefly as indicating the fertilizing value of the stalk and leaf midrib as sources of nitrogen.

MINIMUM NITROGEN REQUIREMENTS

The data in table 18 illustrate the fact that the minimum content of nitrogen in the leaf which will suffice to prevent visible symptoms of nitrogen hunger varies considerably with change in environmental conditions. An abnormally pale or yellowish-green color of the leaves which is the most characteristic symptom of nitrogen hunger in the plant was always evident on the control plots but scarcely could be recognized on the plots receiving 20 pounds of fertilizer nitrogen. Nevertheless, the leaf crop grown on the latter plots in

1925 contained about the same percentage of nitrogen as the leaf grown on the control plots in 1919 and 1929. However, it appears that symptoms of nitrogen hunger are likely to be seen when the nitrogen supply is such that the content of the cured leaf falls below about 1.5 percent. The plant was able to make most effective use of the nitrogen actually absorbed in the crop season of 1925. Disregarding the small quantity of nitrogen in the roots, in that year each pound of nitrogen absorbed by the plant produced 56, 41, 36, and 27 pounds of cured leaf, respectively, on the control plots and those receiving 20, 40, and 80 pounds of nitrogen in the fertilizer.

WATER RELATIONS IN THE PLANT

In 1929 a series of leaf samples covering the growing period up to the flowering stage was taken from the plots of the primary series receiving nitrogen from nitrate of soda at the rates of 0, 20, 40, and 80 pounds per acre. For this purpose three groups of 20 uniform plants each were selected, the successive samples being taken from the three groups in regular rotation. Samples were taken 47, 40, 32, 25, 17, and 11 days, respectively, before flowering and at time of first flowering. For the first sample in each group the fifth leaf from the bottom was used, and for each succeeding sample the next higher leaf was used. At the flowering stage, samples were taken from both the first and the second groups of plants. For each sample only a half of each leaf was collected by cutting the leaf lamina along the midrib, the remainder of the leaf being left attached to the plant. This method of sampling was followed in order to reduce to a minimum any disturbance in the normal processes of plant activity. After obtaining the fresh weight, the area of the leaf material was ascertained by tracing the outlines of each half leaf on paper. The material was then exposed to chloroform vapor to induce plasmolysis and quickly dried at air temperature in a current of air dried over strong sulphuric acid. In ascertaining the true fresh and dry weights, a correction for adhering earthy material, as determined in the ash, was applied. The water content of the several samples is shown in table 20.

TABLE 20.—Effect of increasing the nitrogen supply in the fertilizer on the total area of the lower leaves of the plant, their water content and dry weight per square foot, and the content of total, protein, nitrate, nicotine, ammonia, amino and amide and residual nitrogen in the leaves through a period of 47 days extending up to the time of first flowering, season of 1929

Number of days before flowering	Quantity of nitrogen in fertilizer, per acre	Average area of 10 leaves	Water content of leaf	Weight of dry matter per square foot	Nitrogen in 1 square foot of leaf as—										Nitrogen in 1 leaf as—	
					Total nitrogen	Protein nitrogen	Nitrate nitrogen	Nicotine nitrogen	Ammonia nitrogen	Amino nitrogen	Amide nitrogen	Residual nitrogen	Total nitrogen	Protein nitrogen		
	Pounds	Sq. ft.	Per cent.	Gms.	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams
47	None	5.060	83.71	4.209	142.0	115.6	0.2	—	0.4	5.5	0.9	—	—	—	80.9	66.1
	20	5.125	84.32	4.012	163.8	130.8	—	—	—	4.9	1.3	—	—	—	89.7	71.7
	40	5.240	85.40	3.918	109.5	131.4	2.3	—	—	4.7	1.6	—	—	—	86.3	68.8
40	80	7.438	85.45	4.109	178.1	137.5	3.7	13.3	—	4.9	1.9	15.0	—	—	132.4	102.7
	None	5.840	84.00	4.341	127.5	93.2	1	14.0	—	5.5	1.1	13.3	—	—	74.8	54.3
	20	6.570	85.84	3.957	104.1	117.9	1.5	18.0	—	7.3	1.6	17.1	—	—	107.7	77.5
32	40	6.359	84.68	4.311	101.1	114.3	1.4	17.0	—	6.9	1.7	19.0	—	—	102.7	72.7
	80	7.820	85.83	4.082	149.0	110.1	1.5	15.5	—	6.9	1.4	13.2	—	—	116.5	80.0
	None	5.851	83.08	5.098	126.3	83.5	3	18.3	—	8.5	1.1	16.2	—	—	73.7	49.1
25	20	6.500	83.02	4.729	159.0	103.0	8	24.3	—	8.4	1.4	19.7	—	—	103.4	67.6
	40	6.734	84.28	4.395	168.0	105.9	1.6	—	—	7.2	1.8	—	—	—	111.7	71.3
	80	8.270	85.84	4.060	142.3	93.0	1.4	21.2	—	7.1	1.3	17.1	—	—	117.4	77.7
17	None	6.324	83.84	4.405	109.6	66.9	0.03	18.1	—	4.2	1.0	17.0	—	—	69.5	42.3
	20	6.637	84.88	4.236	137.8	86.0	7	22.5	—	8.0	1.3	15.7	—	—	91.0	59.1
	40	6.710	85.30	4.100	135.8	81.7	1.1	23.2	—	7.4	1.4	20.4	—	—	91.3	55.0
11	80	8.897	85.67	4.216	141.5	88.1	1.6	21.9	—	6.7	1.4	19.7	—	—	126.0	78.1
	None	6.882	83.78	4.331	98.4	50.9	0.00	14.7	—	3.4	—	18.1	—	—	60.5	35.1
	20	7.734	84.44	4.347	104.6	61.2	2	20.5	—	4.5	—	19.9	—	—	81.2	47.2
At flowering	40	7.591	86.53	4.189	120.0	72.6	3	21.8	—	5.1	1.1	19.0	—	—	91.8	55.4
	80	8.071	86.40	3.893	116.2	70.0	4	19.0	—	5.3	1.2	19.2	—	—	105.2	63.5
	None	7.633	82.45	4.610	129.5	51.8	0	15.2	—	4.4	1.0	17.0	—	—	68.7	39.7
Do	20	7.754	82.42	4.992	124.5	71.4	0.08	24.8	—	5.8	1.3	20.3	—	—	96.0	55.0
	40	7.405	83.86	4.477	115.1	67.0	2	21.7	—	4.4	1.2	19.5	—	—	85.9	50.0
	80	8.812	85.35	4.158	114.0	68.0	5	21.2	—	5.1	1.2	17.6	—	—	111.8	66.7
At flowering	None	7.201	83.60	4.533	83.4	48.2	3	15.8	—	5.4	1.1	18.1	—	—	63.4	34.6
	20	8.130	84.51	4.217	94.0	51.2	3	18.3	—	4.8	1.0	17.9	—	—	76.5	41.5
	40	7.963	85.37	4.154	100.8	54.4	4	20.9	—	5.0	1.0	18.8	—	—	80.4	43.0
Do	80	10.061	85.78	4.138	116.7	63.9	6	22.8	—	7.1	1.4	20.4	—	—	124.7	68.2
	None	7.556	83.80	4.335	82.1	44.8	3	12.4	—	4.5	—	18.8	—	—	62.0	34.0
	20	9.283	84.06	4.386	92.9	51.7	3	17.2	—	4.0	—	17.4	—	—	80.3	48.3
Do	40	9.192	86.09	3.794	96.3	51.6	4	20.2	—	6.3	1.0	17.2	—	—	88.2	47.8
	80	10.125	86.90	3.794	102.7	58.9	4	18.3	—	5.8	1.0	17.7	—	—	104.3	59.8

The results show a clearly defined trend toward increased water content with increase in the nitrogen supply. These results have been verified repeatedly in other years and there can be no doubt that the relationship applies generally. It is evident that an increased supply of nitrogen enables the plant the better to withstand excessive transpiration losses and resultant loss of turgidity. In popular language, the plants are more sappy. It is interesting to note that the effect of an increased nitrogen supply on the water content of the leaf is associated with definite effects on the physical characteristics of the leaf (table 20).

A series of observations was made in 1924 on the comparative effects of nitrate of soda, ammonium sulphate, and ammonium chloride on the water content of the green leaf tissue when applied at rates to supply 80 pounds of nitrogen per acre. The results along with those from certain other treatments have been published elsewhere (10). It will be sufficient to point out here that ammonium chloride was distinctly more effective in maintaining a high water content in the leaf than the other forms, an effect believed to be due to the properties of the chloride ion. Apparently the ammonium sulphate was the least effective in this particular of the three forms used, but

the reason for this is not apparent. In some of the treatments studied in 1924 there seemed to be a decrease in water content with increasing age of the leaf, but a similar change was not observed with the heavier rates of nitrogen fertilization.

To ascertain the effect of an increased nitrogen supply in the fertilizer on the hygroscopic properties of the dried leaf material, samples were allowed to come into equilibrium with various fixed vapor pressures or relative humidities. These humidities were obtained by use of saturated salt solutions in closed containers at a fixed temperature of 83° F. The salts employed were ammonium nitrate, sodium nitrate, ammonium sulphate, and potassium chloride, and these supplied relative humidities of approximately 61.4 percent, 74.5 percent, 80.2 percent, and 85.3 percent, respectively. To ascertain the moisture content of the samples at these humidities they were dried at 100° C. for 4 hours. The three series of 1925 leaf samples used for study of nonvolatile organic acids and prepared as described on page 45 were utilized for the present study, and, in addition, the 1925 samples of cured leaves described on page 59 and a 1929 series of samples of cured leaves from the same plots were employed. To obtain direct evidence as to the effect of the curing process on the hygroscopic properties of the leaf, tests were made with representative mature leaves taken from a plot receiving 80 pounds of nitrogen per acre derived from ammonium sulphate. One half of each leaf was exposed to chloroform vapor and quickly dried while the remaining half of each leaf was cured normally. The results of the observations with the several lots of dried leaf material are shown in table 21.

TABLE 21.—Effect of increasing the nitrogen supply in the fertilizer on the hygroscopic properties of the dried leaves of the plant, before and after curing, 1925 and 1929

Year, number of picking, and character of sample	Quantity of nitrogen in the fertilizer, per acre	Water content of tobacco			
		Over NH_4NO_3 solution, relative humidity 61.4	Over NaNO_3 solution, relative humidity 74.5	Over $(\text{NH}_4)_2\text{SO}_4$ solution, relative humidity 80.2	Over KCl solution, relative humidity 85.3
	Pounds	Percent	Percent	Percent	Percent
1925:	0	10.67	14.55	16.80	18.60
First picking, uncured.....	20	11.08	14.63	18.14	19.93
	40	10.68	14.62	17.91	19.73
	80	10.79	14.78	18.96	21.03
	0	10.62	14.12	16.65	18.24
Second picking, uncured.....	20	10.52	14.06	17.18	18.92
	40	10.01	14.06	17.40	19.03
	80	10.17	14.42	17.09	20.55
	0	10.30	13.95	16.30	18.31
Third picking, uncured.....	20	10.48	13.64	16.63	18.81
	40	9.78	14.11	17.25	18.95
	80	10.38	14.67	18.28	19.96
	0	7.19	9.90	12.46	15.71
All leaves on plant, cured.....	20	8.67	11.82	13.59	15.29
	40	8.80	12.35	15.02	17.30
	80	0.49	13.20	15.31	18.04
1929:	0	10.04	14.11	15.20	21.08
Lower leaves, cured.....	20	11.60	15.14	16.07	21.69
	40	11.01	15.17	19.21	21.29
	80	11.59	16.38	19.76	23.38
Do.....	80	12.24	17.63		27.11
Lower leaves, uncured.....	80	10.77	14.13		18.93

The results obtained with the 1925 uncured material show that, as a whole, there is a well-defined tendency toward decreased affinity for water with increasing age of the leaf, although this tendency is less marked with the highest rate of nitrogen fertilization. Except at the lower relative humidities, there is definite increase in affinity for water as the rate of nitrogen fertilization is increased. This effect of an increased nitrogen supply is especially marked in the 1925 and 1929 cured material. The comparison of 1929 cured and uncured leaf shows a decided increase in affinity for water as a result of the curing.

OSMOTIC CONCENTRATION OF CELL SAP

A few observations were made on the osmotic concentration of the cell sap of the leaf during the later stages of development and the ripening process, as affected by the nitrogen supply. In 1926, leaf samples were taken at intervals from the same field plots that were used in 1929 for the preceding studies on water relations in the plant. Each sample was taken from 12 selected plants, one leaf being chosen from each plant. All samples were collected between 10 and 11 a.m. For the first observations the leaf attached to the middle portion of the stalk was used, and for each of the later samples the leaf immediately above the one taken for the preceding sample was employed. One half of each leaf was cut off along the midrib and this was again halved crosswise, using the frontal or distal portion for the sample. The leaf material was frozen and the sap expressed at a fixed pressure. The specific lowering of the freezing point of the expressed sap was determined, and the equivalent osmotic concentration was calculated in the usual manner. The results are given in table 22.

