CHEMICAL INVESTIGATION OF "TRASHY" LEAF PHENOMENON IN AUSTRALIAN-GROWN FLUE-CURED TOBACCO

By R. JOHANSON*

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Summary

Carbohydrates and nitrogen distribution in flue-cured tobacco leaves described as "trashy" were determined. Sugars in "trashy" leaf were as low as 3 per cent. and in "good" leaf as high as 28 per cent. "Trashy" leaf, relatively to normal leaf, had large total N, protein N, and ammonia N contents and low amide N values, while its weight per unit area was about 30 to 50 per cent. less than that of "good" leaf. Uncured "affected" leaf also had low sugar and high N values and low weight per unit area.

It is believed that trashiness is due to processes associated with carbohydrate impoverishment in leaves on the plant and that its development is conditioned by constraints such as soil nitrogen (N), temperature (T), and sunlight (L). It is suggested that effects of the N-L-T constraints on compounds available to the plant for degradation as "foods," can be expressed in simplified form as $\Sigma R \propto NT$, where ΣR is the total energy "spent" irreversibly (i.e. become non-available for various living processes) by the plant in unit time. If ΣE is the total available energy from all sources during the same time, then for certain values of $\Sigma E - \Sigma R$ "sugars" will decrease with corresponding approach to the state of exhaustion of other compounds and onset of trashiness.

In agricultural practice it is suggested that, when considering measures for control of trashiness, attention should be given to plant spacing, nitrogen supply, number of hours of sunshine, and night temperatures.

I. INTRODUCTION

The phenomenon of tobacco leaves becoming worthless brown material during the flue-curing process occurs every year in tobacco-growing areas of the eastern States of Australia. Such leaves are commonly described as trashy. Loss to the tobacco industry through the occurrence of trashy leaf may be considerable,† the amount varying from season to season and in extreme cases including almost the entire crop in some areas.

The mode of occurrence of this trouble clearly indicates that trashiness is not due to diseases, insect pests, or faulty procedure in harvesting and curing.

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

† The season 1932-33 in north Queensland became known as the "black" year. Whole crops were worthless. The economic difficulties of growers were so great that a public inquiry was held and a report (Tobacco Inquiry Committee 1933) presented to the Commonwealth Government. The accumulated evidence showed that the high percentage of trashy leaf in the crop was the major factor affecting production adversely. At that time, tobacco in north Queensland was grown entirely under natural rainfall conditions whereas now a high proportion of the crops are grown under irrigation in the dry season, but trashiness still occurs.

It is developed in the leaf as a result of the conditions under which the leaf was produced, and is a manifestation of intrinsic properties that become more evident during flue-curing. This disposition to trashiness establishes the desirability of obtaining an intimate understanding of the phenomenon and of its relation to known metabolic processes in plants. As far as can be ascertained, no reports on the chemical composition, cause, or occurrence of trashy leaf have been published. Some aspects of the chemical nature of trashy leaf were investigated, special attention being given to nitrogen and carbohydrate distributions.

II. Sampling

The importance of sampling of plant material for chemical studies has been emphasized by many chemists (e.g. Chibnall 1924; Smirnov and Izvoschikov 1930; and others) and discussed extensively by Vickery, Leavenworth, and Bliss (1949). Investigations by Vladescu (1938*a*, 1938*b*, 1938*c*) and Andreadis and Toole (1939) on individual leaves of several varieties of tobacco; Piatnitzky (1927) and Darkis *et al.* (1936) on groups of leaves; and Askew *et al.* (1948) on consecutive harvests, all showed that in normally developed plants there is a small change in composition from leaf to leaf depending on stalk position.

To ensure comparative samples for this work, particular attention was given to leaf position on the stalk and to leaf maturity. The mature leaves were harvested and cured or dried. Trashy and good leaves were obtained from the leaf groups represented by leaf positions 4 to 10 inclusive, from the lower half of the plant. All leaves of this group from all the plants in the plot were in seven groups representative of their leaf positions. Each group was then subdivided into three classes—trashy, semi-trashy, and good. Composite samples of each class were obtained by aggregation of proportionate numbers of leaves from each of the seven groups. This procedure reduced errors due to differences in developmental age, should such have been present prior to onset of "trashiness," and ensured comparability of samples throughout the range of leaf positions.

Ripe leaves were harvested in the morning when the amount of carbohydrates is at the lowest level (Moroz-Morozenko 1935) and when differences in sugar contents due to uneven shading through the day are lowest. The cured or dried and graded leaves were stripped, and both web and midrib were dried at 70°C. in an oven with forced air circulation, then ground to pass through No. 36 mesh (openings 0.0170 in.) sieves, and conditioned to contain about 5 per cent. moisture.

*Samples.**—Tobacco plants were of the flue-cured variety, known locally as Kelly (*N. tabacum*), and were grown in plots on the Tobacco Experiment

* Most of the analyses of plant materials have been done on samples obtained during growth and senesence but with flue-cured tobacco the important part of the plant. the leaf, is removed at maturity and subjected to the flue-curing process. Particular attention has therefore been given to the flue-cured leaf, which is the product of growth as modified by curing. The nature of these modifications is well known but further confirmation, under the conditions of the experiments, was obtained by examination of material both before and after curing.

Station of the Queensland Department of Agriculture and Stock at Clare, north Queensland.

