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U. S. DEPARTMENT OF AGRICULTURE.

Report No. 65.

PHYSIOLOGICAL STUDIES

OF

CONNECTICUT LEAF TOBACCO.

BY

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DETAILED TO THE DIVISION OF SOILS.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF SOILS,
Washington, D. C., March 24, 1900.

SIR: I have the honor to transmit herewith a manuscript concerning some physiological studies of Connecticut leaf tobacco by Dr. Oscar Loew, of the Division of Vegetable Physiology and Pathology. These investigations are in continuation of the cooperative work between this Division and the Division of Vegetable Physiology and Pathology in carrying out in the broadest sense the tobacco investigations authorized by Congress and in the critical and exhaustive study of the possibilities of the soils of the Connecticut Valley.

Respectfully,

MILTON WHITNEY,
Chief of Division.

Hon. JAMES WILSON,
Secretary of Agriculture.

LETTER OF SUBMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., March 20, 1900.

SIR: In accordance with a plan of cooperative work between this Division and the Division of Soils, I respectfully submit herewith the manuscript of a report on some physiological investigations of Connecticut leaf tobacco, by Dr. Oscar Loew. The work deals with a number of questions relating to the physiology of the leaf and the changes which take place during the processes of curing and sweating.

Respectfully,

B. T. GALLOWAY,
Chief of Division.

Prof. MILTON WHITNEY,
Chief, Division of Soils,
In Charge of Tobacco Investigations.

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PHYSIOLOGICAL STUDIES OF CONNECTICUT LEAF TOBACCO.

GENERAL PHYSIOLOGY OF THE TOBACCO PLANT.

THE FACTORS OF DEVELOPMENT.

The interest connected with the tobacco plant is not only of an agricultural and commercial nature¹ but is also to a considerable degree of a physiological and chemical character. First of all the minuteness of the seed strikes us as peculiar when compared with the large plant developed in but ninety days rivaling in extent of leaf surface most other cultivated plants of equal age. This rapid development requires a steady and full nutrition, if the leaves are to become of the desired size and quality. In order to accomplish this result the roots must be able to grow and extend with corresponding rapidity, requiring for this purpose not only a loose humus or a fine sandy soil to the depth of several feet, but also the presence of all necessary mineral nutrients in most easily assimilable form. The soil must, on the one hand, admit air freely to the finest rootlets to satisfy the wants of respiration and, on the other hand, it must be of such quality as not to part with its moisture too easily in a period of dry weather. The nature of the subsoil is also of great importance, since when it is impermeable the stagnant water is just as detrimental to the roots as too great loss of water on account of excessive permeability of the soil.²

The abnormal physiological condition created by the mutilating (topping and suckering) of the plant in order to obtain suitable leaves for the requirement of the tobacco manufacturer is also of interest. Further points of consideration are the production of the enzymes necessary for the processes of curing and sweating and, finally, the production

¹ The annual total tobacco production of the globe is estimated by Darmstädter to be nearly 1,000,000,000 kilograms.

² For full information on tobacco soils see Bulletin No. 11, Division of Soils, Tobacco Soils of the United States, 1898, by Milton Whitney. A tropical temperature might favor the development of the root in humus soil to such an extent that relatively less of mineral nutrient for the unit of soil will suffice for a normal plant development. The fact that some tobacco soils of Sumatra contain less of nutrients than tobacco soils in northern countries and still produce excellent crops is thus explained by A. Mayer.

of nicotine. All these points are of particular interest. Unless all the conditions necessary for the normal development of the plant are united various dangers menace the crop; and there may even be the development of a singular disease which annually causes immense damage, namely, the mosaic disease.

Among the various tobacco soils of Connecticut there are some that are better suited for broad leaf than for Havana seed as, for example, the soil at East Hartford, Conn., where the culture of the latter had to be given up, the fine cinnamon-brown color not having been attained on this kind of soil.

The weather also constitutes one of the most important factors as to the quality of the tobacco. Too moist as well as too dry years are injurious; the latter, however, more so than the former, since the leaves remain smaller and grow thick and become relatively too heavy; and, furthermore, show insufficient elasticity and frequently burn poorly. In too moist years the leaves remain weak, are attacked easily by fungi and, moreover, the aroma of the tobacco is injured. In rainy weather, especially when the temperature is rather low, the amount of acid in the leaves is also increased and this may exert a detrimental effect on the oxidizing enzymes in the curing process when the tobacco is harvested in such a condition. The oxidizing enzymes are the promoters of all desirable changes in the curing and the sweating process. Variable weather is the best. Van Bemmelen¹ says that the weather in Sumatra is generally more favorable than in Java where frequently extremes are observed. In Deli, Sumatra, occasional rains occur even during the dry monsoon. After twenty days of dry weather a rain is much desired there for tobacco. Forty days of dry weather are considered very injurious. The short showers falling through the period of tobacco growing are considered so important that a continuous record is kept on many tobacco plantations. It is principally the great rapidity of development that causes the fineness of the ribs and veins and the elasticity, making the tobacco leaves from Sumatra unsurpassed as wrappers.²

On warm bright days not only is much more carbon assimilated—that is, more starch, the mother substance of all the other organic constituents of the plants, is formed, but also the respiration is increased by the high temperature, although not to such an extent as to consume all the starch increased by the bright light. All processes of metabolism are thus promoted by warm and bright days, undesirable by-products may be destroyed and favorable products formed. How differences of temperature influence the intensity of the respiration process may be seen from the following data. While Behrens observed, for 100 grams fresh tobacco leaf at 21° C. (69.8° F.), a production of 0.254 grams of carbonic acid in ten hours, Déherain and Moissan ob-

¹Landw. Vers. Stat., vol. 37, p. 385.

²Report No. 62, U. S. Department of Agriculture. M. L. Floyd.

served in the same length of time as much as 1.325 grams at 40° C. (107.6° F.)—that is, more than five times as much.¹

Soil, climate, rich and uniform manuring, careful curing and proper fermenting must be combined in order to yield a commercial product of superior quality. The value of tobacco depends probably more largely upon its quality than is the case with any other farm product.

The tobacco seed seems somewhat neglected by nature, since the amount of reserve material to support the young plant in its first stage is exceedingly minute. It takes 21 seeds to weigh 1 milligram; consequently the initial growth is but slow and much in disproportion to the later energetic development, the tobacco after being planted in the field often reaching its full development in forty-five days in Florida. The reserve material of the endosperm of the seed consists, according to Harz, essentially of fat and protein matter, and is, like the 0.6 mm. long embryo, devoid of starch. The young delicate plant soon consumes this small amount of reserve material and is forced to prepare its own organic matter in a much earlier stage than is for instance a corn, barley, or bean plant. Unusual care is required in the seed bed, which if too moist will favor fungous diseases among the young plants. A moderately dry soil, occasionally sprinkled, is preferable.

Much has been written about the proper manuring of the tobacco field. General experience indicates that barnyard manure is here superior to all other fertilizers. An addition, however, of some superphosphate of lime, and carbonate or silicate² of potash (5 parts per 1,000 of barnyard manure), and Chile saltpeter (not over 15 parts per 1,000 of the barnyard manure) the last mentioned to be applied in the spring and the other materials in the autumn, is recommended by some. The advantage of the barnyard manure consists in loosening the soil, increasing its absorptive power by formation of humus and yielding up its insoluble nourishing compounds gradually in a soluble condition. It makes, in short, a uniform nutrition of the plant during the entire season more easily possible than a mere mixture of mineral fertilizers can do.³ The tobacco thus grown is of the best quality and least subject to the mosaic disease.

¹ Sand leaves, which are generally poorer in respiration material, produced only 0.568 grains at 42° C.

² Sulphate and chlorid are to be avoided.

³ Of other materials that have been applied with great advantage should be mentioned cotton-seed meal and castor pomace with cotton-hull ashes. The amount of fertilizers applied per acre of a tobacco field varies from 200 pounds to a ton. Nitrogenous manures applied in very large quantities increase the percentage of nicotine and protein and also, naturally, the total yield, but the quality may be injured. Thick ribs and a poor burn have been observed in such cases.

Behrens (Landw. Vers. Stat. 52, 242) calls attention to the fact that the increase of easily soluble salts in the soil, as is the case when merely mineral manures are applied, leads to the formation of thicker leaves. This modified anatomical structure also caused by very dry seasons, decreases the amount of transpiration from the leaves. Such thick leaves require a longer time to cure, and thus the danger of pole burn and other fungous diseases is increased.