TABLE 22.—Effect of quantity and form of the nitrogen supply on the osmotic concentration of the cell sap of tobacco leaves in the later stages of growth and during the ripening process, season of 1926

Nitrogen applied in fertilizer		Osmotic concentration of cell sap in atmospheres—				
Quantity per acre (pounds)	Form	15 days before flowering	At time of flowering	8 days after flowering	15 days after flowering	16 days after flowering
None			8.793	8.071		0.394
20	Nitrate of soda		8.552	8.071		9.154
40	do.		7.830	7.469		9.093
80	do.		8.071	7.590		9.755
80	do.	8.312	8.432		9.033	
80	Ammonium sulphate	8.191	7.590		8.131	
80	Ammonium chloride	10.240	8.732		9.514	

These data show no clearly defined relationship between the quantity of nitrogen supplied the plant and the osmotic concentration of the cell sap of the leaf, although, on the whole, the tendency is toward a reduced osmotic value with an increase in the nitrogen supply except toward the end of the period of observation. As regards the effects of different forms of nitrogen, the ammonium chloride produces the highest osmotic values while ammonium sulphate gives the lowest values. These relationships are similar to those observed in the studies of relative water content of the leaf. On the other hand, the increased water content of the leaf resulting from an increase in the

quantity of nitrogen supplied the plant is not accompanied by an increase in osmotic concentration of the cell sap.

ACIDITY RELATIONS

In 1919 a series of observations were made on the pH value of the sap of leaves taken from the primary series of field plots, beginning at time of topping, which was also the time of first flowering. Six selected plants were used for each sample, and on each plant one half of the middle leaf, cut off along the midrib, was taken for the first sample. Each succeeding sample was taken from the next higher leaf on the plant. The samples were collected September 5, 10, 15, 20, 25, and October 4, 9, 14, and 18. A second series of samples was taken from the same plants, proceeding downward from the middle leaf in procuring successive samples. These were collected on September 30, October 17 and 25, and November 1. All samples were collected between 9:30 and 11 a.m. The material was thoroughly ground in a tinned meat chopper and passed through cheesecloth. The pH values were determined electrometrically. The results are shown in table 23.

TABLE 23.—Effect of the quantity and the form of the nitrogen supplied in the fertilizer on the pH value of the cell sap of tobacco leaves from plants topped on September 3 and collected at intervals during the ripening period, season of 1919

Nitrogen added in the fertilizer		pH value of expressed sap of—												
		Samples taken immediately above the center of the stalk on—								Samples taken immediately below the center of the stalk on—				
Quantity per acre (pounds)	Form	Sept. 5	Sept. 10	Sept. 15	Sept. 20	Sept. 25	Oct. 4	Oct. 9	Oct. 14	Oct. 18	Sept. 30	Oct. 17	Oct. 25	Nov. 1
None	5.36	6.23	6.17	6.20	6.12	6.23	5.36	6.03	6.12	6.02	5.95	5.89	5.60
20	Nitrate of soda	6.26	6.03	6.05	5.94	5.95	6.03	6.15	5.96	6.00	6.07	5.76	5.60
40	do	6.17	6.13	5.98	5.99	5.85	5.84	6.09	5.76	6.02	5.94	5.78	5.69
80	do	6.33	6.16	6.95	6.83	5.87	6.05	6.19	5.91	5.99	5.88	5.80	5.74	5.60
40	Ammonium sulphate	6.34	6.22	6.02	6.01	5.99	6.03	6.14	5.87	5.94	5.99	5.63	5.64
80	do	5.97	5.88	5.98	6.03	5.79	5.82	5.90	5.80	5.77

With increasing age of the leaves there was a definite downward trend in the pH number of the cell sap during September. This would indicate an increase in active acidity during the ripening process, and this relationship between the pH value and the stage of maturity of the leaf has been repeatedly observed. It is probable, in fact, that the progressive decrease in the pH number may be regarded as an indication of the progress of the ripening process. In the present experiment there was a decided rise in the pH figure during the first week of October, which went far toward restoring the original index of active acidity. During this period it was observed that the color of the leaves, which previously had been gradually assuming a lighter shade of green, changed to a darker green. Growers are familiar with this return toward a darker green color in tobacco leaves, which may occur during the progress of the ripening process as a result of wet weather. Under these conditions the

leaves are said to undergo a second growth in that they tend to return to the original, more vigorous state of vegetative activity. In the present case this change of condition, clearly indicated by the rise in the pH value, probably was due to a period of rainy and foggy weather which prevailed during the first week of October. Subsequently, with a change to clear weather, the increase in active acidity was resumed although a second temporary decrease occurred October 18. Through the remainder of the ripening period the active acidity continued to increase.

As regards the effect of the nitrogen supply, the leaf sap of plants receiving no nitrogen in the fertilizer showed consistently a relatively low active acidity till very late in the season. The form of the nitrogen apparently did not greatly affect the rate of increase of active acidity of the cell sap. Unfortunately, data could not be obtained on total titratable acidity in relation to nitrogen supply.

In 1925 a study was made of the content of nonvolatile organic acids in the leaf during the ripening period in relation to the nitrogen supply of the plant. Leaf samples were collected from the nitrate of soda series of plots 11 days before flowering, at flowering time, and 12 days after flowering. Each sample was composed of a single leaf from each of 15 selected plants, the leaves being taken from the center of the plants. The midribs were removed and the leaf material killed with chloroform vapor and dried. In addition to organic acids, total nitrogen and protein nitrogen were determined in the samples. The results are given in table 24.

TABLE 24.—Effect of the content of nitrogen in the fertilizer applied to tobacco on the content of nonvolatile organic acids, total nitrogen, protein nitrogen, and nicotine of the leaves during the period of ripening, season of 1925

Time of sampling	Quantity of nitrogen applied per acre		Water content of fresh leaves	Composition of water-free leaf material					
	Pounds	Percent		Citric acid	Malic acid	Oxalic acid	Total acids	Total nitrogen	Protein nitrogen
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
11 days before flowering	None	75.49	0.28	1.91	0.94	3.10	1.78	1.57	0.42
	20	79.97	.02	2.11	1.35	3.45	2.47	2.04	.67
	40	82.79	.06	2.35	1.25	3.60	3.30	2.89	.75
	80	82.25	.15	2.54	.82	3.51	3.37	2.94	.73
At time of flowering	None	76.15	.21	1.91	1.05	3.17	1.70	1.40	.42
	20	79.06	.16	2.60	1.12	3.88	1.78	1.05	.60
	40	78.94	.22	3.12	1.01	4.35	2.46	2.17	.85
	80	80.48	.30	3.37	.89	4.56	2.94	2.47	.83
12 days after flowering	None	76.07	.24	2.31	1.39	3.94	1.77	1.40	.61
	20	76.20	.25	2.77	1.28	4.30	1.76	1.43	.83
	40	78.70	.24	3.99	1.25	6.48	2.28	1.84	1.03
	80	79.88	.84	4.29	1.15	6.28	2.85	2.28	1.46

As previously explained, the values for citric and malic acids that were obtained by the Kissling method cannot be considered as being accurate, but the data for oxalic acid are believed to be at least approximately correct and the values for citric probably afford a rough measure of the comparative content of this acid in the different samples. As a whole, the data for oxalic acid do not show any clearly defined trend during the period of observation while the content of citric acid tended to increase except when the nitrogen supply was deficient. There was a more pronounced increase in the fractions of somewhat uncertain composition which are designated collectively as malic acid. It is likely that other acids also were present in appreciable quantities in these fractions. Apparently the content of oxalic

acid was not greatly affected by the nitrogen supply of the plant, and this was true also of the citric acid content except that in the later stages of ripening a liberal nitrogen supply increased the content of this acid. The fraction designated as malic acid, however, showed quite consistently an increase as the nitrogen supply was increased.

NITROGEN METABOLISM

In 1929 a series of 8 samples of leaf was taken at intervals covering a period of 47 days up to the flowering stage of the plants on the primary series of fertilizer plots receiving various quantities of nitrogen in the form of nitrate of soda. The same samples were used for a study of water relations in the plant, the results of which already have been considered, and the details of collecting and handling the material are described on page 40. Data were obtained on the changes in total area of the leaves, the weight of dry matter per unit of leaf area, and the content of total, protein, nitrate, nicotine, ammonia, amino, and amide nitrogen. As in the case of water content, data were secured on samples taken at the time of first flowering in both the first and second of the three groups of plants selected for the observations. (See table 20 for a summary of the results.)

In interpreting the results of the 1929 experiment it should be borne in mind that three different groups of plants were used in rotation as sources of the successive leaf samples. Thus, the samples collected 47 and 25 days before flowering and the first sample taken at time of flowering were derived from the same group of plants and, therefore, are more strictly comparable among themselves. Similarly, the samples taken 40 and 17 days before flowering and the second sample taken at time of flowering are strictly comparable, as are the samples taken 32 and 11 days before flowering. The plants were not topped prior to the beginning of flowering, the treatment in this respect approximating that commonly used in practical culture in Maryland and in the production of some types of leaf in other tobacco-growing districts. The leaves immediately above the lowermost were used in the studies, and these continued to increase in area up to the flowering stage, the increase being greatest with the heaviest rate of nitrogen fertilization, but there was no consistent change in weight of dry matter per square foot during the period of observation. In general, the dry weight per unit of area varied inversely with the rate of nitrogen fertilization. As regards both the physical and the chemical characteristics of the leaf in relation to the nitrogen supply, it should be recalled that, as shown in table 18, for some unknown reason the total quantity of nitrogen absorbed by the 1929 crop was only slightly greater in the 40-pound rate of fertilization than in the 20-pound rate. For practical purposes, therefore, the leaf samples from these two treatments may be regarded as duplicates and actually throughout table 20 the experimental data for the two series are seen to be in rather close agreement.

It is obvious that there was a marked loss of total nitrogen and an even greater proportionate decrease in protein nitrogen per unit of leaf area during the period of study, and these losses began more than a month before the flowering stage was reached. Since the total dry weight per unit of leaf area is recorded in table 20 it is a simple matter to ascertain the percentage content of the nitrogen fractions in the leaf, and the data for total nitrogen and protein nitrogen are presented graphically in figure 9. Here, again, there was in all

treatments a marked, progressive decrease in both total and protein nitrogen. Protein nitrogen accounted for about 79 percent of the total nitrogen at the beginning of the observational period, but at the end of the period the protein nitrogen composed only about

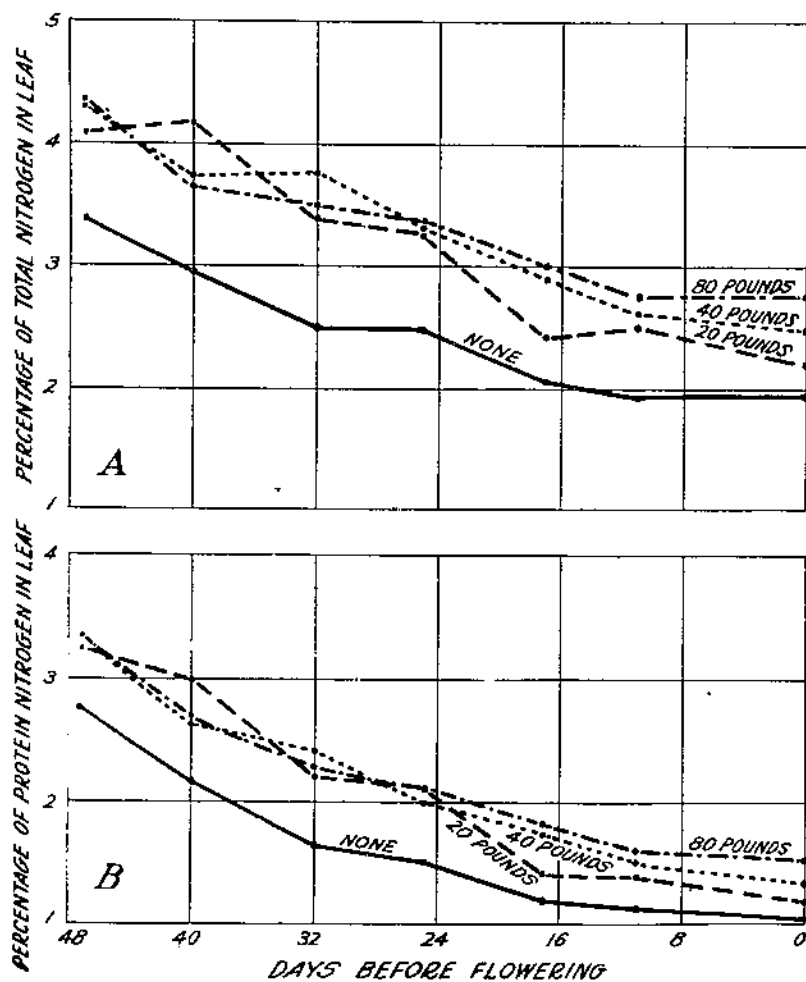


FIGURE 9.—Effect of the nitrogen supply on the percentage content of (A) total nitrogen and (B) protein nitrogen in the lower leaves of the plant during the later stages of their growth. There was a progressive decrease in both of the nitrogen values per unit of leaf area as well as on a percentage basis, which was due primarily to utilization of a portion of the nitrogen for increase in area of the leaves. Where no nitrogen was applied to the soil the total nitrogen and the protein nitrogen content of the leaf were low at all stages of growth, but otherwise differences in the quantity of nitrogen applied had little effect on the percentage content of these constituents until the last stages of growth had been reached.