The samples 1*d*, 2*w*, 3*n*, 4*n*, and 5*n* are from leaf harvested in November 1948, from plots (each 30 plants) that received uniform cultural treatment in all respects except in quantity of water applied by irrigation. Samples harvested in November 1949, came from the same plot area as those of 1948 but the plants followed soon after a peanut crop and probably had extra N from the soil.

Sample 1*d* was obtained from tobacco plants grown under extremely dry conditions. The plants were stunted (2 ft. high—see Plate 1) and produced comparatively small leaves approximately 18 × 10 in. that failed to ripen normally and retained their green colour after curing.

Sample 2*w* was from plants that always had more than sufficient water, the plants (4 ft. high, topped—see Plate 1) being normally developed with leaves approximately 21 × 12 in. that cured well and were of good quality and colour. Plant development was such that all leaves were exposed to direct sunlight during growth.

Samples 3*n*, 4*n*, and 5*n* were obtained from a plot which received what appeared to be the optimum watering as judged by the appearance of the plants, which were large (6 ft. high, topped—see Plate 1) and had more than the average number of leaves, all of which were of good size, being approximately 24 × 16 in. In the field there was considerable shading of the lower part of the plant and some leaves were thin and lacked gumminess, but these characteristics were not sufficiently pronounced to be used as a basis for the separation of good and trashy leaves. During curing, however, trashiness manifested itself clearly.* Some leaves from these plants were of exceptionally good quality, while others were worthless. From this tobacco three grades were selected: "all trash" (sample 3*n*); thin, papery leaves with brown areas, which may be regarded as "semi-trashy" (sample 4*n*); and high-quality leaf (sample 5*n*).

Samples from the 1949 crop consisted of uncured and cured leaf, which was harvested from alternate plants, the uncured leaf, as harvested, being immediately dried at 70-75°C. Uncured leaf from the same positions on the plants was separated into two groups, representative of "good" and "affected" leaves had they been cured. However, the differences in physical characteristics were small, the leaves with patches from dull green to a brown tinge and somewhat thin being taken to be affected leaf.

Flue-cured samples of 1949 crop were graded into three classes, good leaf, semi-trash, and trash, by the method used for the 1948 samples.

* Affected leaves behave normally during the early stages of the colouring process, but later become brown, whereas normal leaves remain yellow. At the completion of curing, trashy leaves are extremely thin, brittle, and dark brown. These leaves are often referred to as "dead" or "perished." They will not absorb moisture readily and shatter during handling. Trashy leaves after curing weigh only 30-50 per cent. of normal leaves of the same area, a factor of importance because it means that the actual loss of yield is much greater than is apparent on a weight basis. Furthermore, leaves from the lower half of the plant are most commonly affected and such leaves are normally expected to be of good quality and of high value.

III. Experimental

Some chemists have reported their data on tobacco constituents on a dry weight basis and others on weight per unit area (e.g. Smirnov and Izvoschikov 1930; Moroz-Morozenko 1935; and others) the former procedure being considered preferable in this work. However, approximate transpositions to a weight per unit area basis can be made on the basis of comparative weights of equal areas of trashy and good leaves from the same leaf groups. In this connection it must be remembered that the proportion by weight of midrib to leaf-web markedly increases with increasing trashiness. Trashy leaves weighed less per unit area than good leaves and the loss of weight can be substantial. Thus 65 good leaves weighed 605 g. whereas 80 trashy leaves weighed only 242 g., a loss of 67 per cent. in weight for equal areas of leaf. Accordingly this loss of weight per unit area of trashy leaf is taken into account in the interpretation of the results of analyses.

(*a*) *Protein Preparations*

These were prepared by removing non-protein nitrogenous compounds, together with non-nitrogenous extractable substances using the method of Lugg (1939) as modified by Lugg and Weller (1944).

(*b*) *Nitrogen*

Estimation of total N was made by the Kjeldahl method (Johanson 1948*a*), using apparatus as described previously (Johanson 1948*b*, 1949).

(*c*) *Sugars and Starch*

The estimation of reducing sugars (glucose and fructose) and sucrose was carried out on the aqueous alcoholic extracts from protein preparations. The aliquots were diluted with water and alcohol was driven off by heating on the water bath. The aqueous extracts obtained were then purified with clearing solution, as described by Doak (1939), and the reducing sugars determined by the procedure of Blick (personal communication; see also Blick 1943; Lepper 1945) by oxidation with Fehling solution, using methylene blue as an indicator. The values for sucrose were obtained by estimating the increase in reducing sugar values after acid hydrolysis. Starch was determined by the method used by Pucher, Leavenworth, and Vickery (1948).

(*d*) *Mineral Matter*

The total ash content and soluble and insoluble silica were determined (Askew 1932) and Mg and Ca were estimated by standard methods adopted by the Plant Analysts Committee, New Zealand Institute of Chemistry.