A Havana seed plant, after topping and suckering, is generally left to develop about 15 leaves, while the Connecticut seed leaf is allowed to develop but 13, the latter, however, being larger than the former.¹ Of one Havana seed plant with 15 leaves, 10 leaves were found by the writer to average 44 cm. in length and 20 cm. in width, while the 5 largest leaves averaged 52 cm. in length and 24 cm. in width. On another field the length and width of all 17 leaves of one plant were measured and the average taken. The average was—length, 45 cm.; width, 28 cm. An unusually large Havana leaf measured 75 cm. in length and 39 cm. in width. The proportion between the length and width of full-grown leaves varies considerably even on the same field.

Two ripe leaves of medium size each measuring 44 cm. in length and 24.1 and 25.5 cm., respectively, in width, weighed 6.55 and 8.40 grams, respectively, in the dried state. In order to determine the weight for 1 square meter 100 square centimeters were cut from different leaves, partly avoiding the midrib and partly taking it in. The fresh weight varied between 3.01 and 4.12 grams, averaging 3.5 grams in the fresh and 0.595 grams in the dry state. This would give for 1 square meter 59.5 or about 60 grams. It nearly agrees with the result obtained by H. J. Patterson, who found for Maryland tobaccos the maximum average dry weight to be 65.5 grams. According to Behrens the weight of Sumatra tobacco leaves varies between 52 and 90 grams per square meter.² Since the quantity of starch differs very much in leaves cut in the morning and those cut in the evening, considerable difference in weight should not surprise us. The figures given above refer to starch-free leaves.

REMARKS ON THE CONTENTS OF THE TOBACCO LEAF.

The epidermis of the tobacco leaf develops partly into noncapitate hairs and two kinds (long and short ones) of capitate gland hairs. The apical cells of the gland hairs contain chlorophyll granules when young and generally in each cell a radiary group of calcium oxalate crystals. Later on the gland hairs contain resin and other matters, and a dilute alcoholic iodine solution stains the contents a deep brown. The dissolved albumin content in the plant juice varies considerably with the age of the leaf. The lower or sand leaves contain, on becoming yellow, but very little or no soluble albumin, while the full-grown green leaves on the same plant are rich in it. This is to be expected, since only the normally green chlorophyll bodies can produce sufficient sugar to make protein production considerable. The "ripe" leaves, however, are richest in soluble albumin. It may also be mentioned that tobacco attacked

¹In Deli, Sumatra, 22 leaves are left when the tobacco is on good soil, but on poor soil only 14 leaves are left. The leaves in Sumatra reach 42-46 cm. in length and from 31-38 cm. in width. (Van Bemmelen.)

²These numbers vary very much from the numbers given by Barth (Landw. Ver. Stat., vol. 39, p. 92) who states that dried coarse leaves may weigh per square meter 200 grams and more. There may be an error in his calculations.

by the mosaic disease contains much less soluble albumin than normal leaves, for an equal surface. The latter give a juice which, upon being heated, forms a large amount of flocculent coagulum; the former, however, hardly more than a turbidity. This is not surprising, since the chlorophyll bodies are affected in the mosaic disease and hence can not furnish a sufficient amount of sugar for an abundant protein formation. The fat content varies considerably, the amount being—other things being equal—probably influenced by the weather, since low temperature will retard growth and respiration more than it does assimilation; hence some sugar that would otherwise have been consumed by respiration may be transformed into fat, if the conditions for protein formation are not all fulfilled at that time. The sugar is the material from which not only the cellulose, starch, fat, and protein, but also the wax, tannin, and resin of the leaves are produced.

The most interest, of course, is attached to the formation of nicotine, which alkaloid, according to Pinner, has to be considered as pyridyl-N-methyl-pyrrolidine. It is probably formed from decomposition products of proteins and plays no further physiological rôle in the plant. The malic acid of the leaf may also participate in the synthesis of nicotine, since nicotinic acid can easily be reached by transformations of malic acid. The object of the nicotine production in the wild plant is possibly to keep off depredating enemies among the insects, but the amount of this poison in young plants cultivated in our climate is too small to kill the insects feeding on the leaves.¹ Even the ripe leaves, richest in nicotine, are attacked with impunity by certain caterpillars, grasshoppers, and the "suck fly," a heteropterous insect (*Dicyphus minimus*). The nicotine, according to Wizsman-Leyden, is contained especially in the cells underneath the hairs.² Adolf Mayer found that the veins contain much less nicotine than the lamina.

The fresh leaves contain relatively but little wax, resin, lecithin, tannin, and calcium oxalate, the latter occurring as a sandy crystalline deposit, especially in mesophyll cells. The oxalic acid, as well as the citric and malic acids of the leaves, is probably an oxidation product of sugar.³ A part of these acids occurs in the form of a neutral, another part as acid salts.⁴ The cell sap, therefore, shows an acid

¹ The amount of nicotine formed depends largely upon climate and weather (cf. A. Mayer, *Landw. Vers. Stat.* vol. 38, 453 [1891]).

² *Südd. Apoth. Zeitg.*, 1898.

³ According to Kissling the amount of oxalic acid varies in commercial tobaccos between 0.96 and 3.72 per cent of the dry matter; that of citric acid between 0.55 and 8.73; that of malic acid (in form of neutral and also acid salts) between 3.49 and 10 per cent. (*Chem.-Zeitung*, 1899, No. 1.)

⁴ Behrens (*Landw. Vers. Stat.* vol. 52) found from 5.79 to 9.79 per cent malic acid in form of soluble salts in the tobacco leaf (dry matter) and infers that it is the hygroscopic quality of these salts that keeps the leaves from drying up too easily, which thus preserve a certain softness and elasticity. The more malic salts are present the more "life" is in the finished tobacco leaf. Stored under equal conditions, leaves rich in malic salts may contain 3 per cent more water than leaves poor in them.

reaction. It seems of special interest to determine here the degree of acidity, since the amount of enzymes of importance in the curing and sweating process may be decreased by acids.¹

ACID CONTENT IN DIFFERENT PARTS OF THE TOBACCO PLANT.

The free acid of the juice was titrated in the usual way by one-tenth normal soda solution and calculated as free malic acid. Although in reality this acid is present as an acid salt and not as free acid, it makes no difference as to the number of unneutralized carboxyl groups, and the data obtained can serve for comparison. The juice was prepared by grinding the objects with the aid of some pure quartz sand and pressing the pulp through thin flannel. The juice of the leaf had to be mixed, however, previously to titration, with an equal volume of strong alcohol to precipitate suspended chlorophyll grains, otherwise the point of neutralization could not be observed with sufficient precision on blue litmus paper, still less distinctly on violet litmus paper. Filtration, after the addition of the alcohol, was not found necessary previous to the titration.

The amounts of free acid in the different organs are, of course, subject to variation within certain limits, according to the atmospheric conditions and the influence of direct sunlight. Thus the writer has observed with the flowers of *Ipomaea triloba* that warm weather with bright sunshine diminishes the acidity, while cool and rainy weather increases it, whereby the blue flowers turn red; and further, the acidity is greater in the morning than in the evening, which the writer also observed in the case of tobacco. The custom of harvesting tobacco on bright, sunny days has therefore a sound physiological foundation.

The following data were observed:

Part of plant.	c. c. $\frac{n}{10}$ soda solution required by 10 c. c. juice	Per cent of free malic acid in juice.
Small roots	0.5	0.033
Pith	.8	.052
Bark	1.4	.092
Midrib of leaf (evening)	1.6	.105
Midrib of leaf (evening)	1.5	.098
Midrib of leaf (morning)	2.1	.138
Lamina of ripe leaf α	4.4	.294
Lamina of ripe leaf	4.2	.277
Lamina of young suckers	2.2	.145
Lamina of young suckers	2.4	.156

α Midrib and large side ribs removed.

We learn from these numbers that the least amount of acid is contained in the small roots and in the pith; also that the lamina contain much more than the rib, and that the young leaves contain less than the ripe old leaves.²

¹ Certain fungi, as, for example, *Oospora Nicotianæ*, will grow on fermented tobacco only in the presence of some free organic (malic) acid (*Splendore*). This shows also in this respect an unfavorable effect of the acidity.

² These relations may differ in different plants. Thus Kraus has observed less of free acid in the leaf of *Sempervivum* than in the root.

RIPENING OF THE TOBACCO LEAF.