54 percent of the total. This change, of course, involved a corresponding relative increase in nonprotein nitrogen. On the basis of unit area of leaf there was no marked change, however, in the actual content of nicotine, ammonia, amino, amide, and undetermined nitrogen during the period, while on the whole there was a tendency toward decrease in nitrate nitrogen. Next to protein, nicotine furnished decidedly the largest nitrogen fraction.

As regards the effect of variation in the nitrogen supply on the content of this element in the leaf, the data for the no-nitrogen or control treatment show in nearly all cases a uniformly low content of total nitrogen and of the various nitrogen fractions except the residual or undetermined portion. This holds true for the values calculated on the basis of unit area of leaf and in percentage of total dry matter. (See table 20, and fig. 9.) On the other hand, there are only small differences in the corresponding values for the various nitrogen fractions as between the 20-, 40-, and 80-pound rates of nitrogen fertilization, although it is worthy of note that frequently the nitrogen values for the intermediate rates of fertilization actually exceed those for the highest rate. In the case of the whole leaf the values for total nitrogen and protein nitrogen content vary directly with the quantity of the nitrogen supplied in the fertilizer. On the basis of unit area of leaf, the losses in total nitrogen and protein nitrogen in percentage of the quantities originally present, as well as the actual losses by weight taking place during the period of observation were highest with the intermediate rate of nitrogen fertilization.

The data obtained in 1924 and 1925 (tables 24 and 25) covering the period of 11 to 13 days before, to 11 to 12 days after, first flowering (and topping) show that in the developmental stages immediately following those covered in the 1929 studies, there was, on the whole, an increase in the content of total nitrogen and protein nitrogen with increase in rate of nitrogen fertilization. As in the latter case, where no nitrogen was used in the fertilizer, approximately the minimum content of total and protein nitrogen in the leaf had been reached several days before flowering. Where more nitrogen was made available to the plant in the soil, there were further sharp decreases in the content of total and protein nitrogen in the later stages of development, the general tendency apparently being toward a decrease to fairly definite minimum values. It seems that the higher the initial content of total nitrogen and protein nitrogen in the leaf, the longer is the time required for decrease of these constituents to the minimum values. Immediately following flowering and topping there was a sharp rise in the content of nicotine. Increase in the rate of nitrogen fertilization also increased the nicotine content.

TABLE 25.—Effect of increasing the nitrogen supply in the fertilizer on the content of starch, sucrose, reducing sugars, total nitrogen, protein nitrogen, and total ash in the lower leaves of the tobacco plant, shortly before flowering, at flowering, and shortly after flowering, season of 1924

Time of sampling	Quantity of nitrogen in fertilizer per acre		Water content of leaf sample	Composition of dry matter in leaves					
	Pounds	Percent		Starch	Sucrose	Reducing sugars (as invert)	Total nitrogen	Protein nitrogen	Total ash
13 days before flowering	20	77.83	31.84	0.12	5.96	2.28	1.86	8.66	
	40	78.60	29.74	.08	6.72	2.51	2.20	8.42	
	80	81.63	20.72	.08	6.65	3.06	3.14	9.39	
At flowering	20	78.75	31.60	.09	5.29	1.81	1.45		
	40	79.66	28.63	.11	6.09	2.01	1.65	9.22	
	80	81.70	15.14	.02	6.37	3.42	2.70	10.38	
11 days after flowering	20	78.07	32.32	.15	3.58	1.67	1.35	8.35	
	40	78.06	26.35	.15	3.74	1.78	1.38	8.63	
	80	83.03	19.27	.14	5.18	2.94	2.21	9.80	

An attempt also was made in 1925 to secure data on the content of soluble protein in the cell sap of the leaf as affected by the quantity of nitrogen supplied in the fertilizer. Leaf samples were taken 10 days before flowering, at flowering, and 7 days after flowering. For each sample, a single leaf located near the center of the plant was taken from each of 15 selected plants. One half of each leaf was cut off and used as a source of the sap for analysis, while a series of disks were cut from the remaining half and, after being weighed, were placed in boiling alcohol for moisture determination. After freezing the leaf tissue, the sap was expressed by applying a fixed pressure to each sample. The official Stutzer method was employed for determining protein nitrogen in the sap and the residual nitrogen in the filtrate also was determined in the usual manner. It is recognized that the freezing treatment may have affected the solubility of the protein, and the Stutzer procedure may not afford an accurate measure of the soluble protein. However, the results probably are of value for purely comparative purposes. The data obtained are shown in table 26.

TABLE 26.—Effect of increasing the nitrogen supply in the fertilizer on the content of soluble protein and nonprotein nitrogen in the cell sap of the leaf, season of 1925

Time of sampling	Quantity of nitrogen in the fertilizer per acre	Water content of leaf sample	Nitrogen in 10 cc of sap as—	
			Protein nitrogen	Nonprotein nitrogen
	Pounds	Percent	Milligrams	Milligrams
10 days before flowering.....	None	76.58	20.2	5.1
	20	79.38	19.0	6.9
	40	79.88	20.7	7.8
	80	82.37	25.0	9.5
At flowering.....	None	77.50	18.9	7.6
	20	77.62	18.9	4.1
	40	79.77	17.7	6.2
	80	82.15	21.5	7.3
7 days after flowering.....	None	78.12	12.4	5.1
	20	77.78	12.4	6.5
	40	80.16	14.1	7.7
	80	82.28	19.5	10.5

The data for the nitrogen fraction of the cell sap designated as soluble protein show much the same relations as apply to total protein and total nitrogen in the leaf during approximately the same stages of development (table 26). There is in all cases a definite decrease in soluble protein as the leaf ages or matures, though the rate of decrease is relatively slow when the maximum rate of nitrogen fertilization is practiced and is highest when no nitrogen is included in the fertilizer. There is no corresponding progressive decrease in the nonprotein nitrogen in the sap. As to the effect of nitrogen fertilization, the first 20 pounds of nitrogen in the fertilizer shows no material influence on the content of soluble protein, but additional increments of fertilizer nitrogen and more particularly the 80-pound rate of fertilization bring about a clearly defined increase in protein content of the sap. Increased nitrogen in the fertilizer also tends to increase the content of nonprotein nitrogen in the sap.

CARBOHYDRATE METABOLISM

In 1926 a study was made of the effect of increasing the supply of nitrogen in the fertilizer on the carbohydrate content of the leaf during the period of growth preceding the flowering stage. A series of leaf samples was collected from the primary series of fertilizer plots receiving various quantities of nitrogen derived from nitrate of soda, the samples being taken 41, 32, 24, and 18 days, respectively, before the date of first flowering. The method of collecting, handling, and preparing the material was the same as for the 1929 study of nitrogen metabolism (p. 46) except that for each fertilizer treatment only a single group of plants was used for all samples. The leaves were taken from the lower half of the plant, the final sample consisting of the twelfth leaf from the bottom in each case. The analytical data are shown in table 27.

TABLE 27.—Effect of increasing the nitrogen supply in the fertilizer on the content of total dry matter, starch, sucrose, and reducing sugars in the lower leaves of the tobacco plant during the period of growth preceding the flowering stage, season of 1926

Number of days before flowering	Quantity of nitrogen in fertilizer per acre	Water content of leaf sample	Weight of dry matter per square foot	Carbohydrate in 1 square foot of leaf		
				Starch	Sucrose	Reducing sugars (as invert)
	Pounds	Percent	Grams	Milli-grams	Milli-grams	Milli-grams
41.....	None	84.47	4.070	938.0	18.6	282.4
	20	86.76	3.455	210.3	5.0	128.7
	40	86.78	3.473	202.5	21.6	177.2
	80	85.38	3.517	231.4	5.5	177.1
	None	85.87	3.356	477.7	4.6	135.4
32.....	20	87.74	3.124	151.5	3.5	87.3
	40	87.74	3.288	184.5	3.9	97.9
	80	85.53	3.370	202.0	6.1	100.3
	None	85.35	3.328	345.3	7.4	153.1
	20	86.39	3.304	168.3	7.7	154.2
24.....	40	86.27	3.417	188.5	2.4	178.0
	80	87.20	3.373	98.3	3.1	136.0
	None	84.89	3.602	309.2	0.2	162.1
	20	83.92	3.943	310.9	2.8	196.8
	40	84.83	3.849	200.0	2.4	205.7
18.....	80	85.49	3.808	119.5	0.8	157.9

In 1924, leaf samples were taken from the same plots, except as to the control or no-nitrogen treatment, as in the 1926 experiment, the samples being collected 13 days before flowering, at flowering, and 11 days after flowering. Determinations were made of total nitrogen, protein nitrogen, and ash in addition to estimating the content of starch, sucrose, and reducing sugars. The results expressed in percentages of total dry matter are summarized in table 25.

The observations made in 1926 show that the starch content was decidedly high when the nitrogen supply was very low, especially in the earlier stages of growth. An addition of only 20 pounds per acre to the nitrogen supply of the soil greatly reduced the content of starch while there was comparatively little difference as to this constituent between leaves of plants receiving 20 pounds of nitrogen

per acre in the fertilizer and those of plants fertilized with 80 pounds of nitrogen. As the flowering stage was approached there was in general a temporary decrease in the leaf content of starch. Differences in content of reducing sugars were less clearly defined either with respect to increasing age of the leaves or increase in the nitrogen supply of the soil.

The data obtained in 1924 show that in the developmental stages immediately following those covered in the 1926 test there may be a decidedly high content of starch in the leaf when the quantity of nitrogen in the fertilizer is low and again the percentage of starch decreases as the nitrogen supply is increased. The content of reducing sugars seems to increase somewhat with increase in the nitrogen supply though the differences are not large. The quantity of sucrose in the leaf is rather small. As in the experiments previously discussed, an increased supply of nitrogen in the fertilizer results in an increased content of total nitrogen and protein nitrogen in the leaf. The limited data available show a slight increase in total ash content of the leaf with increase in the nitrogen supply of the plant.

OBSERVATIONS ON OXIDIZING ENZYMES

In 1925 and 1926 a number of measurements of catalase activity were made on leaf material taken from the nitrogen test plots and from other sources during the period of growth and the subsequent ripening process. Only a portion of the results obtained will be presented in detail. In carrying out the tests a number of small disks of known area were cut from definite positions on the leaf by means of a sharp cork borer. The material was ground in a mortar to a fine cream after addition of a little water and calcium carbonate, the mixture then being washed into a 250 cc Florence flask with a small measured volume of water. After placing the flask in a water bath at 25° C. and connecting it with a shaking device the volume of oxygen liberated in 15 minutes by addition of Oakland hydrogen dioxide that had been previously treated with calcium carbonate was collected and measured in the usual manner. All results were calculated to a uniform basis of 25° C. A duplicate sample of leaf material was treated with hot alcohol and used to determine the approximate content of dry matter and water in the test material per unit of area. In this way the results could be calculated on a basis of unit area or in percentages of total fresh weight and total dry matter.

Preliminary observations were made on the relative catalase activity of leaves located on the lower, middle, and upper portions of the stalk. The results invariably showed the lowest activity in the lower leaves. As between the upper and the middle leaves the results were dependent primarily on the size or stage of development attained by the former. Where the upper leaves had reached a fairly large size they were more active than the middle leaves, but in younger stages they were less active than the latter. The data, as a whole, give definite indication that the catalase activity of a given leaf normally increases up to the later stages of growth followed after a time by a gradual decline as the leaf ages. Catalase activity, however, appears to be subject to rather wide fluctuations from day to day.

In 1925, measurements were made on the activity of middle leaves of selected plants on the plots of the primary series receiving nitrogen at various rates. The plants were topped at the beginning of the flowering stage and samples were collected 1, 9, and 15 days, respectively, after topping. The results are contained in table 28.

TABLE 28.—Effect of variation in the quantity of nitrogen supplied in the fertilizer on the catalase activity of tobacco leaves during the ripening period, season of 1925

Number of days after flowering	Quantity of nitrogen in the fertilizer per acre	Volume of oxygen liberated from—		Number of days after flowering	Quantity of nitrogen in the fertilizer per acre	Volume of oxygen liberated from—	
		1 sq. in. of leaf	0.5 gram of fresh leaf			1 sq. in. of leaf	0.5 gram of fresh leaf
	Pounds	Cc	Cc		Pounds	Cc	Cc
1	None	14.6	55.3	9	40	14.2	54.3
	20	12.0	50.5		80	32.0	126
	40	10.4	44.6		None	15.8	55.0
	80	21.1	85.0	15	20	12.7	50.5
9	None	14.3	55.5		40	22.6	83.3
	20	18.1	72.8		80	39.1	146

It appears that in the 1925 experiment the catalase activity of the middle leaves of the plant had already reached a maximum at the time of first flowering where no nitrogen was used in the fertilizer, while with the heavier rates of nitrogen fertilization there was a marked further increase in activity during the following 2 weeks. At all stages a high nitrogen supply resulted in increased catalase activity.