(*e*) *Ammonia*

The interference by alkaloids in titrimetric and to a lesser degree in colorimetric estimations of NH_3 N (see also Vickery and Pucher 1929), together with the complicating effect of remaining enzymic activity in tobacco leaf even after

flue curing, made it obligatory to determine the most suitable method for estimation of NH_3 in tobacco. A more detailed report of this investigation will be published elsewhere. The procedure adopted was as follows: Before use, the apparatus (see Pucher, Vickery, and Leavenworth 1935; Archibald 1943) is thoroughly aerated with air scrubbed with H_2SO_4. The 100 ml. receiver flask is charged with 2 ml. 1N H_2SO_4 and 10 ml. water. Then 2-4 g. of dry sample are placed in the 250 ml. distilling flask together with 20 ml. of 0.25 molar Na_2HPO_4 buffer solution and 10 ml. of water. The distillation is continued for 22 minutes at the reduced pressure of 5-6 cm. Hg, at a temperature of 40-41°C., and a steady stream of air is maintained during the distillation. As soon as the initial frothing subsides, another 20 ml. of water is added through the thistle funnel. The distillate is evaporated to 40 ml. volume in a 100 ml. beaker with 1 ml. of 10 per cent. silicotungstic acid solution, cooled, and set aside for three hours. The solution is filtered into a 100 ml. standard flask using washed No. 42 Whatman filter papers, and the precipitate is washed with 20 ml. N/70 H_2SO_4. To the filtrate 2 ml. of 1N NaOH is added and 3 ml. of Nessler reagent (Folin 1925; see description by Lepper 1945), diluted to the mark, and allowed to stand for 15 minutes. The colour that develops is then estimated in a photoelectric colorimeter (Hilger) and the concentration of NH_3 N is directly obtained with the aid of a prepared calibration curve.

(*f*) *Amide N*

The usual methods of estimating amide N are based on the relative instability of amide linkages in an acid medium, the amides being hydrolysed and the liberated NH_3 estimated in the usual manner (see e.g. Borsook and Dubnoff 1939; Pucher, Vickery, and Leavenworth 1935; Lugg 1938; and others). In a careful study Shore, Wilson, and Stueck (1936) presented a reasonably clear picture of the formation of NH_3 during the period of acid hydrolysis, showing that the deamidation reaction is accompanied by the slow deamination of peptides and amino acids. It would appear that, strictly speaking, an exact value requires the measurement of the NH_3 produced after consecutive periods of hydrolysis, and "extrapolation" from the curves thus obtained eliminates NH_3 due to the deamination reactions. However, since the deamination error is very small it is scarcely worth while to correct for it when only comparative values are of interest. After several trials the following procedure was adopted: 1-2 g. dry samples were heated with 50 ml. 1N H_2SO_4 for four hours at 100°C. in 250 ml. flasks, made neutral to bromophenol blue with 5N NaOH, and after addition of 20 ml. of 0.25M Na_2HPO_4 buffer the procedure followed that described for the estimation of preformed ammonia.

(*g*) *Nicotines*

The estimation of nicotine alkaloids in tobacco has been extensively considered by many workers (e.g. Bowen and Barthel 1944; Markwood and Barthel 1943; Bowen 1947). It would appear that steam distillation, with subsequent precipitation of the alkaloids (notably nicotine and *nor*nicotine) with silico-

tungstic acid, offers the simplest and most accurate means for their determination. While it is necessary to recognize that the values obtained by such methods are somewhat arbitrary, depending on nicotine-*nor*nicotine ratios and amounts of minor steam-volatile alkaloids (nicotyrine, nicotimine, and anabasine; see Smirnov 1940), the total nicotines within the same variety of tobacco from the same area may be taken, for comparative purposes, as representative of their alkaloid contents.

TABLE 1

NITROGEN IN TOBACCO LEAF-WEB SAMPLES AND IN THEIR PROTEIN PREPARATIONS

Samples*	Fresh wt. Taken (g.)	Moisture (%)	Dry Wt. (g.)	Total N (%)	N Total (mg.)	Coagulable N (mg.)	Protein N (mg.)	N in "Protein" Prep. (%)	Dry Wt. "Protein" Prep. (g.)
1*d* Still green after curing	80.7	5.24	76.5	3.41 1.89†	2608	1525	1500	3.63	41.4
2*w* Good leaf	80.0	7.29	74.2	1.64 1.39†	1218	637	627	1.94	32.3
3*n* All trash	60.0	6.04	56.4	1.83 1.62†	1031	587	582	1.57	37.2
4*n* Semi-trash	75.0	4.55	71.6	1.82 1.58†	1301	688	682	1.73	39.4
5*n* Good leaf	85.0	4.77	80.9	1.81 1.43†	1462	733	724	1.88	38.6
‡Good leaf (uncured)	47.6	7.36	44.1	2.05	906	645	636	2.70	23.6
‡Affected (uncured)	45.3	5.14	43.0	2.25	966	702	697	2.91	24.0

* Grown under — "*d*"—dry, "*w*"—wet, and "*n*"—normal watering conditions.

† Total N in midrib of the corresponding samples.

‡ From 1949 crop; all others 1948 crop.

Procedure.—Samples of 1-2 g. were distilled with 10 ml. 8N NaOH, 5 g. NaCl, and 100 ml. of water the volume of which was maintained, under reduced pressure of about 7 cm. Hg at 90°C. in the apparatus used for the determination of preformed ammonia. The distillate was collected in 20 ml. of 0.5N HCl until no opalescence appeared when some of it was treated with a drop of silicotungstic acid. The distillate was evaporated to 200 ml. with 5 ml. of 10 per cent. silicotungstic acid ($4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$) and allowed to stand overnight. The crystalline precipitate was filtered through No. 42 Whatman papers and washed with N/70 HCl until free from silicotungstic acid. The papers were folded, dried in Pt crucibles to drive off the remaining HCl, and then strongly ignited to constant weight. Weight of residue x 0.1141 was taken as the weight of nicotines. Individual determinations agree within less than 1 per cent.