The ripening process, in a normal physiological sense, refers to the fruit and implies the normal formation of the embryo and the filling up of the reservoirs of the fruit with the proper material for the young growing plant. The ripening of the tobacco leaf on a topped and suckered plant, however, implies an abnormal accumulation of organic matters in the leaves, brought on by preventing their transportation to the forming seeds and shoots or suckers, which would otherwise have consumed a great deal of these substances. The "ripening" of the tobacco leaf is, in other words, not a physiological, but a pathological phenomenon. By the accumulation of nicotine, oxidizing enzymes, and acids a state is finally reached in which the normal deep green color of the chlorophyll grains gives way to a yellowish color. A "ripe" leaf contains per 100 square centimeters an average of 0.164 gram of matter soluble in boiling water, while a young leaf contains only 0.066, the former having, therefore, for an equal surface about 2.5 times as much soluble organic matter as the latter. As to the total weight, a ripe leaf weighs from 1.3 to 1.5, on the average 1.4, times as much as an equal surface of a young leaf of the same plant. It is especially the nicotine and protein compounds that increase in the ripe leaf, a fact of which anyone can soon convince himself by a comparison of the reaction caused by phosphotungstic acid in the juices of ripe and unripe leaves. The amount of water in ripe leaves averages roughly 83 per cent and in young leaves 88 per cent, that is, when compared in nearly starch-free condition after being kept for two days in darkness.

But it is not only the increase (relative and absolute) of reserve materials and of products of metabolism that is brought on by topping and suckering; it is also the enlargement of the leaf surface, which may amount to over 50 per cent above the normal. The suckering exerts these influences to a higher degree than the topping (Behrens). A further influence, due to differences in topping, has been observed, low topping producing thicker leaves than high topping.

An important factor is the proper time for topping and suckering. The suckers must be removed neither too soon nor too late. In the former case, too much protein would be left in the full-grown leaf; in the latter, too much nicotine might be lost from it. Goff observed that in moderate climates the tobacco leaves increased in weight at least to the thirty second day after the topping had been performed. A ripe leaf is recognized by the planter not only by the setting in of a yellowish coloration and a peculiar rough feeling to the touch, but also by the facility with which the lower epidermis peels off and the leaves break ("snap") when folded between the fingers.

As to the so-called "overripe" leaves, they contain, like the young leaves, relatively more water and less organic matter than the ripe leaves. By the stoppage of the functions of the chlorophyll grains, which gradually change in the overripe leaves, new production of

organic matter is prevented, while consumption still proceeds. With the young leaves it is the growth, besides the respiration, that consumes material and prevents its accumulation.

In comparing the undiluted juices of overripe and ripe leaves, great differences can at once be noticed by adding a double volume of alcohol or phosphotungstic acid, the ripe leaves yielding a much more copious precipitate than the overripe ones. The juice of ripe leaves is, further, more slimy and more acid. From the central part of the leaves 100 sq. cm. were cut out of each side of the midrib and ground with some quartz sand and 10 c. c. of water to a fine pulp in a mortar and directly titrated, after the addition of 10 c. c. of strong alcohol. The result was as follows:

	c. c. $\frac{n}{10}$ soda solution for 200 sq. cm. of leaf.	Content of free malic acid in 100 sq. cm. of leaf.
Ripe upper leaf.....	3.8	Gram. 0.012
Sand or lower leaf.....	3.0	.010

From the center of another field, ripe upper leaves and lower or sand leaves of the same plant were collected. The lower leaves were of a lighter green than the upper ones. The juices were prepared as usual, and 10 c. c. titrated. The juice of the upper leaves required 4.2 c. c. of one-tenth normal soda solution, while that of the lower leaves required but 3.2 c. c. Since it is the sugar principally that gives rise to the formation of acid, we should not be surprised at finding more acid in the upper leaves than in the lower, as the production of sugar and starch depends upon the access of light to the chlorophyll bodies.¹

Well-ripened leaves, rich in oxidizing enzymes and in nicotine, and very rich in soluble matter generally, will yield when normally cured the so-called "gummy" leaves with a well-developed "grain," and after a thorough sweating a fine aroma, while unripe leaves will show injuries after the sweating process and yield, like the sand leaves and overripe leaves, an inferior product. Hence, the single-leaf-curing process would be preferable to the method of curing on stalk, since the overripe, ripe, and unripe leaves are in the latter method harvested together, to be cured under equal conditions.

It is not the object of this report to give an exhaustive critical comparison of these two methods.² Where labor is very expensive, the method of single-leaf curing may be out of the question. The same may be said for the priming of the very large leaves of Connecticut seed-leaf tobacco. Here in the removal of the lower leaves at their

¹ In organs free of chlorophyll, as the flowers, the direct sunlight can, on the contrary, exert a diminishing effect on the amount of acid.

² The chemical analysis of fully ripe leaves cured after these two methods does not show essential differences. (Behrens.)

ripening stage the upper leaves may easily be broken or lacerated, thus leading to a considerable loss.

The single-leaf curing, a process that seems to grow more and more in favor with the farmers in certain States, takes one-third less time than stalk curing, permits the harvesting of about double as many leaves as the curing on the stalk, gives a more uniform product, and permits the gathering of top suckers, which may form good wrappers.¹ The stalk is here longer in the field and continues to produce healthy leaves from the suckers.

ENZYMOLGY OF THE TOBACCO PLANT.

The important physiological rôle of enzymes for every organism is well known. Some enzymes are found in all organisms, others only in a few, and some may even occur only in one. Three physiological groups of enzymes can be distinguished: (1) Such as are most intimately and directly connected with the nutritive processes, as diastase and proteolytic enzymes; (2) such as bring on coagulation—the clotting enzymes, as pectase and thrombase; and (3) such as cause oxidation—that is, the oxidases.

It was to be expected from analogy that enzymes of the first and third groups would occur in the tobacco plant, and, since the activity of the enzymes is not only of physiological value to this plant itself, but in this case is also of practical importance to the tobacco planter, it was important to prove their presence beyond a doubt. Two oxidizing enzymes have been shown by the writer to exist in the tobacco plant,² and recently a third one has also been observed. This will be described further on.

DIASTASE IN THE TOBACCO LEAF.

The disappearance of starch in the early part of the curing process is due to the presence of diastase. It has been asserted that diastase does not occur in leaves and that protoplasm itself is capable of doing the same work.³ This appears very improbable, since both starch and protoplasm are insoluble in water. It is, however, somewhat difficult to demonstrate the presence of small quantities of diastase in the juice of leaves, since some can easily be retained by the pressed residue. Brown and Morris have observed that powdered leaves, if directly digested with some starch paste, gave a diastatic action of much greater intensity than was obtained when juice corresponding to that amount of leaf substance was applied.

¹ This method is practiced extensively by Mr. G. Barnes, of Hatfield, Mass.

² Report No. 59, U. S. Department of Agriculture.

³ Wortman observed that leaves killed with chloroform or kept in an atmosphere of carbonic acid show no decrease of starch, and hence inferred the absence of diastase in leaves; but this is not justified, for several reasons. Recently it has been shown by A. F. Woods that diastase is easily changed by oxidizing enzymes. Furthermore, Vines, and also Jentys, have given new proofs of the presence of diastase in leaves.

The presence of diastase in the tobacco leaf was demonstrated by the writer as follows: Four large healthy leaves were well crushed in a mortar with some sand and pressed. The filtered juice was precipitated with alcohol, and the precipitate, after being washed with alcohol of 90 per cent and pressed between filter paper, was digested with 10 c. c. of highly diluted starch paste at 60° C. (140° F.). The iodine test, applied to a drop of the liquid on a white porcelain plate, showed that the starch gradually diminished and disappeared after two hours. Leaves kept for eight days in darkness and near death by starvation did not, when so examined, show any diastase content. This would probably also prove the case with leaves from the curing barn. The destructive effect of oxidase on diastase (Woods) comes into play here.

PROTEOLYTIC ENZYM IN THE TOBACCO LEAF.

The decrease of protein in the tobacco leaf, while curing is going on, can be ascribed only to a proteolytic enzym. The amount of it, however, is probably small, since the coagulable albumin contained in the juice of tobacco leaves decreases very slowly; even after several weeks leaves taken from the barn may show some of it. It may be possible that the oxidizing enzymes injure this enzym, as they do the diastase, and thus prevent an accumulation beyond mere traces in the fresh leaf. Many authors have encountered difficulties in attempting to prove the presence of proteolytic enzymes in green leaves and fruits. One of the causes of this failure may be that blood fibrin, or coagulated egg albumen, has been used for the test; while certain proteolytic enzymes may exist which can easily peptonize dissolved albumen without being capable of dissolving fibrin or coagulated albumen. Indeed, there may exist a great variety of proteolytic enzymes. Thus, Bourquelot and Hérissey have shown an enzym to exist in fungi which is capable of easily digesting casein, while it can not dissolve fibrin. The writer's experiments with casein and fibrin, in attempting to prove the presence of a proteolytic enzym in the fresh tobacco leaf, were not conclusive, no visible solution of these proteins taking place when digested for several days at 40° C. The method of Fermi and Buscaglioni also proved unsuccessful. These authors recommend a gelatin solution that solidifies at the ordinary temperature. The liquid to be tested is, in the presence of an antiseptic substance, left upon the gelatin in the test tube for several days. The presence of even a trace of proteolytic enzymes will soon be indicated by the liquefaction of the gelatin below the line of demarcation. The principal objection which may here be raised is that proteolytic enzymes may exist which can act on gelatin only at a higher temperature—that is, above the temperature 20° to 22°, at which the usual gelatin preparation liquefies by melting.