In 1927, studies were made of peroxidase activity in the fresh leaf of plants from the same plots that were employed for the experiments dealing with catalase activity. The method of sampling the leaves was the same as for the catalase tests, the disk samples being taken from one half of each leaf and the other half left intact for tests on curing (p. 58). Leaves located approximately at the middle of the stalk were used for sampling. The samples were taken 5, 13, and 18 days after first flowering and topping. The fresh leaf material was finely ground in a mortar with a little water and calcium carbonate and the peroxidase determined according to the method of Willstätter (28). The results expressed in milligrams of purpurogallin on a basis of 0.2 gram of both the fresh and the dry leaf tissue are shown in part in table 29.

TABLE 29.—The peroxidase number of tobacco leaves during the ripening period, season of 1927, as affected by the quantity of nitrogen supplied in the fertilizer

Quantity of nitrogen in fertilizer per acre (pounds)	Purpurogallin obtained from 0.2 g of leaf material—					
	5 days after topping		13 days after topping		18 days after topping	
	Fresh leaf	Dry leaf	Fresh leaf	Dry leaf	Fresh leaf	Dry leaf
	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams
None	8.41	33.48	10.40	39.64		
20	8.64	34.58	13.58	46.55	13.60	47.58
40	8.36	36.58	14.37	55.62		
80	12.41	61.83	16.50	78.62	16.83	80.54

Increase in the nitrogen supply of the plant tends to increase the peroxidase number of the leaf, this effect being especially evident in the highest rate of nitrogen fertilization. The peroxidase value also increases considerably through the period of 2 weeks immediately following first flowering. In experiments with plants fertilized with 80 pounds of nitrogen per acre derived from nitrate of soda, ammonium sulphate, and ammonium chloride, respectively, the results were somewhat variable and would hardly justify definite conclusion as to possible significant differences in the effects of nitrogen from these three sources.

EFFECTS OF HIGH AND LOW TOPPING ON GROWTH AND METABOLISM

In some important tobacco-growing sections it is the usual practice to top the plants low and the topping is done several days before flowering would begin. It is desirable, therefore, to consider briefly the primary effects of low topping on the ripening process. Observations were made on two plots in 1930 which were fertilized with 100 pounds of nitrate of soda, 300 pounds of 16-percent superphosphate, and 200 pounds of high-grade sulphate of potash per acre. The season was an extraordinarily dry one. One plot was located on a slight elevation, with the result that the crop was more severely affected by the drought than that of the other plot which was located on a lower area. For convenience, the plots will be referred to as dry land and moist land. The tobacco made much better growth on the latter soil than on the former. On each plot three groups of 25 uniform plants each were selected for study. On the dry land one group was topped to 16 leaves and a second group was topped to 8 leaves, while on the moist land the respective groups were topped to 18 and 9 leaves. In each case the control groups remained untopped. The topping was delayed until flowering had begun, in order to maintain comparison with all of the preceding experiments. Under these conditions the maximum effects of low topping probably were not obtained. The first samples, consisting of the fifth leaf from the bottom of each plant on the dry land and the sixth leaf of each plant on the moist land, were taken at time of topping. Subsequently samples consisting in each case of the next higher leaf were taken at intervals of 10 to 12 days. The last sample taken on the moist land was subjected to curing, while all others were exposed to chloroform vapor and dried in the usual way. The average area, the water content, and the dry weight per unit area were ascertained and protein, nicotine, and total nitrogen were determined in the dried material. The results are indicated in table 30.

TABLE 30.—Effect of high and low topping on the ripening process in the leaf in the field and on the cured product, season of 1930

Type of soil and treatment of plants	Time of sampling	Area of 10 leaves	Water content of leaf	Dry weight of leaf per square foot	Nitrogen in 1 square foot of leaf as—			Nicotine in dry leaf
					Total nitrogen	Protein nitrogen	Nicotine nitrogen	
Dry land:								
Not topped	At topping	Sq. ft. 7.82	Percent 78.70	Grams 5.754	Milli-grams 135.8	Milli-grams 97.8	Milli-grams 17.8	Percent 1.70
Topped high	do	8.02	77.54	5.669	137.7	96.9	18.0	1.03
Topped low	do	7.71	76.95	5.800	138.6	98.0	18.2	1.82
Not topped	10 days after topping	7.59	77.86	5.362	130.0	87.8	20.8	2.26
Topped high	do	7.53	77.32	5.539	135.7	88.6	23.4	2.44
Topped low	do	7.59	76.72	5.929	163.6	103.7	29.2	2.85
Not topped	21 days after topping	6.71	76.04	5.822	128.7	85.6	20.5	2.04
Topped high	do	7.05	74.82	6.349	149.8	92.1	29.1	2.65
Topped low	do	7.04	77.32	6.360	192.1	117.7	38.0	3.40
Not topped	31 days after topping	5.87	76.14	5.506	135.8	90.2	21.3	2.24
Topped high	do	6.42	76.80	6.228	167.5	101.6	36.9	3.43
Topped low	do	6.79	78.84	6.700	251.9	144.0	68.5	5.05
Moist land:								
Not topped	At topping	11.33	80.22	5.205	161.3	120.2	16.4	1.83
Topped high	do	11.14	80.37	5.109	163.5	121.1	18.2	2.06
Topped low	do	11.57	80.26	5.190	168.7	127.1	17.0	1.90
Not topped	12 days after topping	11.32	80.65	4.814	144.4	101.1	19.7	2.37
Topped high	do	11.09	79.57	5.178	156.4	107.7	24.2	2.71
Topped low	do	11.71	78.37	5.728	192.5	127.2	32.0	3.23
Not topped	24 days after topping	10.92	79.81	4.915	152.4	104.2	22.6	2.67
Topped high	do	11.02	78.93	5.216	168.3	106.3	31.0	3.45
Topped low	do	11.54	78.39	6.349	246.3	146.7	50.7	4.62
Not topped	28 days after topping	10.05		4.309				
Topped high	do	10.14		4.571				
Topped low	do	10.86		6.023				

* After curing.

The abnormally dry weather greatly restricted the growth of all leaves except the lower ones on the plants, with the result that in successive samples there is a definite decrease in area which ordinarily would not be the case. The smaller size of the leaves in the dry-land series as compared with those in the moist-land series is associated with a decreased water content. On the other hand, the dry weight per unit of area is decidedly greater on the dry land, especially for the untopped and the high-topped plants. The nitrogen content of the leaves from the moist land is considerably higher than that of the leaves from the dry land, particularly on a percentage basis.

Topping the plants increased the area of the leaf during the period of observation when comparison is made with the untopped plants, that is, there was less decrease in area of the leaves in advancing up the stalk when the plants were topped. The weight of dry matter per square foot of leaf was decidedly increased as a result of topping, the low topping producing the greatest increase. In the plants not topped there was an actual decrease in dry weight per unit of area. The effect of topping on dry weight was especially marked after the leaf had been cured, the increase over the untopped plants being nearly 40 percent. On a percentage basis the data in table 30 do not indicate any considerable, consistent increase in water content as a result of topping because of the definite increase in dry matter. The absolute quantity of water per unit area of the leaf, however, is definitely increased by the topping.

During the period of observation there was a tendency toward decrease in total nitrogen and protein nitrogen in the leaves of the

plants that were not topped although the losses were partly recovered in the late stages of ripening. In the plants topped high there were no important changes in total or protein nitrogen though there was a moderate gain in total nitrogen in the dry-land series and a small loss of protein nitrogen in the moist-land series. In the low-topped plants, on the other hand, there was a very striking, progressive increase in total nitrogen and a somewhat less pronounced increase in protein nitrogen. On the dry land the increase in total nitrogen per unit of area reached approximately 80 percent and that of protein nitrogen more than 45 percent. Nicotine showed only a slight increase in the untopped plants during the period of observation. In the high-topped plants the increase was much greater and in the low-topped plants it amounted to more than 200 percent.

Opportunity was found for making observations in 1931 on a different series of plots pertaining to the effects of high and low topping in conjunction with variations in rate of nitrogen fertilization on the catalase activity of the leaf during the ripening period. All plots were fertilized uniformly with 64 pounds of phosphoric acid and 96 pounds of potash per acre while one group of plots received no nitrogen and the other two groups received 32 and 128 pounds, respectively. In each group one plot remained untopped, a second was topped to the lowermost leafless sucker, and the third was topped to 10 leaves. The low topping was done when the flower bud appeared above the leaves, and the high topping was done on date of first flowering. Except where no nitrogen was used in the fertilizer the low topping was carried out on August 4 and the high topping on August 13. Because of delayed development where nitrogen was omitted from the fertilizer the respective dates of topping were postponed until August 24 and September 9. The methods of sampling and conducting the analysis were the same as previously employed. For the first series of readings in each treatment the fifth leaf from the bottom was chosen, and for the second series the eighth or ninth leaf was selected. Where no nitrogen was used in the fertilizer the first sample was taken 9 days after the low topping and 7 days prior to the high topping and the second sample was taken 13 days later. In the other two treatments the first samples were taken 2 days after the low topping and 7 days prior to the high topping and the second samples were taken 26 or 27 days later. The results are summarized in table 31.

TABLE 31.—Effect of height of topping and variation in rate of nitrogen fertilization on the catalase activity of tobacco leaves during the ripening period, season of 1931

Quantity of nitrogen in the fertilizer per acre (pounds)	Date of sample	Volume of oxygen liberated from 1 square inch of leaf in—			Quantity of nitrogen in the fertilizer per acre (pounds)	Date of sample	Volume of oxygen liberated from 1 square inch of leaf in—		
		Controls	Plants topped high	Plants topped low			Controls	Plants topped high	Plants topped low
None.....	Sept. 2	Cc	Cc		None.....	Sept. 16	Cc	Cc	
32.....	Aug. 6	21.9	16.0	23.2	32.....	Sept. 2	33.7	22.1	41.0
128.....	do.....	26.2	29.3	30.9	128.....	Sept. 1	33.5	31.8	53.0
		43.0	45.6	44.3			49.9	58.0	74.4

It is apparent that low topping of the plants produced a decided effect in increasing the catalase value, especially when the rate of nitrogen fertilization was high. However, high topping produced no effect except possibly a slight increase in the case of the heavy rate of nitrogen fertilization.

THE NITROGEN SUPPLY AND THE CURING PROCESS

The data bearing on the effects of the nitrogen supply on nutritional and growth processes during the development of the tobacco plant make it plain that at time of harvest the physical and chemical characteristics of the leaf will depend to a considerable extent on the quantity of nitrogen absorbed and assimilated by the plant. The regulated rate of drying to which the freshly harvested leaves are subjected, commonly known as curing, involves complex, deep-seated changes in composition in addition to the loss of water. Radically different methods of curing are applied to the various types of tobacco, the air-curing and the flue-curing methods perhaps providing the widest contrast. Air curing is a relatively slow procedure conducted at ordinary temperatures, while flue curing is a rapid process involving use of comparatively high temperatures. In Maryland air curing is generally applied and the leaves remain attached to the stalk until the cure has been completed.

In the present investigations no attempt was made to follow through the different stages of the curing the effect of the nitrogen supply on all of the various chemical processes that are involved, but a limited number of observations were made on certain features. In 1927, leaf samples were collected from the middle portion of plants on the plots receiving various quantities of nitrogen derived from nitrate of soda, the samples being taken just before harvest. After obtaining the fresh weights and areas of the leaves, the midribs were removed and one half of each leaf was chloroformed and quickly dried, while the remaining leaf halves were cured in the usual manner. The loss in total dry matter was ascertained and the material was utilized for determining the resin content before and after curing, according to the method of Degrazia (8). The results are brought together in table 32. The several components of the resin mixture were estimated separately, but no clearly defined differences in the various fractions were found so that only the total content of resins is shown in the table.

TABLE 32.—Effect of the nitrogen supply on the loss of total dry matter and on the content of total resins in the leaf during the air-curing process, season of 1927

Quantity of nitrogen in the fertilizer per acre (pounds)	Average area of leaves		Moisture in fresh leaf		Weight of dry matter in leaf per square foot		Loss of dry matter in curing	Resin content of dry leaf	
	Sq. ft.	Percent	Fresh	Cured	Percent	Fresh			
						Grams	Grams	Percent	Percent
None.....	0.7879	75.14	0.053	4.777	21.07	3.63	4.42		
20.....	1.1005	75.87	5.486	4.341	20.86	4.50	5.27		
40.....	1.2419	78.35	5.520	4.311	21.99	4.82	5.06		
80.....	1.4099	81.53	4.691	3.574	22.33	4.96	5.23		

¹ Data calculated on a basis of dry matter in the fresh leaf.