IV. Results

The reported values in Tables 1, 2, 3, and 4 are the means of closely agreeing duplicate or replicate estimations. In Table 1 are shown the percentages of total N, N in "protein" preparations, the amounts of total N, coagulable N, protein N, and other data concerning the preparations. The variation trend of total N in the tobaccos from the 1948 crop is clearly affected by the conditions of growth. The total N and protein N (see Table 2) in 1*d* are greater than any values recorded elsewhere for flue-cured tobaccos (see e.g. Frankenburg 1946) and are correlated with extremely poor smoking quality. The total N content is least in 2*w* whilst in 3*n*, 4*n*, and 5*n* the total N values are similar when expressed on a dry weight basis. It is also evident that the N values of the 1948 crop are much lower than those for 1949 crop (Table 4).

Table 2

NITROGEN DISTRIBUTION IN CURED TOBACCO LEAF-WEB SAMPLES, 1948 CROP

Samples	Protein N in Dry Sample (%)	Total Extractables (%)	Ratio Extractables/Protein N	Total N (%)	NH_3 N (% × 100)	NH_3 N of Total N (%)	Amide N (%)	Amide N of Total N (%)	Nicotines (%)
1*d* Still green after curing	1.97	45.9	23.4	3.41	3.20	0.94	0.316	9.27	3.11
2*w* Good leaf	0.84	56.5	67.0	1.64	0.021	0.013	0.128	7.80	0.86
3*n* All trash	1.03	34.1	33.0	1.83	0.104	0.57	0.085	4.64	1.72
4*n* Semi-trash	0.95	44.9	47.1	1.82	0.050	0.028	0.130	7.15	1.65
5*n* Good leaf	0.89	52.4	58.6	1.81	0.020	0.011	0.155	8.58	1.77

In Table 2 the contents of nicotines, NH_3 N, and amide N are given, and the last two are expressed as percentages of total N; the values of protein N and total extractables are expressed as a percentage of dry samples. Incidentally, the criterion for inclusion of substances in the class of extractables (comprising compounds of three major groups, viz. static, dynamic, and non-protein nitrogenous — see Frankenburg (1946)) is based entirely on their property of remaining in plasmolysing and aqueous alcoholic solvents during the preparation of "proteins." From the N distribution of samples 3*n*, 4*n*, and 5*n* it is apparent that, chemically, trashy leaf is not "dead" in the usually accepted sense because total N and protein N (see Table 2) when expressed on a dry weight basis are normal. This trend is also apparent in total N (1.39 to 1.62 per cent.) in the corresponding midrib samples (Table 1). The development of trashiness is shown even more clearly by the notable decrease of the percentage total extractables (viz. from 52.4 to 34.1 per cent.) also by the ratio

of extractables to protein N. Similar trends are evident in leaf from the 1949 crop (Table 4) where percentages of N increase with trashiness in both cured and uncured leaf.

It must be noted, however, that the increase in N values is only relative to other constituents. Had the data been expressed on a weight per unit area basis the absolute amount of N would have shown a marked decrease. A much greater rate of decrease in other constituents, mainly carbohydrates, produces the effect of an apparent increase in N. Furthermore, these changes in constituents are of such magnitudes that variations due to leaf position (Vladescu 1938*a*, 1938*b*, 1938*c*), within the range selected, become unimportant.

The amounts of NH_3 N (Table 2) are much smaller in the cured leaf than those reported by other workers (e.g. Vickery *et al.* 1940) for the fresh tobacco leaf. Some loss of ammonia, depending on pH of the tissues, must be expected during curing since a temperature of 85°C. is often attained. However, a definite trend to higher NH_3 contents with the progress of trashiness is apparent. The amide N values in samples 3*n*, 4*n*, and 5*n* clearly indicate that there is progressively less of this nitrogen as the degree of trashiness increases. Possibly a greater proportion of amide N is formed in good leaf than in trashy leaf during curing, depending on relative amounts of carbohydrates present. Such amide formations in detached leaf would be in accordance with reports made in the literature (see e.g. Vickery *et al.* 1937; Chibnall 1939; Street 1949). The value of nicotines appears to vary mainly with respect to water treatments and to be less per unit area in trashy than in the good leaf.

TABLE 3

SUGARS AND MINERAL MATTER IN CURED TOBACCO LEAF-WEB, 1948 CROP

Samples	Starch as Glucose (%)	Reducing Sugars (%)	Sucrose (%)	Total "Sugars" (%)	Non-Sugar Extractables (%)	Ca (%)	Mg (%)	Soluble Silica (%)	Insoluble Silica (%)	True Ash (%)
1*d* Still green after curing	—	3.59	1.64	5.31	40.7	2.54	0.60	0.54	1.39	13.4
2*w* Good leaf	1.71	24.4	3.62	28.2	28.5	1.90	0.39	0.45	0.93	10.0
3*n* All trash	0.00	3.06	0.23	3.30	30.8	—	—	—	—	—
4*n* Semi-trash	0.40	11.9	0.32	12.2	32.7	—	—	—	—	—
5*n* Good leaf	0.80	19.3	0.44	19.7	32.7	2.17	0.44	0.63	1.30	12.6

In Table 3, percentages of sugars and mineral matter are given. The values for Ca, Mg, true ash, and silica are lowest in sample 2*w* and highest in 1*d* except for the soluble silica. Thus the general distribution of the mineral fractions clearly indicates a trend toward increases in values with drier growth conditions.