In the following experiment the test recommended by Märtz was adopted. Ten fresh and 10 starved leaves, of as nearly the same size

as possible, were rubbed with sand to a fine pulp and the juices pressed out. A portion of each of these juices was kept separate as control, while the chief portions were mixed with an equal volume of a solution of albumin. This solution was obtained by pressing the white of an egg through linen, diluting the liquid with fifteen times its bulk of water and filtering. A portion of the albumin solution was also diluted with an equal volume of water and kept for control. All liquids were kept at from 18° to 22° C. in closed test tubes which had received an addition of ether to exclude bacterial development. After nine days the filtered liquid received an addition of some nitric acid and the precipitate formed was left one day to settle. The volume of the precipitate showed a moderate decrease in the juice of the fresh leaves and a considerable decrease (about one-half) in that of the starved leaves; therefore the existence of a proteolytic enzyme can not be doubted, but it differs from the ordinary trypsin, inasmuch as it can not attack fibrin and casein under ordinary conditions, while it can attack dissolved albumin.

As to the first-mentioned control cases of the juices which had not received the addition of egg albumin, the soluble albumin originally present as reserve matter in the juice was found fully digested in the juice of the starved, but not yet completely so in that of the fresh leaves.

IS A CELLULOSE-DISSOLVING ENZYM (CYTASE) PRESENT IN TOBACCO LEAVES?

Cytohydrolytic enzymes, especially such as easily attack hemicelluloses, occur in phanerogamous plants and are not identical with diastase, as Newcombe¹ has demonstrated. He found such an enzyme in the cotyledons of seedlings of *Phoenix dactylifera* and of *Lupinus albus* and confirmed its presence in the malt of *Hordeum vulgare*.

Parasitic fungi, such as eat through wood, must contain enzymes which dissolve the genuine cellulose with great facility. Various observations, especially those of Dr. Erwin F. Smith on bacteria acting as plant parasites, leave no doubt as to the occurrence of such enzymes also in certain species of bacteria.

The fact that tobacco leaves lose more or less of their original degree of tenacity and cohesion, and even show holes when subjected to sweating in a too moist condition, appears to find a simple explanation in the action of bacteria, which may occur in the presence of too much moisture. But it would have to be assumed that cellulose dissolving bacteria, of which not many kinds are known, are always present whenever too much water is applied to the tobacco. It was therefore of interest to test whether cytase, which has been shown to be present in certain germinating seeds, was also contained in the tobacco leaf itself, or whether it was eventually formed from another enzyme in the

¹ Annals of Botany, March, 1899.

process of curing; in other words, whether the abnormal softening of the leaves was accomplished without the action of bacteria. Parts of cured tobacco leaves and of fresh leaves, the latter previously dried at 45°C ., were therefore moistened with water and left for twelve days at from 45° to 50°C . in test tubes with as little 20 per cent alcohol as sufficed to cover them. Bacterial development was thus excluded, but not the action of enzymes. Vapor of chloroform in closed flasks was also used in place of the 20 per cent alcohol. The result was that the green leaves (healthy as well as mosaic diseased leaves) did not show any decrease of tenacity, but the cured leaves did, although only to a slight degree. From sweated leaves a concentrated extract was now made at the ordinary temperature, a second portion of leaves being extracted with the first extract obtained, and so on until the fourth portion was extracted with the same water. The concentrated extract thus obtained was saturated with ammonium sulphate, whereby a copious precipitate of proteins of a very dark brown color was formed. This precipitate was freed of most of the adhering salt solution by pressure between filter paper, dissolved in a little water, and in presence of chloroform permitted to act for five days on a small piece of fermented leaf at 50°C . Although bacterial growth was here successfully prevented, the leaf was very much softened and lost most of its former tenacity. Nevertheless, the writer is not yet fully prepared to give a decisive answer as to the nature of the cellulose injuring agency in the sweating leaf.¹

TEST FOR OXIDASE AND PEROXIDASE IN THE FRESH TOBACCO LEAF.

Although in report No 59 (p. 28) the way of testing for the oxidizing enzymes of tobacco was mentioned, some further remarks on this subject will not be superfluous for those who intend to make further observations on these enzymes. The principal reagent, as has been mentioned, is guaiac resin in absolute alcoholic solution, which gives a blue coloration when oxidase is present and, in connection with hydrogen peroxide, a blue coloration when peroxidase is present.

While the test for oxidase and peroxidase in the stalk and ribs of leaves can be directly applied to the objects themselves, there is some difficulty encountered in the lamina, since the green color of the chlorophyll interferes with the recognition of the pure blue reaction.² Furthermore, when the juice is prepared by rubbing the leaf to a pulp and pressing this through flannel, it will contain so many chlorophyll granules in suspension that the juice has to be filtered through filter paper.

¹Bumke and Wolfenstein have recently found that cellulose is comparatively easily changed, under certain conditions, hydralcellulose being formed. Perhaps a similar action takes place in the sweating leaf when too much moisture is present and the oxidizing enzymes act upon the cellulose walls.

²The blue reaction may be distinguished to some extent in the leaf by applying the reagent to the spongy tissue after the removal of the lower epidermis.

This filtering, however, proceeds so slowly that a brown coloration is meanwhile developed by oxidation. By mixing the juice, however, with an equal quantity of 90 per cent alcohol, or by rubbing the lamina in a mortar with an addition of 50 per cent alcohol—whereby proteins and other matters are precipitated—a larger amount of clear filtrate ready for the test is soon obtained.

It is often preferable to dry the leaf at a temperature of from 40° to 45° C., grind it to a fine powder, extract this for a short time with cold water, and apply the tests to the filtrate. It need hardly be mentioned that the juices of the pith or of the main ribs of the leaves can serve directly for the tests. They filter easily and do not darken as readily as the juices from the lamina.

In regard to the guaiac blue, it may be mentioned that it sometimes fades out soon after its formation. This may be due to tannin and other reducing compounds; it seems not to be due to another enzym.

ARE THE TOBACCO OXIDASE AND PEROXIDASE KINDS OF PROTEINS?

These enzymes belong neither to the coagulable albumins nor to the nucleoproteids. The latter are precipitated by dilute acids, but since the juice of green tobacco leaf is of moderate acid reaction, it can not contain ordinary nucleoproteids in solution. Even upon the addition of 1 per cent of acetic acid to the juice, no nucleoproteids are precipitated. If, as Spitzer has recently claimed, the peroxidase of mammalia is a nucleoproteid, we must assume a considerable difference between the peroxidase of mammalia and that of tobacco, and probably of other vegetal sources.

The following tests show, further, that oxidase and peroxidase do not belong to the coagulable albumins. The freshly expressed and filtered juice of the tobacco leaf yields upon heating to 54° C. a copious coagulum of albumin, while the filtrate therefrom gives a strong reaction for oxidase and peroxidase. On further heating this filtrate to 65° C. for a second, another but a very insignificant coagulation is observed. The filtrate of this precipitate still yields both reactions, but it may now be heated to the boiling point without giving a coagulum. Further, when green tobacco leaves are dried at a temperature of 50° C., pulverized, and extracted with ten times the weight of cold water for thirty minutes, a filtrate of weak acid reaction is obtained which contains mere traces of albumin. Nevertheless, it gives the peroxidase reaction with guaiac, even after heating for a second to 85° C. (not to 90° C.). This solution was heated for a few seconds to a temperature of 66° C. and the slight turbidity removed by filtration, after which the cooled filtrate was saturated with ammonium sulphate, whereby a dark precipitate was formed. This contained the oxidase and peroxidase and a part of the brown coloring matter of the original extract, while the filtrate yielded no trace of the guaiac reactions. After being freed as far as possible from the adhering liquor, by pressing between sheets of filter

paper, the precipitate was easily dissolved in water. This solution not only gave intense reactions for oxidase and peroxidase, but yielded also a precipitate with alcohol and gave Millon's reaction; the biuret reaction, however, was impeded by the dark color of the liquid. Both oxidase and peroxidase, therefore, seem to have the main character of albumoses. It follows, then, that the animal oxidase must differ from the vegetal, since Abelous and Biarnès believe the former to be a kind of globulin.¹ When the aqueous extract of dried tobacco leaves is neutralized, a small amount of precipitate is obtained, containing, among other things, lime salts, and a part of the oxidase mechanically carried down.