Variations in nitrogen supply did not greatly affect the loss in total dry matter in curing although the loss was somewhat greater in the leaves of plants grown with an increased supply of nitrogen. The values for so-called resins apply to a group of poorly defined constituents determined by an empirical procedure and their exact significance is not known. The values for resin content of the cured leaf, when calculated on the basis of the original weight of the uncured leaf, seem to indicate a definite increase in some of the constituents included in this group during the curing process. Although in this particular experiment the tobacco which received no nitrogen in the fertilizer shows a relatively low content of resins, the data for the leaf from the other treatments do not indicate that the nitrogen supply is an important factor in the content of total resins in tobacco leaf.

Since the effect of the curing process on the content of oxidizing enzymes previously had not been studied extensively it seemed desirable to obtain additional information on this subject, particularly since there is reason to believe that these enzymes play an important role in the fermentation and ageing processes which follow the curing. In this connection it should be noted that Smirnow and Drboglav (23) found that the catalase and peroxydase activity increases in green leaves of oriental yellow tobacco subjected to regulated conditions of starvation. A number of preliminary tests were made to ascertain the normal course of catalase activity in mature leaves through the various stages of air curing. The data uniformly showed an appreciable rise in activity in the first stages of curing, this usually occurring in the first 2 or 3 days. Thereafter a sharp decline in activity was observed, and at the end of 10 days or 2 weeks the decrease was such that in some instances the order of magnitude of the catalase activity coefficient had changed. In a representative case the initial coefficient of catalase activity of 56 per square inch of leaf increased to 78 on the second day and at the end of 9 days had fallen to 9.

In connection with the studies on catalase activity during the curing process it was found that exposure of the fresh, green leaf to vapors of chloroform results in a rapid and very marked decrease in catalase activity. For example, in a sample of the fresh leaf dried rapidly in a current of air at ordinary temperature the coefficient of catalase activity was 48.9 while in a similar sample exposed to chloroform before drying the catalase value had fallen to 3.5. The action of chloroform vapor in diminishing catalase activity was found to proceed much more slowly after the leaf has been dried and again moistened. Exposure of the above-mentioned dried-leaf sample to chloroform vapor for 2½ hours produced no measurable effect on the coefficient of activity, while an exposure of 42 hours decreased the coefficient to 7.5 as compared with a value of 14.2 for the control sample not treated with chloroform.

In 1926, groups of plants were selected on the plots receiving in the fertilizer 80 pounds of nitrogen per acre derived from nitrate of soda, ammonium sulphate, and ammonium chloride, respectively, and on a control plot, and leaf samples were taken from the middle portion of the plants for observations on relative catalase activity during the curing. At time of sampling, the plants on the plot receiving no nitrogen showed a uniform light yellowish-green color and in this respect would be regarded as fully mature. The plants fertilized with nitrate of soda appeared to be only slightly less mature than the

controls, while those fertilized with ammonium sulphate appeared to be somewhat less mature and those receiving nitrogen from ammonium chloride seemed to be the least mature of all in the series. After cutting small disks from the leaves for determination of catalase they were allowed to cure in the usual way, four successive disk samples being taken at intervals of 2 to 3 days. The results are given in table 33.

TABLE 33.—Effect of the nitrogen supply on catalase activity of the leaf during the curing process, season of 1926

Nitrogen supplied in fertilizer		Sept. 14 ¹	Sept. 16	Sept. 18	Sept. 21	Sept. 24
Quantity per acre (pounds)	Form	Cc	Cc	Cc	Cc	Cc
None.....		46	10	24	9	4
80.....	Ammonium chloride.....	116	74	125	57	15
80.....	Ammonium sulphate.....	98	62	49	19	4
80.....	Nitrate of soda.....	87	67	84	36	7

VOLUME OF OXYGEN LIBERATED FROM 0.1 G OF DRY LEAF		Sept. 14 ¹	Sept. 16	Sept. 18	Sept. 21	Sept. 24
None.....		136	51	72	27	13
80.....	Ammonium chloride.....	321	217	389	183	40
80.....	Ammonium sulphate.....	311	188	193	64	17
80.....	Nitrate of soda.....	269	189	259	100	26

¹ Date of harvest.

From the results of this experiment it appears that although initially the mature leaves grown with a high nitrogen supply possessed a relatively high catalase activity, as the curing progressed the rate of decrease in activity was correspondingly greater than in the leaves grown with a low nitrogen supply (the controls). The initial activity was greatest and the rate of loss of activity was the least in the leaves of plants fertilized with nitrogen derived from ammonium chloride. This result was possibly due in part to the fact that the leaves from this treatment were less mature when harvested than the others, and in part to the effect of the chloride ion in slowing down the rate of drying.

In 1927, studies were made on the effect of the nitrogen supply on peroxidase activity during the curing process in continuation of the observations made during the ripening period in the field (p. 52). The same leaf samples were utilized, and the method of determination was the same. The leaves were allowed to cure for 22 days. The results are assembled in table 34.

TABLE 34.—Effect of the nitrogen supply on peroxidase activity of the leaf during the curing process, season of 1927

Quantity of nitrogen in the fertilizer per acre (pounds)	Purpurogallin obtained from—			
	1 square inch of leaf		0.2 g of dry leaf material	
	Fresh	Cured	Fresh	Cured
None.....	Milli-grams 7.4	Milli-grams 5.7	Milli-grams 39.6	Milli-grams 29.2
20.....	9.4	7.5	49.6	40.4
80.....	11.9	9.6	78.6	59.1

The data in table 34 show that there is an appreciable decrease in the peroxidase value during the curing although this decrease seems to be much smaller than that of catalase. The results are much the same whether the data are based on equal areas or equal weights of the leaf. In both the cured and the green material the peroxidase number is increased by an increased supply of fertilizer nitrogen.

CHEMICAL COMPOSITION OF CURED TOBACCO AS AFFECTED BY THE NITROGEN SUPPLY

In 1925, groups of 32 representative plants each were selected from the plots fertilized with 20, 40, and 80 pounds of nitrogen per acre derived from nitrate of soda and from a control plot receiving no nitrogen. The plants were topped, harvested, and cured according to the usual practice in southern Maryland. When the curing was completed the leaves were stripped from the stalks, ground, and preserved for chemical analysis. The midribs of the leaves were not removed in preparation for analysis. The prepared samples were composed of all the leaves secured from the cured plants, so that in each case the composition represents the average for all the grades of leaf produced under the conditions of the experiment. In the analysis nearly all organic constituents were determined insofar as analytical methods were available even though in some cases it was recognized that the methods employed are not entirely satisfactory. The principal ash constituents also were determined. The tobacco samples were found to contain large percentages of fine earth, and proper correction for this material was applied to all analytical data. The results are shown in tables 35 and 36.

TABLE 35.—Effect of quantity of nitrogen in the fertilizer on the composition of cured leaf tobacco on a water-free basis, crop of 1925

Constituents	Percentage of constituents found in leaves from plants fertilized with—			
	No nitrogen	20 pounds of nitrogen per acre	40 pounds of nitrogen per acre	80 pounds of nitrogen per acre
	Percent	Percent	Percent	Percent
Starch.....	4.34	3.34	2.42	1.52
Reducing sugars (as invert).....	1.48	1.40	1.28	.59
Sucrose.....	.32	.20	.15	.20
Cellulose (crude fiber).....	16.07	17.20	16.89	16.60
Pectic acid (as calcium pectate).....	14.93	16.01	14.00	15.05
Protopectin (as calcium pectate).....	1.24	.70	1.63	.09
Protein.....	7.50	9.06	8.63	11.50
Nicotine.....	.03	.73	1.24	1.46
Nitric acid (N ₂ O ₅).....	.09	.09	.11	.58
Ammonia.....	.08	.06	.10	.19
Chloric acid.....	1.17	1.46	1.67	3.36
Malic acid.....	3.16	4.07	4.98	5.32
Oxalic acid.....	1.53	1.77	1.68	1.50
Plant wax.....	.34	.20	.31	.17
Total resins.....	0.55	5.60	6.57	5.81
Resene.....	4.40	3.76	4.08	4.04
Alpha-tobaccoic acids.....	.70	.74	.67	.69
Beta-tobaccoic acids.....	.75	.62	.45	.09
Gamma-tobaccoic acids.....	.55	.37	.37	.31
Resin alcohol.....	.06	.11	.00	.08
Total ash.....	14.70	12.88	14.47	14.96
Potash.....	3.36	2.96	2.19	2.23
Calcium oxide.....	4.90	5.11	5.58	6.12
Magnesium.....	1.73	1.58	1.62	1.73
Phosphoric acid (P ₂ O ₅).....	.64	.48	.48	.43
Total sulphur (as SO ₂).....	1.48	1.60	1.45	1.30
Chlorine.....	.10	.37	.28	.35
Total nitrogen.....	1.81	2.18	2.30	3.06

TABLE 36.—Effect of quantity of nitrogen in the fertilizer on the distribution of the nitrogen in cured leaf tobacco, season of 1925

Form of nitrogen	Distribution of nitrogen found in leaves from plants fertilized with—			
	No nitrogen	20 pounds of nitrogen per acre	40 pounds of nitrogen per acre	80 pounds of nitrogen per acre
	Percent 100	Percent 100	Percent 100	Percent 100
Total.....	56.30	66.51	60.60	60.13
Protein.....	6.11	5.95	9.13	8.17
Nicotine.....	3.31	2.02	4.09	5.26
Amide.....	8.23	5.65	7.52	7.90
Nitrate.....	1.32	1.10	1.22	4.87
Ammonia.....	3.70	2.34	3.74	5.10
Undetermined.....	11.03	16.53	14.30	8.51

On the whole, the influence of increasing the nitrogen supply in the fertilizer on the chemical composition of the cured leaf is not particularly striking, but some clearly defined effects of importance are apparent. With increasing quantities of nitrogen applied to the soil there is a progressive decrease in content of starch and sugars. Insofar as the values for crude fiber are indicative of the content of cellulose, change in the nitrogen supply seems to have no significant effect on this constituent of the cell wall. The values for pectins are somewhat irregular, but there is indication that a high nitrogen supply tends to reduce slightly the pectin content. None of the samples contained appreciable quantities of water-soluble pectin. The data for protein also are somewhat irregular, but it seems clear that a high nitrogen supply results in a decided increase in protein. It is evident that the nicotine content tends to follow the nitrogen supply of the plant, while the highest rate of nitrogen fertilization also produced a significant increase in content of nitrates and ammonia. The total nitrogen in the leaf increases as the rate of nitrogen fertilization is increased, and the major increase in percentage content is obtained in the increase in fertilization rate from 40 to 80 pounds per acre.

With respect to nonvolatile organic acids the analytical data indicate a definite increase in citric and malic acids as the rate of nitrogen fertilization is increased, while the oxalic acid values are not materially affected. The results for citric and oxalic acids are believed to be fairly reliable, but those for malic acid probably are too high and no method was available for checking the data for the latter. The figures for content of plant wax are irregular and do not seem to be especially significant. The values for tobacco resins obtained by the empirical procedure of Degrazia do not indicate significant effects of the nitrogen supply with respect to either the content of total resins or the distribution of the several resin fractions. Under the conditions the content of total ash is not much affected by rate of nitrogen fertilization. The contents of potash and phosphoric acid seem to be somewhat reduced by an increased nitrogen supply, while the contents of calcium oxide and magnesia remain approximately constant.

As regards distribution of the nitrogen in the cured leaf (table 36), the insoluble or protein fraction, which constitutes about two thirds of the total, decreases somewhat with increase in nitrogen fertilization. The product grown with a supply of 20 pounds of nitrogen per acre runs relatively low in the soluble fractions determined, for reasons that are not known. There is a general tendency toward increased proportions of the several soluble fractions as the rate of nitrogen fertilization is increased, with the exception of the amino-nitrogen component. In no case, however, is this increase particularly striking.

INTERPRETATION OF RESULTS AND CONCLUSIONS

YIELD AND VALUE OF CROP

It is believed that the soil areas employed were sufficiently uniform to leave no doubt as to the significance in yield and value obtained with various quantities and forms of nitrogen, at least when the successive increments of nitrogen used were as much as 20 pounds per acre. (See tables 5, 7, and 10.) Interpretation of the data is somewhat less certain with 10-pound increments of fertilizer nitrogen, although even in this case the results in the main probably are at least approximately correct. With a 10-year combined average yield (table 5) of 614 pounds of leaf and 325 pounds of stalk per acre for the several plots receiving no nitrogen in the fertilizer and without applying corrections for variations in the individual control plots, the increases in the yield of leaf for each pound of nitrogen in the fertilizer in the nitrate of soda series were 13, 13, 11, 9, and 5.5 pounds, respectively, for the 10-, 20-, 30-, 40-, and 80-pound rates of fertilizing. The corresponding increases in yield of stalk were 13, 14, 10, 8.5, and 4.5 pounds. Analysis of the results shows that the effectiveness of the 80-pound rate of nitrogen fertilization was dependent to a considerable extent on the method of application employed. The data as a whole indicate that under the conditions the yield with 40 pounds of nitrogen per acre is not far below the maximum obtainable unless split applications of nitrogen are made, in which case 80 pounds per acre give a decided additional increase.