Trends in nitrogen are of significance, but the more spectacular chemical indication of trashiness is given by the carbohydrates. Thus, as shown in Table 3 (1949 crop), 28.2 per cent. of total sugars are present in good leaf (*2w*) and only 3.30 per cent. in trashy leaf (*3n*). Sugars may amount to half of the total extractables, depending on the "quality" of the leaf, total sugar contents increasing with decrease in trashiness. The percentages of non-sugar extractables (Table 3) in good and trashy leaf are somewhat similar. This means that in the flue-cured leaf the leading role in the major quantitative changes in the extractables must be conceded to the sugars which, of all the carbohydrates, are the major constituents of the dynamic group (see Frankenburg 1946).

The above trends in sugars are also present in the 1949 crop, in both cured and uncured leaf samples (Table 4). Total sugars in cured leaf (expressed as glucose) drop from 23 per cent. in "good" leaf to 2.5 per cent. in trashy leaf, while the uncured "affected" leaf has 8 per cent. less total sugars than its counterpart uncured "good" leaf.

V. Discussion

When the data in the previous sections are considered in conjunction with the extensive evidence available in the literature, and with observations of conditions under which tobacco is grown, they suggest the probable causes of trashy leaf. In evaluating the causes, the effects, if any, of flue-curing have to be taken into account. Results of extensive investigations (e.g. Frankenburg 1946) show that total changes are such that percentage compositions of a leaf before and after curing are somewhat similar and the total loss of dry matter is of the order of 10 per cent. Thus it has been shown that the final composition of the flue-cured leaf is dependent on the initial composition of the green leaf, therefore trashiness does not develop as a result of flue curing.

The results of chemical analysis (Table 4) of cured and uncured leaf of both groups (trashy and "good" leaf) proved beyond doubt that the identification of affected leaf before curing was possible. Physical characteristics used in 1949 as a basis for separation of uncured leaf into "affected" and "normal" leaf were observed in 1950 in the field on plants that produced trashy leaf, thus confirming the association of trashiness with conditions of growth. These findings indicate that affected uncured leaf (or at least most of it) is lower in sugars, has high N content, and has less weight per unit area than normal uncured leaf. Consequently, it must be concluded that trashiness is due to intrinsic properties of affected uncured leaf and is not produced by the flue-curing process. These intrinsic properties, whatever they are, are associated with low carbohydrate and high N contents on a dry weight basis or weight per unit area basis.

Incidentally, such a conclusion would explain, in part, the higher ammonium N and lower amide N contents in trashy leaf, as these substances are likely to form in such proportions to one another during the starvation period of curing in a leaf low in carbohydrate.

As it has been shown that uncured affected leaf is low in "sugars" and high in N, it is reasonable to assume that processes during growth and ripening of the leaf are responsible for this occurrence. From observations made in tobacco fields during the past 20 years, it appears that trashy leaf comes from areas where the soil is high in N and where night temperatures are relatively high, and particularly from shaded portions of plants in high crops. Occasionally cloudiness may persist over a wide area for relatively long periods, thus simulating shaded conditions within a crop. In the disastrous 1932-33 season in north Queensland, persistent clouds and light rain for a period of three weeks when the crops were about to mature provided extremely favourable conditions for the development of trashiness. In 1950, trashiness developed under similar conditions and persisted until rain and clouds were replaced by brilliant sunshine.

Evidence has accumulated to show that with tobaccos grown on soils high in N, carbohydrate contents are lower than for tobaccos grown in low N soils and also that high N content is invariably associated with relatively low sugar values. An interesting example of this relationship was reported by Askew *et al.* (1948) where, with a sudden uptake of N from the soil, the general level of 28 per cent. sugar dropped to 16 per cent. while N increased from 1.9 to 3.0 per cent. Similarly, Woltz, Reid, and Colwell (1948) found that sugar content in the cured leaves of flue-cured tobacco was inversely related to total N applied to the soil. In an experiment during 1949, plants (including controls and guard rows) were distinctly deficient in N (as was verified by additions of N to fully grown plants and observations on the radical change that followed the applications), the leaf was smaller and thicker than usual and yellow in colour. Analysis of this leaf revealed exceptionally high sugar content, up to 43 per cent., while N values were of the order of 1 per cent. (expressed on dry weight basis). In general, flue-cured varieties grown on soils excessive in N have leaf high in proteins and other nitrogenous constituents and are very low in sugars. These tobaccos are at best of "poor quality," often will not cure but "decompose," and represent a loss to the tobacco industry.

How N as a constraint through metabolic reactions causes depletion of sugars in the leaf is in the field of speculation and remains to be determined. However, processes removing carbohydrates from the leaf are respiration, formation of organic N compounds, cell wall material, pigments, phenols, essential oils, etc., and translocation from the leaf to other parts of the plant. Therefore the carbohydrate content of a leaf at any time is a measure of the rate of formation of "sugars" (mainly by photosynthesis) and rate of loss. These considerations would explain how environmental conditions conducive to loss of sugars (e.g. such as excessive rate of respiration at high temperature etc.) or interruptions of photosynthesis (e.g. insufficient sunlight etc.) would lead to formation of affected leaf, while retarded consumption of carbohydrates and prolonged photosynthesis would lead to accumulation of sugars.