BEHAVIOR OF OXIDIZING ENZYMES TOWARD INJURIOUS COMPOUNDS.

The supposition might be entertained that oxidase and peroxidase are chemically closely related, the latter representing, perhaps, a derivative of the former. Peroxidase, however, resists not only a higher degree of heat than oxidase, but also is less easily injured by poisons.

In comparing tobacco oxidase with tobacco peroxidase in their behavior toward noxious compounds, a solution of the former was prepared in the manner formerly described,² and its reaction compared with the oxidase reaction in fresh tobacco juice after addition of various solutions of known strength.³ Before making the tests care was taken to have a neutral or only slightly acid reaction, as guaiac blue is easily altered by various influences, and especially by mineral acids and alkalis. Also, certain organic substances of comparatively weak acid qualities, like tannin, can prevent the reaction. Acetic acid, in higher concentration than 2 per cent, interferes with the production of the guaiac blue.

The following table shows the results obtained:

Reagent.	Oxidase.	Peroxidase.
Acetic acid, 3 per cent.	Killed within 5 hours.	Reaction much weaker after 24 hours.
Acetic acid, 1 per cent.	Killed within 24 hours.	Reaction weaker after 24 hours.
Hydrochloric acid, 6.5 per cent.	Killed immediately.	Killed immediately.
Sulphur dioxide, 0.25 per cent.	do.....	Unchanged after 24 hours.
Ammonia, 0.3 per cent. <i>a</i>	But little altered after 24 hours.	Unaltered after 48 hours.
Potassium hydroxide, 0.3 per cent. <i>a</i>	Considerably decreased after 2 days.	Do.
Free nicotine, 1 per cent.	Killed in 24 hours.	Do.
Formaldehyde, 5 per cent.	do.....	Do.

a In these cases the fresh juice was neutralized before adding the above percentage.

¹ Compt. Rend. Soc. Biol., vol. 49, p. 493.

² Report No. 59, U. S. Department of Agriculture, p. 29.

³ A colorless solution of the tobacco oxidases can not be obtained from the lamina of the tobacco leaves, since the juice turns brown very soon, but it may be obtained from the midrib by grinding it, expressing the pulp, and filtering the liquid. By heating a moment to 65° C. (149° F.), the albuminous matters coagulate and a clear, colorless filtrate is obtained which turns yellow but slowly.

It may be added that ammonium oxalate (5 per cent) had no deleterious effect upon oxidase after two days; but nicotine tartrate in a 5 per cent solution, and quinine acetate in a 0.5 per cent solution, seemed to damage the oxidase after four days, although but very little.

KILLING TEMPERATURE OF OXIDIZING ENZYMES.

The temperatures thus far observed which render enzymes inactive are not constant under all conditions; the acidity of the plant juice, the degree of dilution, the duration of the heating, and the presence of certain salts have a modifying influence on the height of the temperature at which the change to the inactive modification takes place. It is to be regretted that authors have not always mentioned the conditions and the duration of the heating. The oxidase of the stalks of the sugar cane is killed at 60° C. (140° F.), the peroxidase of it at 95° C. (203° F.), according to Raciborski. Tyrosinase, an oxidase easily oxidizing tyrosine to a dark substance, is injured at 50° C. (122° F.) and killed below 70° C. (158° F.), according to Bertrand. Laccase, an oxidase of widespread occurrence, is killed at 63° C. (145.4° F.). Oenoxidase loses half of its oxidizing power at 72° C. (161.6° F.) within four minutes, and at 55° C. (131° F.) in one and one-half hours. The oxidase of mammalia is killed at from 80° to 85° C. (Abelous and Biarnès.)

The influence of the duration of the heating is shown by the following test. The killing temperature of the oxidase in the juice of the tobacco leaf was repeatedly found to be from 66° C. to 67° C. when the juice was diluted (one part dried leaf to twenty parts of water) and the solution heated for three minutes. But in heating it for a second only a much higher temperature may be endured. Certain salts, as ammonium sulphate, and also traces of free ammonia, increase the resistance, while acids, even in small quantities, decrease it. The juice of the midrib contains less acid than that of the lamina, and consequently it takes a temperature several degrees higher to kill the oxidase in the former than in the latter, but on being neutralized the latter may be safely heated for a moment to 75° C. The addition of alcohol decreases the power of resistance. Thus an addition of 33 per cent of alcohol to a neutralized solution of oxidase will depress the killing temperature about 2° C.

The following tests were made with peroxidase: Fresh tobacco leaves were ground, with the addition of some sand and a little water, and the pulp expressed. The residue was now left for some hours in contact with water to which 0.5 per cent acetic acid was added, and expressed again. The filtrate thus obtained was exactly neutralized with dilute caustic soda. It gave only a very faint reaction for oxidase, but an intense reaction for peroxidase. To one portion (20 c. c.) was added one-half its volume of absolute alcohol; to another 10 per cent of ammonium sulphate. Upon heating in a water bath it was found

that the peroxidase in the alcohol mixture was killed at a temperature of 70° kept up for a few seconds, while in the mixture containing the ammonium sulphate the enzyme was not killed after heating the mixture for a short time to 93° C. In the control solution the enzyme was killed at 87° C. after three minutes.

Grüss encountered in some vegetable objects an oxidase that resists the action of boiling absolute alcohol. He designates it as γ -oxidase.

That such an oxidizing enzyme exists which can resist even boiling absolute alcohol might appear strange. But when we consider that the action of absolute alcohol consists in the first place of depriving the objects of water, and, further, that dried enzymes resist a much higher temperature than enzymes in the moist or dissolved state, that observation loses its singularity. It may here be pointed out that recent investigations of bacteriologists have proved that certain kinds of bacteria also succumb much more easily to alcohol of 50 per cent than to absolute alcohol. Protoplasm of a high density which can resist drying is much less accessible to poisonous actions when dry than when moist.¹ The writer has also observed that the oxidase in the tobacco leaf resists a higher temperature when the leaf is itself warmed with some absolute alcohol than when the dilute leaf juice is. However, a prolonged contact with absolute alcohol will doubtless kill the oxidase. Here, also, the peroxidase resists much better than the oxidase.

CAN THE AMOUNT OF OXIDASE BE INCREASED BY SPECIFIC MANURING?

Since Bertrand and Villiers have found some manganese is a regular ash constituent of the oxidases² and since it seems justifiable to attribute to that circumstance an essential influence on the specific actions of the oxidases, it was of some interest to try to discover whether an increase of manganese in the soil would lead to an increase of the oxidizing enzymes in the tobacco plant. Therefore a 0.1 per mille solution of manganese sulphate was repeatedly used to water some tobacco plants, each of which received altogether 0.6 gram of that salt.³ The examination of the ripe leaf, however, showed no noticeable increase of oxidase and peroxidase on colorimetric comparison with the plants not so treated. Possibly the soil contained a sufficient amount of manganese for the formation of the normal maximum amount of these enzymes.

A THIRD OXIDIZING ENZYME IN THE TOBACCO PLANT.

The unfiltered juice of fresh tobacco leaves energetically decomposes hydrogen peroxide with evolution of oxygen. The clear filtrate

¹Also seeds resist a higher temperature when dry than when soaked in water.

²Compt. Rend. vol. 124, pp. 1032, 1349, 1355.

³These experiments were conducted by Dr. E. H. Jenkins on the experimental field in Poquonock, Conn.

obtained from the turbid juice shows this property only to a very small degree while the suspended matter acts as powerfully as the unfiltered juice. It is easy to prove that neither particles of the living protoplasm of the leaf cells nor an admixture of bacteria are the cause of this action, since contact with chloroform does not essentially alter that power after a day. Certain observations of the writer, not agreeing with the prevalent views, led him to a further examination of this phenomenon. Several authors have observed this property with various organisms and ascribed it to the known enzymes contained in them, following Schoenbein,¹ who expressed this opinion in the year 1863.