As regards applicability of the Mitscherlich formula to the yield data, the principal question involved is whether the numerical value of 0.122 which Mitscherlich has assigned to the so-called constant, c , holds true under the conditions of these tests. Investigators are sharply divided on the question of whether c really remains constant under all conditions. Mitscherlich and his followers (18) have presented extensive data in support of the affirmative side, while a number of investigators have advanced experimental evidence tending to show that the numerical value of c may vary widely under sufficiently diverse conditions. Among the latter may be mentioned particularly Lemmermann, Hasse, and Jessen (17) who found that in general the value of c tends to increase as the general environment becomes less favorable for growth, and in this connection in field plot tests with nitrogen as a fertilizer the value of c was found to vary through a wide range, attaining a maximum of 1.03.

Inspection of figure 6 and table 9 shows at once that Mitscherlich's value for c leads to inconsistent and abnormally high values for A , the maximum yield obtainable. These values are far in excess of the

maximum yields indicated by the curves representing yields actually obtained at various rates of fertilization. The indicated quantity of the soil's reserve supply of nitrogen, b , also appears to be too high. The relative quantities of nitrogen recovered by the crop on the control plots and on those receiving 20 pounds of nitrogen per acre as fertilizer (table 18) indicate a content of 20 to 25 pounds of available nitrogen in the soil. As a necessary consequence of unsatisfactory values for A and b , the computed yields for various rates of fertilization utterly fail to agree with those actually obtained. It seems clear that Mitscherlich's value for nitrogen is much too low to fit the present data and, in fact, the computations in table 9 show that the numerical value of c must be raised to unity or higher in order that the formula may yield results even roughly agreeing with those actually obtained. On the whole, best results are obtained by assigning to c a value of about 1.2.

A second difficulty encountered in applying the Mitscherlich equation is the fact that the curves representing the actual yields are not uniform throughout their course as is required by the Mitscherlich formula, but, on the contrary, the lower increments of yield approximate a linear series followed by a rather abrupt decrease in the increments from intermediate rates of fertilization, especially in the case of the stalk yields. For this reason there can be no fixed value for c capable of accurately portraying the relationship between the nitrogen supply and the yield for both low and high rates of fertilization. Finally, as shown in figure 7, the proportions of stalk and leaf in the plant are materially influenced by the nitrogen supply and consequently no single formula can give equally satisfactory results for these two organs of the plant. It appears that in the case of the stalk there is no value for c in the Mitscherlich formula which will give satisfactory agreement with the yields actually obtained, and this situation applies in a somewhat lesser degree to the yields of leaf and stalk combined.

The data on recovery of fertilizer nitrogen by the crop in 1919, 1925, and 1929 in the nitrate of soda series (table 18) supply one reason for the sharp falling off in the yield increment in passing from the 20- or 30-pound rate to the 40-pound rate of fertilization and for the marked variation in yield from year to year in a given treatment. While recovery of the first 20 pounds of nitrogen was comparatively good (40 to 90 percent), the additional nitrogen recovered from the 40-pound rate of fertilization was surprisingly small. The 40-percent recovery from the first 20 pounds of fertilizer nitrogen and the relatively low yield in 1919 were associated with a rainfall of 11 inches in July and August, the 60-percent recovery and a good yield in 1925 were associated with a rainfall of 6.7 inches in July but only 2.6 inches in August, while in 1929 the 90-percent recovery of nitrogen and the heavy yield were accompanied by a rainfall of only 4.2 inches for the 2 months. It seems very probable that the midsummer rainfall figured prominently in these results. The proportionately higher recovery of nitrogen in 1925 and 1929 than in 1919 from the 80-pound rate of fertilization as compared with the 40-pound rate doubtless was due largely to fractionation of the fertilizer application. With only one exception for the leaf and two for the stalk increasing rates of nitrogen fertilization resulted in progressive increase in percentage content of nitrogen in the plant, showing that the additional nitrogen

absorbed with increasing rates of fertilization becomes progressively less effective in promoting growth. In other words, the decreasing increments of yield with increasing rates of fertilization apparently are due partly to a decrease in the proportion of the fertilizer nitrogen absorbed by the plant and partly to decreased effectiveness of the nitrogen actually absorbed in promoting growth.

The results obtained with the various sources of nitrate and ammonia nitrogen (table 6), on the whole, indicate that under the conditions of the tests these two forms of nitrogen were about equally effective in increasing the crop yield provided due consideration is given to the disturbing effects of other ions contained in the nitrogen compounds or in the soil, more particularly those of magnesium, calcium, chlorine, and sulphur. The somewhat superior results with ammonia nitrate suggest, however, that a combination of ammonia and nitrate nitrogen may be more effective than either form alone. Prianischnikow (21) found that under conditions unfavorable to nitrification the relative efficiency of nitrate and ammonia nitrogen cannot be expressed by a fixed coefficient, since it is dependent on a number of factors internal and external to the plant. For example, with a low or a negative hydrogen-ion value and a relatively high concentration of calcium ions, ammonia nitrogen may give superior results, while under reversed conditions of hydrogen- and calcium-ion concentration nitrate nitrogen was more effective. Tiedjens and Robbins (25) found that ammonia nitrogen was readily assimilated by several plants which were studied if the medium had a neutral or alkaline reaction, whereas nitrate nitrogen was most efficiently assimilated in a distinctly acid medium. Contrary to conclusions reached by numerous other investigators, these authors found that in the plants studied although ammonia accumulated in considerable quantities in the tissues there was no evidence of toxic effects.

In the case of tobacco, Haas (12) has reported development of pathological symptoms from continuing applications of ammonium nitrate solutions to the soil in pot cultures. Beaumont and his associates (5) found that in approximately neutral water cultures tobacco was unable to assimilate ammonia nitrogen as readily as nitrate nitrogen and sooner or later toxicity resulted from all ammonium salts used. In field tests (6), however, ammonium sulphate gave good results. In the present field tests, in which presumably there was free opportunity for nitrification, no specific symptoms of ammonia toxicity were observed. The reduced yields that were obtained with a combination of the product Ammo-Phos and sulphate of potash prior to the application of dolomitic limestone to the soil (table 7) and the eventual crop failure obtained with a mixture of monoammonium phosphate and nitrate of potash (table 8) have been shown to be due primarily to deficiency of magnesium and calcium in the soil and not to the form of the nitrogen used (fig. 5). These results have been briefly referred to in a previous publication (11) in which it was shown by means of supplementary plot tests and chemical analysis of the plant material that there is marked deficiency of both magnesium and calcium in the soil used in the experiments reported in this bulletin. The most striking feature of these particular tests is that omitting both calcium and magnesium from the fertilizer mixture had a much greater effect in suppressing growth of the crop than omission of

nitrogen, and other tests on the same soil show that this effect also is greater than that of omitting phosphoric acid or potash.

The increased yield obtained with ammonium chloride at the lower rates of fertilization as compared with other forms of nitrogen is believed to be due to the stimulating action of the chloride ion, an effect which has been discussed at some length elsewhere (10). It has been shown that in favorable concentrations the chloride ion may stimulate growth by enabling the plant to resist drought. Since a high nitrogen supply will be shown to be capable of exercising a similar effect the stimulating action of chlorine may be expected to diminish as the nitrogen supply is increased. These relationships are clearly shown in figure 4.

During the period 1921 to 1929 the average increase in yield from urea somewhat exceeded that from ammonium sulphate at two different rates of application (table 6). However, this series of plots is being continued and in 1930, in which the growing season was extraordinarily dry, ammonium sulphate gave a considerably better yield than urea, although analysis of the plants showed that the recovery of nitrogen from the urea treatments was fully equal to that from the ammonium sulphate. The only sulphur supplied in the basal fertilizer treatment in these tests is that contained in the 80 pounds per acre of sulphate of potash used, and the pale-green color observed in 1930 in both leaves and stems of the plants fertilized with urea indicates that the reduced yield from this treatment under the dry weather conditions probably was due in part to sulphur deficiency. One of the distinctive effects of sulphur deficiency in the soil under normal weather conditions is delayed growth in the early stages of development and it is believed that the hastening effect of ammonium sulphate on date of flowering shown in table 16 was due at least in part to the additional sulphur supplied. There is definite evidence, therefore, that in considering relative fertilizing values of the various synthetic forms of nitrogen on soils of the type here used due consideration must be given to the supply of magnesium, calcium, chlorine, and sulphur made available to the plant.

As regards the effect of the nitrogen supply on the commercial value of the cured tobacco, it should be understood that the data and discussion on pages 22 to 24 relate primarily to Maryland tobacco and do not necessarily apply to other commercial types of leaf. For the Maryland type, best results as to acre value and price per pound of leaf were obtained with 20 to 40 pounds of nitrogen per acre. For best results with respect to quality of product the nitrogen requirements of the various types of tobacco differ materially as regards both the quantity and the form of the nitrogen. It is probable that under comparable conditions the nitrogen requirements of burley tobacco would be somewhat greater than those of Maryland tobacco, while those of the flue-cured type would be only slightly less. On the other hand, the cigar types, especially the binder and wrapper grades, require for best results much larger quantities of nitrogen and this fact, together with the light character of the soils most used in growing the binder and wrapper grades, tends to complicate the question of best forms or sources of the fertilizer nitrogen. Some of the reasons for these contrasts in nitrogen requirements of the different types are brought out in later paragraphs.

GROWTH RELATIONS AND LEAF CHARACTERISTICS

The rate of growth as measured by increase in height of the plant is markedly affected by the amount of the nitrogen supply, and under the conditions of the test the form of the nitrogen also appears to be of some significance (table 13, fig. 8). Although more or less in evidence in most of the treatments, the familiar sigmoid form of growth curve is best developed with the slow rate of growth which occurs on the control plots receiving no nitrogen in the fertilizer. While the heaviest rate of nitrogen fertilization, 80 pounds per acre, produced a somewhat lower average rate of growth than moderate rates of fertilization, there was no pronounced change in form of the growth curve. The most rapid average increase in size of leaf was produced by the highest rate of nitrogen fertilization. The low rate of growth produced by a deficient nitrogen supply was accompanied by a marked delay in flowering (table 16), and the highest growth rate, which was induced by a moderate rate of nitrogen fertilization, also was associated with earliest flowering.

As regards the effects of the nitrogen supply on comparative development of the principal organs of the plant, it appears that under the conditions of the test the relative growth of the root was not greatly affected, while the development of stem and leaf was decidedly influenced. Nitrogen deficiency materially reduced the proportion by weight of stalk to leaf in the plant, and a high nitrogen supply as compared with a moderate supply also tended to reduce the relative weight of stalk (fig. 7).

From a practical standpoint, the most important effects of the nitrogen supply on development pertain to characteristics of the leaf. The total number of leaves produced by the plant is only slightly influenced by the rate of nitrogen fertilization except that the low growth rate and delayed flowering caused by a markedly deficient nitrogen supply apparently favor a moderate increase in number of leaves produced (table 13). In the case of Maryland tobacco it has been repeatedly observed that various conditions causing delayed growth commonly result in increased number of leaves developed by the plant. The effects of nitrogen deficiency in increasing the number of leaves produced therefore can hardly be considered as specific. The ratio of midrib to lamina in the leaf is of some practical importance. The effect of the nitrogen supply on this ratio was found to be much the same as that on the ratio of stem to leaf in the plant. A deficient nitrogen supply produces a relatively low percentage by weight of midrib, as does also a high supply of nitrogen, while a moderate quantity of nitrogen increases the proportion of midrib to lamina.

The data summarized in tables 14 and 15 show that the effects of an increased supply of fertilizer nitrogen in producing a decidedly broader and larger leaf are coupled with a definite decrease in weight per unit of area, and similar relations are well shown by the data contained in tables 20 and 32. Not only is the increase in yield that is obtained with an increase in the nitrogen supply due to the production of a larger leaf, but the gain in size is even greater than the gain in weight. The reduced weight per unit of area resulting from a high nitrogen supply apparently is due at least in part to a decrease in thickness of the leaf, though it is possible that a less dense type of structure of the tissues also is involved. The data in table 15 indicate

that the average specific gravity of the solids of the leaf is not greatly affected by the amount of the nitrogen supply. The action of nitrogen as a fertilizer on the size, shape, weight per unit area, and thickness of the leaf is of considerable practical importance in relation to the fertilizer requirements of the different commercial types of tobacco as well as being of interest to the plant physiologist. These effects indicate that a liberal supply of nitrogen is desirable when it is sought to produce a broad, thin, light-bodied leaf such as is required for use as cigar wrapper or binder. In this connection it is interesting to note that as much as 200 pounds of nitrogen per acre is commonly used in growing these types of leaf.