Since environmental factors, at any one time, would favour either increase or loss of sugars; depending on the sum total of these effects, various contents of carbohydrates in the affected leaf must be expected. Closer examination

of analytical data suggests that in Figures 1 and 2 correlation between sugars and "extractables," and protein N, originate from various degrees of "trashiness." On a dry weight basis the protein N content of trashy leaf is relatively high compared with normal leaf and as "trashiness" progresses, removal of sugars and "labile" compounds (e.g. essential oils, phenols, etc.) produces an apparent increase in N and hence the correlation.

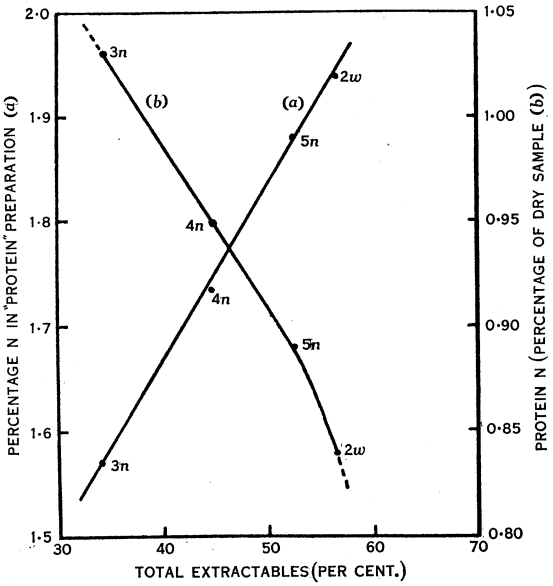

Fig. 1.—Changes in protein N and total extractables with development of "trashiness."

From this, the distribution of 3*n*, 4*n*, 5*n*, and 2*w* samples about a straight line (Fig. 1, curve (*a*)) could be interpreted as showing that these widely varying samples come (or would have come) from leaf with somewhat similar composition and affected by somewhat similar environmental constraints. The environmental conditions under which 1*d* was grown produced "tobacco" of an entirely different nature which, with its extremely high non-protein N and low sugars, could be regarded as a typical product of drought (see also Petrie and Arthur 1943). Further along the line $2w \rightarrow 5n \rightarrow 4n$ trashiness is latent and small adverse changes in environment may produce semi-trashy and trashy leaf, viz. 4*n* and 3*n*. This is again apparent from Figure 1, curve (*b*), with perhaps an additional indication that, as trashiness progressess, the relative rate of decrease of extractables is greater than that of protein N. On the other hand (see Fig. 2) the rate of decrease of total "sugars" with advance of trashiness is somewhat constant in 5*n*, 4*n*, and 3*n*, with the possibility that 2*w* is still further removed from the tendency to become trashy. The difference of 8.5 per cent. for total sugars, between 2*w* and 5*n*, indicates that 5*n* may lie at the limit where infinite "gradation" is represented by a linear function of the curve.

Weather conditions during 1948 and 1949 were not the same, nevertheless values for N and sugars for the 1949 crop (Table 4) conform to the nitrogen-sugars relationship established for the 1948 crop, high N values being associated with low sugars contents.

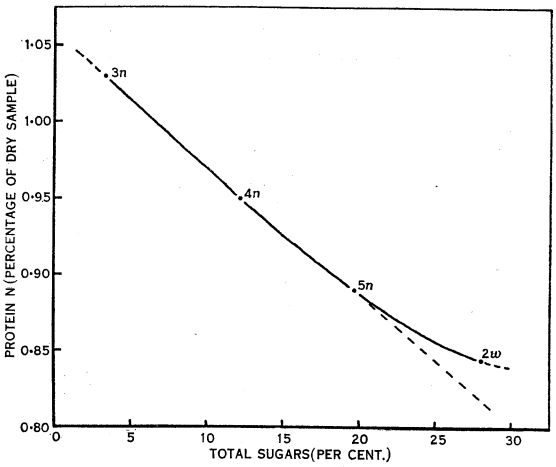

Fig. 2.—Changes in protein N-sugars relationship with development of "trashiness."

The quantitative chemical changes of constituents in the leaf and field observation of conditions with which trashiness is associated show that the

TABLE 4

NITROGEN AND SUGARS IN CURED AND UNCURED LEAF, 1949 CROP

Samples	Total N (%)	Protein N in Dry Sample (%)	Reducing Sugars (%)	Sucrose (%)	Starch as Glucose (%)	Total Sugars as Glucose (%)
Good leaf (cured)	2.03	1.16	14.9	4.70	3.12	22.7
Semi-trash (cured)	2.38	1.34	8.62	3.81	1.18	13.6
Trash (cured)	2.49	1.49	1.47	0.31	0.76	2.53
Good leaf (uncured)	2.05	1.45	17.4	1.55	5.64	24.6
Affected (uncured)	2.25	1.62	9.24	2.20	5.26	16.7

results of this phenomenon have striking similarities to occurrences in other field crops, e.g. sugar cane, cotton, sugar beet, pineapple, etc. Ulrich (1942)