This view was not even doubted after it was shown by Jacobson that this property of the common enzyme preparations may be destroyed without injury to the true enzyme nature, i. e., to the specific activity.²

While pyralin, pepsin, and myrosin preparations gave Jacobson only small quantities of oxygen by decomposition of hydrogen peroxide, emulsin, pancreatin, and diastase, in freshly prepared condition, yielded considerable quantities. Jacobson proved that by heating to a certain temperature, by "exhaustion of the catalytic power" (of decomposing hydrogen peroxide), by dilute acids, and by various poisons, this property could be either destroyed or considerably weakened without the true enzyme character being materially injured.

Since it seems very doubtful that the molecule of an enzyme can be changed to a great extent without destroying the specific property, the writer finds it much more natural to explain the observations of Jacobson by the presence of another enzyme, to which this decomposing action on hydrogen peroxide is due. In the preparations of the common enzymes the presence of other enzymes as impurities can not be surprising. The further observations of the writer have confirmed this view. A full account will be given later in a separate bulletin. Here it may suffice to sum up the main results:

1. The property of developing oxygen by decomposition of hydrogen peroxide is due to a separate enzyme.

2. This enzyme, to which the name catalase may be given, occurs in two modifications, one insoluble and one soluble in water. They may be distinguished as α and β catalase. The insoluble or α catalase is probably a combination of the enzyme with a nucleoproteid.

3. This enzyme is killed at 72–75° C. (161–167° F.). It is an oxidizing enzyme, since it easily produces quinone from hydroquinone. It does not produce guaiac blue from a tincture of guaiac, not even in the presence of some hydrogen peroxide; hence it differs essentially from the ordinary oxidase and peroxidase.

4. The insoluble modification is soluble in dilute alkaline liquids.

5. In the curing process of the tobacco leaf there is a small increase

¹ Journ. Pract. Chem. vol. 89, p. 326.

² Jacobson, Zeitsch. Physiol. Chem. vol. 16, p. 340, 1892.

of the soluble modification, probably formed from the insoluble. This increase is larger in the sweating process.

6. This enzym is the most durable of those in the tobacco leaf under ordinary circumstances, since it occurs in tobacco even over 6 years old, in which no trace of other enzymes, not even of the peroxidase, can be found.

7. It is certainly an important factor in the heating up of the tobacco pile, as recent experiments with fermenting tobacco have shown.

The general occurrence of this enzym in plant as well as in animal cells suffices to indicate a highly important physiological rôle. It is probably intimately connected with the process of respiration. The following two hypotheses as to its function appear the most probable to the writer:

1. Since many oxidative processes lead to the formation of hydrogen peroxide as a by-product, it is important that such a poisonous by-product be at once destroyed when accidentally formed in the cells in the course of the respiratory oxidations.

2. This enzym may have the office of loosening affinities in fatty acids and sugar in order not to tax the chemical energy of the protoplasm itself too heavily when these compounds are consumed for the purpose of respiration.

REMARKS ON THE MOSAIC DISEASE OF THE TOBACCO PLANT.

The mosaic disease, in the Southern States called "frenching" and in the Northern States "calico," "brindle," and "mongrel" disease, is of special pathological interest. For a discussion of the causes, theories, and scientific investigations on the development of this disease the reader is referred to a special and extensive bulletin by Albert F. Woods, of this Division, which will make its appearance at no distant date. In this place will be mentioned chiefly a number of observations made by several planters.

The disease is easily recognized by the contrast of the dark-green regions, mostly near the veins, and the light-green regions of the lamina. The former, being better nourished on account of containing more chlorophyll than the latter, grow faster. This causes the leaves to curl and assume a wavy shape, like corrugated iron, rendering the leaves worthless for wrappers. Highly diseased leaves have to be entirely discarded. The disease sometimes affects as much as 10 per cent of the plants of an entire field. At times it attacks only a few leaves of a plant, and at other times the entire plant is affected. Broad-leaf tobacco is asserted to be more liable to the disease than Habana seed tobacco. The annual damage thus done in the United States is estimated to be more than a million dollars.

Various causes seem to create a tendency to the disease. Some act in the seed bed, some depend upon the mode of planting in the field,

and others seem to depend upon changes in the weather.¹ Solely a surplus amount of water in the soil, involving a deficiency of root aeration, does not produce the mosaic disease, as the writer observed. In a field one corner of which was continuously moist, the plants were of a uniformly yellowish-green all over, and they were only about half the height of the healthy plants, but there was no trace of symptoms of mosaic disease visible.

The diminished amount of chlorophyll in mosaic diseased plants, leading to a diminished production of organic matter, soon shows its effect in the more or less retarded development of the whole plant. There is also less coagulable albumin and less acid in the leaves of the diseased plants than in those of healthy ones of about equal age. The juices were titrated with one-tenth normal soda solution and the acid calculated as malic acid.

The following amounts of malic acid were found in a diseased and in a healthy plant:

	Diseased plant.	Healthy plant.
	<i>Per cent.</i>	<i>Per cent.</i>
Pith	0.033	0.033
Midrib of leaf	.048	.105
Lamina of leaf <i>a</i>	.210	.277

a After removal of midrib and large side ribs.

The relative amounts of oxidizing enzymes also are different in diseased and healthy leaves. The observations of the writer agree essentially with those of Woods.² Not only the midrib and the pith, but even the dead spots of the diseased leaves contain very much oxidase, while the lamina contains much more peroxidase than that of healthy leaves. The writer had occasion to observe tobacco fields, just before harvesting time, in Connecticut and Massachusetts, and was surprised at the great differences in the extent of the disease even on neighboring fields. Some planters entertain the belief that a too extensive use of mineral fertilizers favors the disease, and, indeed, those fields had the least number of diseased plants which had received chiefly organic manure. Mr. Du Bon, of Poquonock, Conn., whose crop of Habana

¹One cause may be an insufficient distribution of the manuring material. A tobacco grower at Poquonock set his plants in a field in which one inch of earth covered the cotton-seed meal employed as manure, the latter not being uniformly mixed with the earth. On further development more than 50 per cent proved calico plants.

²Paper read before the American Association for the Advancement of Science at Columbus, Ohio, August, 1899. Other papers on this disease have been published by Dr. Wm. C. Sturgis, Conn. Agr. Exp. Sta. Report for 1898; further by Adolf Mayer, Landw. Vers. Stat., vol. 32 (1886); by Beijerinck, and by Jwanowski, Centr. Bakt., vol. 5.

seed tobacco was in excellent condition, had applied per acre the following amounts of fertilizers in the drill:

5 cart loads horse manure,
20 hundredweight of cotton-seed meal,
500 pounds of "dissolved bone black,"
500 pounds cotton-hull ashes, and every other year
300 pounds of lime.

On a field near East Hartford, Conn., many more calico plants were to be seen than on another field on the opposite side of the road—on the former about 8 to 10 per cent, on the latter only about 3 to 4 per cent. This difference might be ascribed to differently manured seed beds or to the different manuring in the field. Both farmers applied horse manure—the first, 5 cart loads to the acre; the second, 9 cart loads to the acre. The former also used 10 hundredweight of bone dust, while the latter applied 800 pounds per acre of the following mixture:

150 pounds of ammonium sulphate,
450 pounds of potassium sulphate,
640 pounds pulverized bone and meat,
600 pounds of "dissolved bone black."

The latter had therefore applied not only much more horse manure to his field, but also more of the easily accessible phosphoric acid and more potash than the former.¹

Mr. Du Bon, a good observer, reported to the writer the following:

The calico disease may appear even in the seed bed, especially when it is too rich in nitrogenous manure, but the first two or three leaves of the attacked plants are generally normal. Fresh soil that has never served for raising crops does not produce the disease in the seed bed nor does an excess of moisture, but too high temperature is likely to produce it. Yellow plants, stunted in the seed bed by cold weather, never show mosaic disease in the field. In one case it was observed that an acre and a half planted with such yellow plants did not show a single calico plant, while the neighboring acres, planted with vigorous seedlings, developed a great many. Very vigorous plants that had been manured heavily with castor pomace and were looking very well showed much calico later on.

The disease is not easily propagated through the soil of the field. Even twin plants may be found where one is much attacked and the other bears hardly a trace of the disease.² Near Poquonock fields have been used for twenty-five years uninterruptedly for the culture of tobacco, and upon this land thoroughly healthy crops are now produced, although formerly the disease was often observed. One year, on a piece of land that contained 12,000 plants, 3,200 calico plants were cut out with a hoe when the plants had reached about 2 feet in height. The following year hardly half a dozen calico plants were noticed on that same plot, but these plants came from a seed bed containing new soil. The great influence of the growth in the seed bed on the

¹ Koning (*Zeitschrift für Pflanzenkrankheiten*, 1899, p. 65) asserts that manuring with kainit and Thomas phosphate diminishes the extent of the disease.