The effects of the nitrogen supply on the color and luster of the leaf are of practical importance since the shade, the evenness, and the brilliancy of the color may largely determine the commercial value of the cured product. It appears that when the abnormally pale yellowish-green color characteristic of nitrogen hunger is seen in the plant in the field the cured leaf is likely to be uneven in color and markedly deficient in luster, producing mainly the inferior so-called "dull" grade of product. With a nitrogen supply just sufficient to prevent definite deficiency symptoms there is likelihood of premature ripening and drying or firing of the lower leaves. With a moderate additional increase in the supply of nitrogen a cured leaf of light reddish-brown color and bright luster may be expected. Under the conditions of the test, best results for the Maryland type of tobacco were obtained with 20 to 40 pounds of nitrogen per acre. Heavier rates of fertilization yielded a cured leaf with purer brown or slightly greenish-brown color, with good luster, a result more desirable in cigar leaf.

A thin, light-bodied leaf usually has better burning qualities than a thick, heavy-bodied, close-textured leaf, and since a high nitrogen supply tends to produce the former type its adverse action on combustibility (table 17) probably is due to its effects on the chemical composition rather than the structure of the leaf tissue. It was suggested long ago (19) that proteins and related complex compounds of nitrogen tend to injure the burning qualities of tobacco because of the fact that relatively they are difficultly combustible. The results of the present tests are in accord with this theory, for it is shown that an increase in the fertilizer supply of nitrogen results in an increased content of protein and its derivatives in the leaf.

INTERNAL PROCESSES OF NUTRITION AND METABOLISM

In general, the results obtained with material from the no-nitrogen treatment are regarded as representing nutrition conditions of moderately severe nitrogen deficiency, those from the 20- and 40-pound rates of fertilization conditions of a medium or balanced nitrogen supply, and those from the 80-pound rate conditions of a high and slightly excessive supply of nitrogen under the conditions of the tests. Broadly speaking, the later stages of growth and the early phases of progressive senescence immediately following, which constitute the so-called ripening period of the lower and middle leaves of the plant, involve predominantly katabolic processes that are closely associated with marked translocation of nutritive materials to the upper parts of the plant. Smirnow and his associates (24) have made a comprehensive study of the general nature and results of these processes.

The present biochemical and physiological studies deal chiefly with the effects of the nitrogen supply on the course of these metabolic processes in the fresh leaf prior to harvest and their ultimate effects on the characteristics of the cured leaf.

One of the outstanding physiological effects of the nitrogen supply is that on the water content of the leaf tissues and associated growth phenomena. The data in tables 20, 24, 26, and 32 show that an increased supply of nitrogen materially increases the water content, and the resultant increase in turgidity apparently produces important results in the development of the leaf. It has been pointed out that an abundant nitrogen supply produces a decidedly larger and broader but thinner leaf weighing less per unit of area than is obtained with a low supply of nitrogen. Aside from the mode of action of the nitrogen in reducing or overcoming transpiration losses, it is evident that the effects on the morphology of the leaf are essentially those produced by various other factors which operate to maintain increased turgidity, such as reduced illumination, increased atmospheric humidity, or increased soil moisture. For example, Hasselbring (13) who made a careful analysis of the effects of partial shade on the leaves of tobacco found that their water content was definitely increased, and in association with this effect the leaves were increased in size but weighed less per unit of area. The osmotic concentration of the cell sap seems not to contribute materially to the nitrogen effect on water relations and associated growth phenomena in the leaf (table 22). It appears likely that an increase in content of hydrophilic colloids resulting from an increased intake and assimilation of nitrogen is an important factor, but no studies were undertaken in this direction.

The effect of the nitrogen supply on water content was found to extend to the hygroscopic properties of the cured leaf (table 21). Here again the effect is of considerable importance from a practical standpoint. The capacity of the cured leaf to attract and retain a sufficient quantity of moisture to render it pliable and more or less elastic is of special importance in cigar-wrapper and binder types. The effect of an increased nitrogen supply in this direction suggests that the nitrogenous component of the leaf contributes directly to its hygroscopicity. It appears that the process of curing, at least when the leaves are detached from the stalk in harvesting, tends to render the product more hygroscopic. This result probably is due in part to loss of relatively nonhygroscopic material and in part to accumulation of hygroscopic products of protein decomposition during the curing process. On the other hand, the tendency of the leaf to attract moisture decreases with increase in age, so that during the ripening period the leaf becomes less hygroscopic.

Observations on the active acidity of the cell sap of the leaf (table 23) show that as the ripening or ageing of the green leaf advances there is a definite decrease in the pH value. Since the lower leaves are the first to reach maturity their active acidity usually will be greater in later stages of development of the plant than that of leaves higher up on the stalk. The delayed growth and maturation resulting from a deficient supply of nitrogen is clearly reflected in the slow rate at which the active acidity of the leaf sap increased after the plants had been topped. On the whole, the pH value seemed to fall most rapidly in the plants receiving 40 pounds of nitrogen per acre. Under the conditions of the test the pH value of the immature

leaf was approximately 6.35, or higher, and this value decreased to as low as 5.60 in the fully ripe leaf. The data secured on the content of malic, citric, and oxalic acids (table 24) are not adequate for obtaining a clear picture of the changes taking place during the ripening period in relation to the nitrogen supply. When these data are considered in connection with those for cured leaf (table 35), however, the conclusion seems justified that as the leaf approaches full maturity an increased supply of nitrogen tends to increase the content of citric acid, and the indications are that this relation holds true also for malic acid.

In attempting to obtain information as to the internal mechanism through which variations in the nitrogen supply affect the processes of nutrition and growth the nitrogen metabolism of the plant naturally is of special importance. The fact that during a period of 7 weeks immediately preceding the flowering stage there was a marked progressive decrease in content of total nitrogen and protein nitrogen in the lower leaves of the plant (table 20) might be taken to indicate a very active physiological translocation of the nitrogen from these leaves. For the entire period the decrease in total nitrogen per unit of area averaged nearly 40 percent of that originally present, and the decrease in protein nitrogen was about 58 percent of the original content. Despite these large decreases in total and protein nitrogen there was comparatively little change in content of nitrate, ammonia, nicotine, amino, and amide nitrogen or in the undetermined fraction, thus suggesting that the protein nitrogen undergoes translocation almost as rapidly as it is transformed into soluble forms. However, the leaves increased their area by 25 to 50 percent during the period of observation, and the data in table 20 show that the greater portion of the nitrogen disappearing from the older tissues did not actually depart from the leaves but was utilized in increasing the leaf area. Incidentally these studies show the danger of employing analytical data on a basis of either the percentage content or the content per unit of area in drawing conclusions involving such processes as physiological translocation. In this instance it is essential that the entire area or the mass of the leaf be taken into account.

In association with the slow growth and visible symptoms of nitrogen hunger seen in the plants which received no nitrogen in the fertilizer, the leaf was distinctly low in content per unit of area and on a percentage basis in total, protein, nicotine, nitrate, amino, and amide nitrogen throughout the 7 weeks preceding the flowering stage. While the addition of 20 pounds of nitrogen per acre to the soil produced a decided increase in the content of total and protein nitrogen in the leaf, further additions to the soil showed very little effect on the concentration of nitrogen in the leaf except in the late stages of development (table 20 and fig. 9). On the other hand, in the flowering stage of the plant and subsequently, the content of total nitrogen and protein nitrogen in the leaf increased with each increase in the rate of nitrogen fertilization (tables 20, 24, and 25).

To interpret correctly the foregoing results it is necessary to take into consideration the relative increase in size of the leaf and its total nitrogen content under the different treatments (table 20). The quantity of nitrogen entering the individual leaf increased with increase in the quantity made available in the soil. The addition of 20 pounds of fertilizer nitrogen to the soil's reserve supply effectively

overcame deficiency symptoms in the leaf, and the increased nitrogen content per unit of leaf area that resulted is explainable perhaps on the assumption that the additional nitrogen thus supplied was largely needed to meet immediate nutritional requirements, leaving only a relatively small portion available for expansion of the leaf surface. Further additions to the nitrogen income of the leaf, on the other hand, were chiefly utilized in increasing its surface without further increasing the content of nitrogen per unit of area. After the immediate nutrition requirements had been met the effectiveness of additional nitrogen in promoting leaf expansion increased as the nitrogen supply was increased, as evidenced by a reduction in thickness of the leaf and even a reduction in the content of nitrogen per unit of area. The changes in content of soluble protein in the expressed sap of the leaf taking place during the ripening or ageing process, as measured by the Stutzer method, and the effects of the nitrogen supply on this value tend to follow those applying to nonsoluble protein.

At all rates of fertilization a portion of the nitrogen of the leaf (10 to 20 percent) was returned to the stem of the plant even during the period of rapid growth. It is significant that the quantity thus surrendered was least in the leaves that received the most liberal supply, indicating that a relatively high nitrogen supply tends to cause the leaf to retain its vegetative vigor longer. The very low nitrate values obtained indicate that even at the highest rate of fertilization here employed the nitrogen supply was not in excess of that which could be assimilated. However, where very large amounts of nitrogen are made available to the plant, nitrates may be stored in the leaf in important quantities, as demonstrated by Vickery and Pucher (26) for Connecticut cigar leaf. Whether under these conditions nitrogen metabolism would depart from the course traced in the present experiments cannot be stated. Increase in the rate of nitrogen fertilization had but little effect in increasing the nicotine content of the leaf during the period of most active growth and synthesis of protein, but this effect became more pronounced as the flowering stage was reached. Subsequently there was only a slow increase in nicotine content with a deficient or a low supply of nitrogen, but as the rate of nitrogen fertilization was increased there was an increase in the rate of nicotine accumulation in the leaf (table 24). Consequently, as the leaves reached maturity those that had received the highest supply of nitrogen were decidedly highest in nicotine.

The above results bearing on nitrogen metabolism apply to conditions of high topping. Data presented in table 30 show that in the lower leaves metabolism is profoundly affected by very low topping. Reduction in the transfer of mobile materials from the leaves into the stem as a result of removing approximately the upper half of the plant in the topping operation largely accounts for the decided thickening of the lower leaves even though, under the prevailing conditions of drought, there was not much increase in their size. In these circumstances there was a marked increase in content of total nitrogen per unit of leaf area, accompanied by a somewhat less pronounced gain in protein nitrogen which is in sharp contrast with results under conditions of high topping. Perhaps the outstanding effect of the low topping on nitrogen metabolism was the remarkable increase in nicotine content amounting to nearly 200 percent for a period of 25 to 30 days following the topping.

The observations on carbohydrate relations in the leaf recorded in tables 25 and 27 show that in general the effect of an increased supply of fertilizer nitrogen on the content of starch in the leaf both during the period of active growth and in the later stages of development is the reverse of that on the content of nitrogen, for with a very low nitrogen supply the starch content of the leaf is high, while only a moderate increase in the supply of nitrogen materially lowers the starch content. In other words, there is a complementary relationship between the nitrogen content and the starch content in the leaf. This relationship apparently does not apply to the content of sugar which occurs in the leaf chiefly in the form of monosaccharide. While the nitrogen content tends to constantly decrease during the growth and development of the leaf until a minimum is reached during the ripening or aging period, the content of carbohydrate decreases only temporarily as the plant attains the flowering stage, after which it tends to increase again.

Observations on oxidizing enzymes indicate that they attain their maximum activity at about the time the leaf reaches its greatest vegetative activity. A high nitrogen supply, which increases and prolongs vegetative activity, also increases the catalase activity of the leaf and tends to defer the stage in its life history at which decrease from the maximum catalase activity begins (table 28). Again, low topping of the plants, which increases the nitrogen content and stimulates vegetative vigor, likewise results in increased catalase activity (table 31). Data obtained on peroxidase activity (table 29) show essentially the same relationships that apply to catalase.

Although the nitrogen supply has been found materially to influence metabolic processes in the leaf and its composition in the fresh mature state, the data in table 32 indicate that in the curing process the loss in total dry matter is not greatly affected. The effect of the nitrogen supply on the weight per unit of area of the cured leaf was about the same as that on the leaf in the fresh state. There was an appreciable increase in the content of so-called "tobacco resin" during the curing, but the practical significance of this group of constituents is not definitely known. The results obtained in following the catalase relations through the curing process are of considerable interest. It is known that the greater portion of the loss in dry matter occurs in the first few days of the curing, and it was found that during this period there may be an appreciable increase in catalase activity. In later stages, however, catalase activity decreases sharply so that at the end of the curing the leaf retains but a small fraction of its initial activity. Although, in line with results previously discussed, leaves grown with a high nitrogen supply showed a relatively high catalase activity in the early stages of curing, at the end of the process the activity of such leaves tended to approach that of leaves grown with a low supply of nitrogen (table 33). There was a small decrease in the peroxidase number during the curing. As in the fresh leaf, the peroxidase value in the cured leaf was increased by increase in the rate of nitrogen fertilization (table 34).