stressed the importance of sunlight and warm days but cool nights as conditions favouring the storage of sugar by high-nitrogen sugar beets; conditions conducive to rapid synthesis of sugars and limited loss of them by respiration. Gardner and Robertson (1942) in a study of the effect of N fertilizers on beets concluded that over-fertilization with N results in a relatively low percentage of sugar in the storage roots and in excessive leaf growth. Dunlap (1945) concluded that conditions unfavourable for the synthesis of sugars, such as cloudy weather, artificial shade, close spacing of plants, or reduction in the number of hours of sunlight increased abscission of cotton bolls, while Eaton and Rigler (1945) found that shedding of bolls was associated with limited carbohydrate reserve. The extensive work on cotton (e.g. Wadleigh 1944; Dastur and Ahad 1941; Eaton and Rigler 1945; Dunlap 1945) reveals that the supply of N available for protein synthesis may be critical in relation to the carbohydrate contents of plants and conditions favouring the synthesis of sugars. Wadleigh (1944) states that, with high N supply, cotton plants may become too vegetative for optimum boll production, and the lower the N the higher may be the carbohydrate reserve of the plants. It may be noted that carbohydrate reserves were directly related to the formation of cellulose fibres and to the oil content of the cotton seeds. Again, Ulrich (1950) reports that nitrate concentrations of the petioles were correlated inversely with sugar concentrations of beet roots; the higher the nitrate values, the lower the sugar concentrations.

High N supply from the soil possibly stimulates the utilization of carbohydrates but subsequent analysis of a trashy leaf may not indicate high organic nitrogen content owing to later degeneration changes and translocation arising from the low carbohydrates level. Thus with trashiness a limit may be reached in the absence of carbohydrates when not only further synthesis of organic N compounds cannot proceed normally, but overall degradation of stored products, as a source of energy for respiratory processes, may become intensified.

Again, investigations with sugar cane (see Brink and Van Den Honert 1940; Rosenfeld 1937) show that a critical balance exists between supply of N and accumulation of sugar in cane. In environments where sunlight is low, decreased yields of sugar resulted if the N applications were too high (see Borden 1940, 1944). The work of Clements (1940), Clements and Moriguchi (1942), and Clements and Kubota (1943) with sugar cane showed that, in locations where soil moisture and soil nutrients were essentially the same, the marked difference in yields of sugar is correlated with differences in sunlight. It would thus appear from numerous reports (see Nightingale 1948) that variants such as soil nitrogen (N), sunlight (L), and temperature (T) (see also Foster and Tatman 1938; Went 1944, 1945; Gregory and Sen 1937; Nightingale 1942) exert a powerful influence on metabolic processes in plants and that unfavourable combinations of these variants (N-L-T) may be detrimental to the production of a crop. The effect of adverse N-L-T magnitudes may appear different in unrelated crops, but the operative constraints are the same.

In tobacco, as far as is known of conditions under which affected leaf occurs, the affected plants appear to be normal (relative to those with good leaf) and there is no circumstantial evidence nor suspicion of any trace element deficiencies or toxicity, nor is there evidence of any deficiencies in major inorganic nutrients or in water requirements. Thus, of the possible environmental constraints that would produce affected leaf, the findings of these investigations, when examined in the light of data presented in the literature, lead to the belief that this phenomenon is a direct outcome of unfavourable magnitudes of *N-L-T*. The relative importance of temperature as a major constraint should not be underestimated. In an extensive study on bean, milkweed, and tomato plants, Hewitt and Curtis (1948) found an average of 15 per cent. total loss of dry matter by respiration and translocation in a 13-hour period with increase of temperature from 10° to 30°C., while in the same period the carbohydrate (starch and soluble sugars) content of the bean leaves dropped by 50 per cent. Thus in addition to the effect of *N* on depletion of "sugars" in the tobacco plant as a whole it must be expected that such depletion would be accelerated by an increase in *T*, which would also directly affect the rate of respiration and consequently the total available energy from carbohydrates. In addition to this general effect an increase in *T* accelerates translocation and thus further aggravates the tendency to trashiness in affected leaves already low in "sugars."

Incidentally, the impoverishment of carbohydrates in a leaf, as was found in careful investigations by workers in this field (e.g. Moroz-Morozenko 1935; and others), may be very rapid. Under unfavourable conditions, leaf can become completely devoid of its "sugars" within 24-48 hours, reaching a state of exhaustion.

As already stated, processes responsible for sugar exhaustion in the leaf are respiration, formation of cell wall materials, organic N compounds, essential oils, pigments, etc., and translocation from the leaves to other parts. Some of these processes would make "sugars" (or products of "sugar") partially or totally non-available to carry on life requirements should the fresh supplies from photosynthesis be insufficient or stopped. At the stage when supply of "sugars" is insufficient or exhausted and the plant begins to "starve," other compounds such as pigments, essential oils, phenols, etc., disappear (possibly degraded as "foods") and in tobacco leaf "trashiness" develops.

Since the "sugar" exhaustion and degradation of products has such far-reaching effects, attention is drawn to the associated loss of energy (*E*). Plants may have all the inorganic salts and elements necessary to build components, yet, in the absence of *E*, synthesis of "foods," structural and auxiliary units, enzymes, etc. necessary for life processses will not be possible. If *E* is not stored in readily available compounds for degradation, for use in 'repair jobs,' for removal of mechanical strains, etc., life cannot persist.

Thus a consideration of the *E* requirements of a plant suggests that *N-L-T* as constraints, by producing effects on living processes also produce immediate corresponding effects on total *available E* supply for these processes; remembering that increase in *N* increases demands on carbohydrates, increase in

T increases rate of respiration, and L through photosynthesis is stored in "foods" as chemical energy.