² This fact seems to be in contradiction to the behavior of the plants in the seed beds. A satisfactory explanation is wanting.

later development of the disease is shown by the following observation of Mr. Du Bon: "It happened once that in a portion of a field every other plant was a calico plant and the alternating ones were healthy plants. This remarkable circumstance found its explanation in the fact that in the process of machine planting two baskets were used containing the plants of two different seed beds, one bed being in a condition to favor the disease, the other containing new soil. Two planters worked here at the same time. The neglect of proper precaution in taking the young plants from the seed bed may create a tendency to the disease." In this connection Mr. Barnes, of Hatfield, Mass., related the following: "Young plants were taken from the same seed bed by two different farmers, and, although the soil of the fields was the same, one farmer had many calico plants in his field and the other had almost none. Inquiry revealed the fact that one farmer took the plants from the seed bed when it was quite dry, the other when it was well moistened. In taking the plants from a dry bed one can not help lacerating the roots, while from a well-moistened bed the roots may be withdrawn in an almost intact condition. Lacerated roots do not generally develop again to such a vigorous state as is required by the quickly growing tobacco plants."

For the prevention or diminution of the disease, Mr. Du Bon recommends keeping the plants in the seed bed not too warm and also changing the seed bed. Beds that produce the disease might be grown over for one year with grass, in order to put them in proper condition for the production of healthy tobacco plants. While many planters seem convinced that the diseased plants can not recover, others claim that by hoeing and stirring the soil well the disease may be cured in the initial stage. Mr. Barnes, of Hatfield, Mass., claims to cure the disease by lifting the whole plant a little from the ground. In both cases a better aeration of the soil is reached. The writer is not prepared to confirm or contradict these statements.

OBSERVATIONS ON CURING.¹

INTRODUCTION.

The curing period is a time of continuous anxiety for the tobacco planter. Too dry weather stops all changes in the leaves, particularly the development of the proper color, while too moist weather, especially at a temperature above 24° C. (75° F.), favors the disastrous pole burn. The great desire of the tobacco planter to become entirely independent of the weather by such improvements in the barn construction as will permit the proper regulation of the temperature and moisture is therefore reasonable.

The general introduction of an ideal tobacco barn can be only a question of time. If the tobacco is becoming too dry, steam should be turned into the building; and if it is becoming too moist, dry heat

¹ These observations were made with leaf cured on the stalk.

should be applied. An agricultural journal describes such a barn as follows:

The barn is what is called a "three-bent" shed. There are three rows of beams, one through the center and one on either side. A 2-inch steam pipe follows the outside beams around the shed. This is believed to be sufficient to supply the necessary heat to the whole building. The tobacco in the extreme outside of the shed rarely suffers from the pole sweat. The tobacco through the center, and especially that low down, is always the worst affected, and the pipes are arranged to throw the most heat into that part of the building. If the experiment proves a success in preventing pole sweat, the time is soon coming when artificial heat will be used entirely in curing tobacco, for the even temperature and moisture that can be obtained are just what is needed for a perfect cure.

As the curing proceeds an alternating change from the moist to the dry condition of the leaves takes place, according to the atmospheric conditions. Practical experience has proved that a frequent change of this kind is desirable.¹ This agrees also with the theoretical inference. There is more oxidase in the ribs than in the mesophyll, and by the alternate drying and moistening the evaporation from the surface causes a current of juice to pass from the ribs to the mesophyll cells. This migrating juice finally distributes most of the oxidase from the bundle sheath and sieve tissue uniformly through the leaf. It is therefore desirable to have the ribs keep alive much longer² than the rest of the leaves, since dead and dry ribs would not support the process indicated.

As long as all the cells of a leaf are alive a current of organic matter—especially sugar, and also to a small degree, amido compounds—takes the opposite direction, namely, from the lamina to the ribs. Gradually this function stops, since a state of starvation sets in when the starch, in the form of sugar, has been transported to the stalk and consumed by respiration. The decrease of soluble albumin in the curing process is much slower than the decrease of starch. Even after a fortnight a moderate amount is left, and with single cured leaves, even after four weeks, some traces of it were found in the tissues.

DECREASE OF ACIDITY.

It is an interesting fact that the acid reaction decreases in the lamina as the curing proceeds, as shown by the following table. For 200 sq. cm. there was required the following amounts of one-tenth normal soda solution:

Date.	Uppermost leaves.	Lower leaves.
August 1	c. c.	c. c.
August 8	3.8	3.0
August 14	3.3	2.3
August 26	2.5	1.8
September 26	1.7	.9

¹ Report No. 62 (p. 12), U. S. Department of Agriculture, by Marcus L. Floyd. This report also contains views on the best construction of the usual barns.

² The midrib may remain alive for three weeks and over.

At the end of the curing process the amount of free acid for a given surface was therefore reduced considerably.¹ A portion of the acid may have passed into the stalk, another portion may have been completely oxidized, and another may have been neutralized by ammonia formed in the destructive action of oxidizing enzymes on nitrogenous compounds. In the subsequent sweating process the liberation of ammonia is more energetic, and neutralizes the rest of the acid. The decrease of acidity in the curing process is of practical importance, since the following sweating process will set in with sufficient energy only when the reaction is nearly neutral. The practice of the tobacco manufacturers in Florida of applying a spray of ammonium carbonate solution when the "heaps will not heat up well" is therefore in accordance with the scientific inference.

BEHAVIOR OF THE OXIDIZING ENZYMES IN THE CURING PROCESS.

In a normally conducted curing process, whether curing single leaves or curing on stalk, the oxidase and peroxidase are well preserved. This is not the case under conditions unfavorable to a normal curing. The oxidase especially loses its active character more or less. Flue curing seems just as injurious² as prolonged moist weather. The acidity in the leaves is, according to the writer's observation, generally too weak to cause injury; but the occasional entrance of too much sunlight into the barn may diminish the oxidase in the exposed leaves. Reynolds Green, and Brown and Morris have shown that enzymes, as, for example, diastase, are injured by exposure to direct sunlight, while with regard to the action of water it has been shown by Laborde that a gradual destruction of oenoxidase takes place within twelve days by self-oxidation when it is dissolved in water and exposed to air. There can hardly be any doubt that much water retained for a certain time in the curing leaves of the barn will favor self-oxidation of the tobacco oxidase. This is also shown by the following test.

A large upper leaf was placed in a covered glass cylinder containing some water, to insure an atmosphere saturated with water vapor. After being kept in darkness for a week at from 22° to 28° C., comparison with a normal leaf showed less oxidase in the parenchyma of the leaves so treated than in that of equally old fresh leaves of the same plant, while with the veins the difference was not so marked. On the other hand, no decided decrease of peroxidase could be noticed.

¹In the pith and bark no decrease of acidity, but, on the contrary, a slight increase was observed during the first two weeks of curing. Further it may be mentioned that the acidity seems to decrease more slowly when the leaves are single cured than when cured on stalk. Only one test was made, however, in regard to this.

²Flue curing is mainly used in the curing of the bright yellow tobacco of Virginia, Tennessee, and North and South Carolina. The temperature reached thereby—71–79° C. (160–175° F.)—is detrimental to the oxidase. Such tobacco, however, is not subjected to sweating.

Furthermore, according to Mr. Marcus L. Floyd, it is a known fact that tobacco too long "in case" because of prolonged moist weather will sweat but very imperfectly. The poorly sweated crop of 1898 in Connecticut also showed much "canker" and "pole burn," conditions favored by too moist weather.

When it happens that tobacco does not sweat well, or even not at all, it may be too acid or may have lost its oxidizing enzymes through irregularities in the curing barn, or it may be too poor in the proper oxidizable material. The writer was assured by tobacco growers of Connecticut and Massachusetts that tobacco grown in very dry years is incapable of sweating; 1871 was such a year. Even the transportation of the tobacco to the West Indies and back, an experiment tried by Mr. Barnes, of Hatfield, Mass., to enforce a natural sweat, did not change the rank smell of the cured to the aromatic odor of sweated tobacco.

The writer was informed in Lancaster, Pa., that the crop raised there in 1881 required nine years to reach the proper quality by means of the natural sweat. Here probably a gradual oxidation without oxidases has produced finally a result similar to that reached in a short time in presence of the oxidizing enzymes.