In contrast with the result in normal curing, rapid drying at ordinary temperatures preserves in large part the catalase activity of the fresh leaf. The fact that if the leaf is exposed to chloroform vapor before the drying most of this activity is rapidly lost, may well be due to the action of the chloroform as a protoplasmic poison,

thus permitting diffusion of the cell sap and rapid contact between the catalase and the constituents of the cell sap with which it reacts.

CHEMICAL COMPOSITION OF CURED LEAF

In the cured state the Maryland type of leaf is characterized mainly by its peculiar dry, light, chaffy nature and its mildness, and it normally possesses good burning qualities. The better grades possess a light reddish-brown color. These characteristics of Maryland tobacco are explainable in part on the basis of the chemical composition of the leaf as disclosed in tables 35 and 36. It is exceptionally high in both cellulose and pectins, constituents of the cell wall, the combined content of these two groups of carbohydrate accounting for about a third of the chemical make-up of the leaf. Most other domestic types of leaf usually contain less than 25 percent of these constituents. The high content of cell-wall material undoubtedly contributes to the dry character and the free combustibility of the Maryland type. The content of the more plastic forms of carbohydrate, starch, and sugars is comparatively low in the cured leaf. Cigar-leaf types in the cured state normally are quite low in starch and sugar and when cured under favorable conditions more commonly are practically free of these constituents. Flue-cured tobacco, on the other hand, contains considerable quantities of starch and is very high in sugar, the better grades usually containing 10 to 20 percent. The mildness of Maryland tobacco is accounted for by its low nicotine content (less than 2 percent), most other types usually containing 2.5 to 5 percent.

The nitrogen content of Maryland tobacco is low, averaging perhaps 2 to 2.5 percent, in line with the data shown in table 35. This average probably is about the same as, or only slightly higher than, the average for the flue-cured type of tobacco. However, the cigar-leaf types (1, 4, 16), burley, and the fire-cured types (22) usually contain 3 to 5 percent of nitrogen. These types frequently though not always contain considerable quantities of nitrate nitrogen, while the flue-cured and Maryland types are characteristically low in nitrates. Maryland tobacco contains less total ash and decidedly less potash than some of the cigar-leaf types but does not differ greatly in these respects from flue-cured leaf.

The nitrogen supply apparently is not an important factor in the leaf content of cellulose and pectin, but it is evident that an increase in the supply of this element tends to decrease the content of starch and sugar. Increase in the rate of nitrogen fertilization produces a decided increase in total nitrogen and in all of the nitrogen fractions determined. Addition of 80 pounds of nitrogen per acre to the small supply already present in the soil increased the protein nitrogen of the leaf more than 50 percent and the nicotine nitrogen more than 100 percent. In view of these effects on the composition and those on the color of the leaf it can be seen that only a moderate supply of nitrogen will tend to produce cured leaf of normal characteristics for the Maryland and the flue-cured types, while a high nitrogen supply will favor production of leaf resembling the cigar-wrapper and binder types. To the extent that the tobacco resins may be a factor in providing the aroma of cured tobacco, the data obtained would indicate that this property is not dependent primarily on the quantity of nitrogen made available to the plant. The ash content, which

is of considerable importance in relation to combustibility and other characteristics of the leaf, seems not to be much affected by the rate of nitrogen fertilization at least under the conditions of these tests. The Maryland cigarette type of leaf, after curing, lacks capacity to undergo the active fermentation characteristic of cigar types. The former is a low-nitrogen type and the latter are high-nitrogen products, and since a high-nitrogen content is associated with high catalase and peroxidase activity it seems likely that the content of the oxidizing enzymes is an important factor in the fermentative capacity.

SUMMARY

Field-plot tests have been conducted for periods of 4 to 11 years on fine sandy loam and on loamy sand types of soil of the Collington series in southern Maryland to ascertain the quantity of nitrogen required for best results with the southern Maryland type of tobacco and to compare the fertilizing value of the older inorganic forms of nitrogen and some of the new synthetic products. As another major feature of the work, the tobacco grown on these plots was utilized for conducting fairly extensive physiological and biochemical observations on the effects of variation in the nitrogen supply on growth and developmental relations and associated internal processes of nutrition and metabolism. The sources and quantities per acre of nitrogen employed in the tests were: Nitrate of soda, supplying 10, 20, 30, 40, and 80 pounds; ammonium sulphate and ammonium chloride, supplying 20, 40, and 80 pounds; ammonium nitrate, cyanamide, urea, urea phosphate and Ammo-Phos, supplying 20 and 40 pounds; a combination of nitrate of potash and monoammonium phosphate, supplying 40 pounds. The basal fertilizer used in most instances furnished 60 pounds of phosphoric acid and 40 pounds of potash per acre.

With respect to crop yields, the 80-pound rate of nitrogen fertilization proved to be excessive when all of the nitrogen was applied in the row prior to transplanting, and it produced no significant increase over the 40-pound rate. However, a decidedly larger yield was obtained with the 80-pound rate when one half of the nitrogen was applied after transplanting the crop. Taking average results for all sources except cyanamide, 20 pounds of nitrogen per acre gave an increase in yield of about 300 pounds of leaf tobacco over the yield of approximately 600 pounds on the control plots. The corresponding average increase in yield of leaf from 40 pounds of nitrogen per acre was about 400 pounds. With nitrate of soda as the source, 40 pounds of nitrogen per acre gave only slightly better results than 30 pounds.

The results obtained with the various sources of nitrate and ammonia nitrogen show that under the conditions of the tests these two forms of nitrogen were about equally effective in increasing the crop yield when proper consideration is given the effects of other ions contained in the nitrogen compounds or in the soil. Ammonium sulphate and nitrate of soda were equally effective while ammonium nitrate, supplying both ammonia and nitrate nitrogen, gave somewhat superior results. Ammonium chloride gave relatively high yields because of the stimulating effect of the chloride ion. The soil used in the tests was decidedly deficient in magnesium and calcium and in dry seasons was deficient also in available sulphur. For these

reasons very poor results were obtained with the combination of nitrate of potash and monoammonium phosphate unless the elements in question were added to the soil and somewhat similar results were observed with the product Ammo-Phos. Urea gave better results than ammonium sulphate except when the sulphur supply was a limiting factor. Cyanamide gave fairly good results when used in limited quantities.

Attempts to apply the Mitscherlich law to the yield data for leaf and stalk and the two combined gave very unsatisfactory results. The numerical value of 0.122 assigned by Mitscherlich to the proportionality factor, c , of his equation for the fertilizer element, nitrogen, proved to be far too low to fit the yields actually obtained at various rates of fertilization. Although Mitscherlich considers that the validity of his law rests on the constancy of the numerical value of c under all conditions, it was found necessary to increase this value approximately tenfold (to 1.2) in order to obtain yield data for the leaf agreeing approximately with the yields actually obtained. The increments of yield actually obtained at lower rates of fertilization tend to form a linear series, but at intermediate rates of fertilization a rather abrupt decline in yield increments appears in the yield curves, especially in the curve for yields of stalk. For this reason the Mitscherlich type of curve cannot accurately portray the results at both high and low rates of fertilization. Again, the relative yields of leaf and stalk are modified by the amount of the nitrogen supply, so that no single formula of this type will satisfactorily fit the data for both leaf and stalk.

The best average grade of leaf for the southern Maryland type was obtained with 20 or 40 pounds of nitrogen per acre and in the nitrate of soda series 30 pounds gave slightly better results than 40 pounds. Considering both yield and quality of product, the most profitable returns were obtained with 30 to 40 pounds of nitrogen per acre. When no nitrogen was used in the fertilizer the so-called "dull" grades of leaf, lacking in luster, were chiefly obtained. Ammonium chloride gave a poor quality of leaf as did cyanamide at the higher rate of application, but there were no marked differences in the effects of other forms of nitrogen. The ammonium chloride had a decidedly unfavorable effect on the combustibility of the leaf.

The ratio by weight of leaf to stalk in the plant was relatively high with a very low as well as with a high supply of fertilizer nitrogen as compared with results obtained with a moderate nitrogen supply. The same relationships apply to the ratio of lamina to midrib in the leaf. Through its effect on water relations in the plant, the chloride ion of ammonium chloride produced a high ratio of leaf to stalk. The nitrogen supply apparently did not greatly affect the ratio by weight of root to stalk and leaf.

The growth rate, as measured by increase in height, was slow when the nitrogen supply was deficient, but a moderate rather than a high supply produced the most rapid increase in height. The sigmoid type of growth curve was best developed in association with the slow rate of growth obtained under conditions of nitrogen deficiency. A high nitrogen supply produced the most rapid increase in size of leaf. Early flowering and early ripening of the leaves were favored by a moderate supply of nitrogen.

The effects on leaf development obtained by varying the amount of the nitrogen supply are of considerable importance. The effect on the number of leaves produced was slight, but an increased supply of fertilizer nitrogen decidedly increased the area and the ratio of width to length of the individual leaf, while the dry weight per unit of area and the thickness of the leaf were reduced materially.

Increase in the supply of nitrogen reduced the combustibility of the leaf, probably because of the effect in increasing the content of protein or its derivatives.

With nitrate of soda as the source of the fertilizer nitrogen, the quantity recovered by the crop ranged from 40 to 90 percent when only 20 pounds per acre were applied to the soil, the recovery depending to a considerable extent on the midsummer rainfall. The additional nitrogen recovered by the crop from the 40-pound rate of fertilization was small. The percentage content of nitrogen in the stalk slightly exceeded that in the leaf, while in the leaf the content of the midrib was only a little more than one half that of the web. There was a progressive increase in nitrogen content from the ground leaf upward to the top leaf of the plant. The most characteristic symptom of nitrogen deficiency was a pale yellowish-green color of the lower leaves of the plant, and this symptom usually appeared when the nitrogen content of the leaf fell below about 1.5 percent.

One of the outstanding effects of an increased supply of nitrogen was markedly to increase the water content of the leaf throughout its development. This increase seems to be an important factor in the increase in area and decrease in weight per unit of area of the leaf produced by the increase in nitrogen supply. These effects are much the same as those produced by partial shade and other factors favoring increased content of water in the leaf. A high nitrogen supply also increased the power of the cured leaf to absorb moisture.

The active acidity of the leaf was affected by the nitrogen supply only in so far as the rate of maturation was modified, the general tendency being for the pH value of the sap to decrease with advance of the ripening or ageing. A high content of nitrogen tended to increase the content of nonvolatile organic acids in the mature leaf. The starch content was found to stand in an inverse relation to the nitrogen supply and the nitrogen content of the leaf, but the content of sugars was little affected by the supply of fertilizer nitrogen. Catalase and peroxidase activity appear to stand in direct relation to vegetative activity in the leaf and were found to reach their maximum in the later stages of leaf growth and thereafter gradually to decline. The content of catalase and peroxidase was considerably increased by increase in the rate of nitrogen fertilization.

During the period of growth preceding the flowering stage of the plant, the content of total and protein nitrogen in the lower leaves, on the basis of equal area, was materially increased only by the first 20 pounds per acre of fertilizer nitrogen, and the additional nitrogen absorbed as a result of heavier fertilization was mostly used in increasing the leaf area. Likewise, the progressive decrease per unit of area in total and in protein nitrogen observed during the growth period at all rates of nitrogen fertilization was due chiefly to transfer of nitrogen from the older tissues to the growing portions of the individual leaf. During this period the content of nicotine, nitrate, ammonia,

and amide nitrogen was relatively low and with the exception of the first 20 pounds of fertilizer nitrogen was not materially increased by increase in the nitrogen supply. On the other hand, in the mature leaf the total, protein, and nicotine nitrogen contents increased with each increase in rate of nitrogen fertilization. In contrast with results in high topping of the plants, with low topping there was, during the ripening period of the leaf, a large increase in total and protein nitrogen and a very large increase in nicotine nitrogen. The studies in nitrogen metabolism developed the fact that, in following the nitrogen exchange of the leaf during growth, neither the values based on unit area nor those on a percentage basis are adequate, it being necessary to take into account the total area or mass of the individual leaf.

The plant's supply of nitrogen did not greatly affect the loss in total dry matter of the leaf in the curing process, which amounted to 21 to 22 percent. In the later stages of curing there were in all cases marked losses in catalase and smaller losses in peroxidase activity.

In the cured leaf the content of total protein, nicotine, and nitrate nitrogen was decidedly increased; the content of other forms of nitrogen and of nonvolatile organic acids were increased to a lesser extent, and the contents of starch and sugars were reduced by an augmented supply of fertilizer nitrogen. There was no decided effect of nitrogen on the contents of cellulose and pectin, the tobacco resins, and total ash. The amount of the nitrogen supply is one of the important factors determining not only the grade or quality but also the type of the leaf produced, that is, its adaptability to specific manufacturing purposes.

Maryland tobacco, which is primarily a cigarette type, is characterized by a high content of cellulose and pectin to which are due to a large extent its outstandingly light weight and dry character and in part, at least, its good burning qualities. It is also characterized by a low content of nicotine, to which it owes its mildness. These characteristics are best developed with a moderate nitrogen supply.

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This bulletin is a contribution from

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END