The rapidity with which "sugars" disappear and magnitudes to which they accumulate suggest their important role in available E change in the plant. Let ΣE be the total *available* energy from all sources, including light, during a unit time and ΣR be the total energy "spent" irreversibly* (i.e. become non-available) for the same unit time. Taking S as a value such that $\Sigma E - \Sigma R = S$ when contents of "sugars" and similar compounds in a plant are just adequate, then if $\Sigma E - \Sigma R > S$, "sugars" will increase and if $\Sigma E - \Sigma R < S$, "sugars" will decrease with a corresponding approach to the state of exhaustion in the plant. Further, the effect of N or T variation on ΣR, for small increments when simplified may be written as $\Sigma R \propto NT$. Thus with increase of T, the magnitude of ΣR may be kept somewhat unaltered if N is decreased, thereby tending to maintain the carbohydrate level and ward off trashiness.

From these considerations it is concluded that conditions favourable for suppression of trashy leaf development would be associated with practices that ensure a small ΣR and large ΣE. In agricultural application it would appear that the former could be decreased by providing the minimum necessary nitrogen supply and by producing the crop in areas where the rate of respiration is retarded by relatively low night temperatures, while the latter could be increased by wider spacing of plants and by growing them in areas where days are relatively long. In this connection it may be that in flue-curing areas of North America, where temperatures are somewhat similar to those in north Queensland, the greater number of hours of sunshine is of major importance in maintaining carbohydrates at a level above which trashiness is not apparent.

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* Here irreversible expenditure of E is associated with processes or that part of processes that result in compounds becoming totally or partly non-available to the plant for degradation as "foods" (e.g. by respiration and formation of cell wall material) while on the other hand formation of essential oils, oligosaccharides, etc. is regarded as a reversible process.

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"TRASHY" LEAF IN AUSTRALIAN TOBACCO

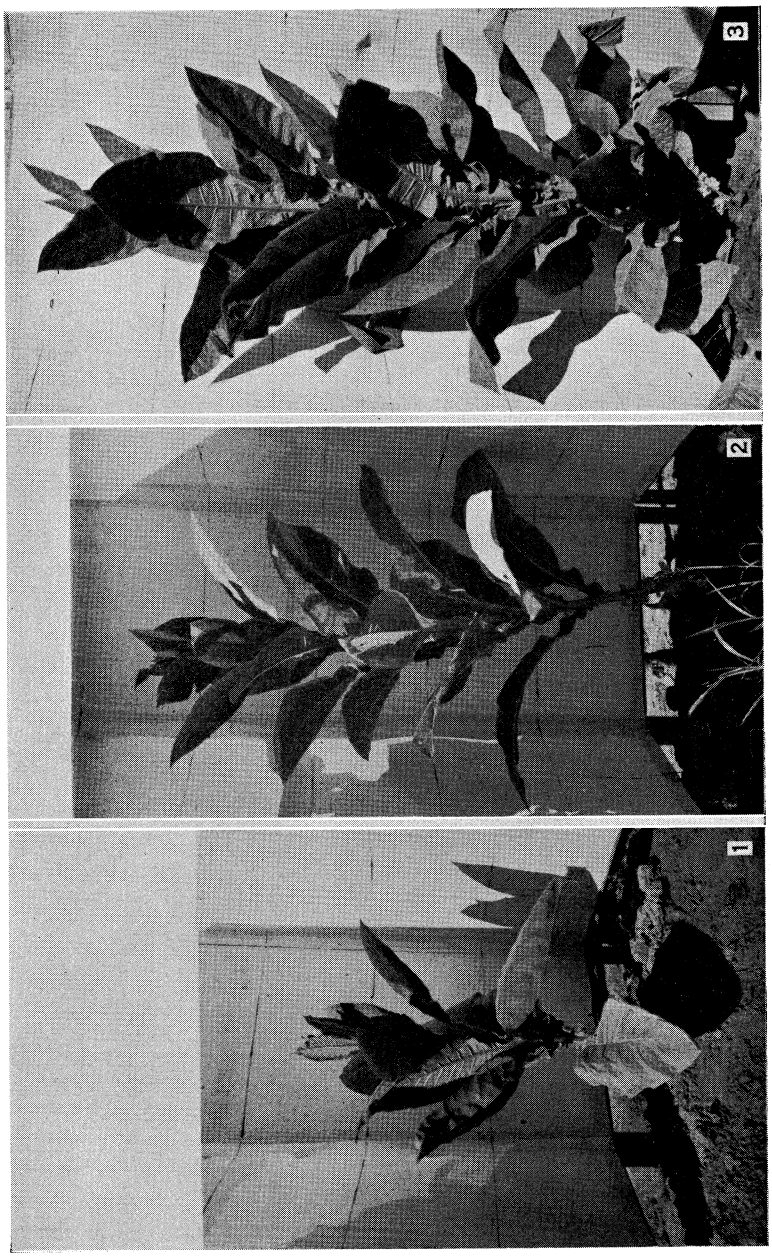

Fig. 1.—Typical tobacco plant grown under dry, "*d*", conditions.
Fig. 2.—Tobacco plant grown under wet, "*w*", conditions.
Fig. 3.—Tobacco plant grown under normal, "*n*", conditions.

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