Since it is the energetic oxidation in the sweating process that yields a cigar leaf of superior quality, it must form one of the principal aims of a tobacco farmer to preserve the oxidizing enzymes while the tobacco is curing in the barn. In the process of sweating, however, there is generally a gradual decrease of oxidizing enzymes going on, the high temperature favoring not only the action of these enzymes on oxidizable material, but also the oxidation of the enzymes themselves. Practical experience long ago found it necessary to take apart and rebuild the fermenting piles as soon as the temperature reached about 60° C. (140° F.). Indeed, if the temperature be allowed to rise much higher there would be a rapid decrease of oxidase, and all the improvements hoped for would come to a premature end.¹ The relative amount of water in the fermenting piles or cases will also exert an influence. The peroxidase suffers less than the oxidase, but the former is probably of very much less importance than the latter in the sweating process. Investigations on this point will be carried on at some later time when occasion offers.

TESTS FOR OXIDASE AND PEROXIDASE IN CURED AND FERMENTED TOBACCO.

In regard to the tests for these enzymes it must be pointed out that from the absence of oxidase in a tested sample it does not necessarily follow that all of the leaves from the same curing barn would be

¹A full account of the temperature changes in fermenting piles of cigar leaf tobacco is given in Report No. 60, U. S. Department of Agriculture, by Milton Whitney and Thomas H. Means.

devoid of it. It may be that the leaves tested came from near a barn door which was perhaps frequently opened, thus freely permitting the destructive action of the sunlight. Therefore in controlling a curing crop in the barn leaves should be tested not only from near the wall, but also from the more central part of the barn.

The test was at first made in the following manner: The tobacco leaf was deprived of the large ribs, and after drying at a temperature of 40° C. (104° F.) was very finely pulverized. One gram of this dry powder was mixed with 20 c. c. of water and left to stand for five hours in an Erlenmeyer flask at the ordinary temperature. To 10 c. c. of the filtrate a few drops of an alcoholic solution of guaiac resin were added and the mixture was left to stand for ten minutes, with moderate shaking. A blue coloration indicated the presence of oxidase. Since, however, the brown color of the tobacco extract is often so dark that it interferes with the recognition of a weak blue reaction, it was found preferable in such cases to pour guaiac tincture cautiously on the surface of the tobacco extract, avoiding a thorough mixing. The blue color will soon appear as a ring on the surface of contact of both liquids. If, however, no blue coloration appears, a few drops of peroxide of hydrogen are added. A blue coloration in this case is due to the presence of peroxidase. In order to test for the peroxidase in presence of the oxidase in the tobacco extract, it is necessary to heat the liquid for three minutes to 70° C. (158° F.) in order to first kill the oxidase before the test for the peroxidase is made. Guaiac solution in presence of peroxide of hydrogen is now left to act on the cooled solution. The blue reaction for peroxidase sets in at once.

Some additional remarks may be made on the tests with guaiac resin. A solution of guaiacum resin is prepared by dissolving at the ordinary temperature 2 grams in 100 c. c. of absolute alcohol. This tincture must be kept cool and in the dark, and be renewed from time to time, since the active principle, the guaiaconic acid, is otherwise gradually oxidized. Old tincture will soon turn bluish on addition of hydrogen peroxide alone. Attention must be paid to the reaction of the liquid to be tested, which must not be alkaline but neutral or slightly acid, and also to the quality of the resin, since worthless kinds exist in the market. A normal resin must yield an intense blue color with highly dilute nitrous acid. Only the interior parts of transparent pieces of guaiacum resin should be selected for preparing the tincture of guaiac. It is of no particular advantage to use the guaiaconic acid, instead of the resin itself.

An attempt has been made to disqualify guaiacum resin as a reagent for oxidizing enzymes on the ground that nitrous acid, ferric chloride, and other oxidizing mineral compounds also yield a blue reaction. Such objections are groundless when fresh organized tissues are under investigation. Other oxidizing influences are easily recognized by control tests, which the careful worker will not omit to make. The

assertion that albumin and gelatin give the reaction is erroneous. On the contrary, egg albumen impedes or prevents the oxidase reaction on guaiacum, as does also much tannin. It may be added here that among other substances silver nitrate also yields a blue coloration, especially in presence of a very small amount of ammonia. Such blue reactions are not restricted to certain limits of temperature as the reactions of oxidizing enzymes are.

Still more attention to control tests must be paid when materials which are not fresh are to be tested; for, while the fresh tobacco leaf never contains nitrites, sweated tobacco may contain them. This must be considered when the tests are made with sweated tobacco. (See on this point also page 55 of this report.)

The following tests were made with samples, not collected personally, but received by mail.

Tobacco cured but not sweated.

A.—SAMPLES FROM MARYLAND.

[The samples were grown in 1898 on the farm of the Maryland Agricultural Experiment Station. Only the last mentioned was from another locality.]

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899.				
Mar. 13	Connecticut seed leaf....	None	Moderate	Cured without artificial heat.
	(a) Lowland plot	do	Trace	
	(b) Upland plot	do	Much	
13	Habana seed	Moderate	Much	
13	Sumatra seed	Less than Habana	do	
13	Vuelta	None	Much	
13	Pardidos	Moderate	do	All subjected to the flue-curing process.
18	Maryland broad leaf	Slight	Moderate	
18	Virginia	Moderate	Intense	
18	Sumatra seed	None	Moderate	
18	Connecticut broad leaf	do	Trace	
18	Habana seed	do	Moderate	
18	Pardidos	do	do	
Apr. 4	Maryland tobacco	do	do	

B.—SAMPLES FROM KENTUCKY. (CROP OF 1898.)

Mar. 13	White burley lugs	None	Moderate	Single-leaf cured.
13	do	Moderate	Much	Cured on stalk.
20	Burley grade red leaf	None	Little	Do.

C.—SAMPLES FROM PENNSYLVANIA. (CROP OF 1898.)

Mar. 20	Pennsylvania Habana seed.	Moderate	Much	Seed wrapper.
20	Habana seed	Trace	Moderate	Seed filler.
20	Pennsylvania seed leaf	Moderate	do	Wrapper.
20	Seed leaf	do	do	Filler.

Tobacco cured but not sweated—Continued.

MISCELLANEOUS SAMPLES.

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899. Feb. 22	Wisconsin tobacco (Soldiers' Grove).	None	None	
24	Florida Habana seed (Quincy).	Much.....	Much.....	Crop of 1898.
Aug. 13	Hatfield, Mass	do	do	Single-leaf cured (1898).
Sept. 23	Poquonock, Conn	do	do	1899 crop, stalk cured.
Oct. 20	Ada, Tex	None	Trace.....	1899 crop. This sample had white veins.
20	do	do	Moderate	This tobacco was cut after being "drowned or scalded," α and was stripped before it was completely cured.
Dec. 12	Raleigh, N. C	do	Trace.....	Was flue cured; crop of 1899.
Oct. 18	Elmira, N. Y	Moderate	Much.....	Medium weight and color; cigar type; crop of 1899.
18	do	do	do	Dark, heavy; cigar type.
18	do	Trace.....	do	Light weight and color; cigar type.
18	Virginia	Little.....	do	Dark, heavy; plug type.
18	do	do	Moderate	Medium dark; crop of 1899.
18	do	Trace.....	Much.....	Light weight, dark.

α This drowned or scalded state is produced when after several days' heavy rain very warm and sunny days follow; the leaves soon wilt.

Tobacco, cured and sweated.

A.—SAMPLES FROM KENTUCKY. (CROP OF 1898.)

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899. Mar. 7	Tobacco from Hopkinsville, Ky.	None.....	Trace.....	New-ground tobacco; planted May 14; cut Aug. 10; fired lightly; bulked Nov. 21.
7	do	Slight trace.....	Moderate	New-ground tobacco; fired heavily; bulked later.
7	do	do	Much.....	Old-ground tobacco; fired heavily; bulked Dec. 10.
7	do	do	do	Same as former, but bulked later.

B.—SAMPLES FROM OHIO. (CROP OF 1898.)

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899. Sept. 24	Zimmer Spanish	None	Trace	
24	do	do	Moderate	

C.—SAMPLES FROM CONNECTICUT. (CROP OF 1898.) α

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899. Sept. 21	Gastonbury broadleaf ..	None	None	Light wrappers.
21	South Windsor broad-leaf.	do	Much	Do.
21	East Windsor Hill broad-leaf.	do	Trace	Do.
21	South Windsor broad-leaf.	do	Much	Dark wrappers.
21	Windsor Habana seed ..	do	Moderate	Do.
21	Portland Habana seed ..	do	Much	Do.
21	Poquonock, Conn., Habana seed.	Moderate	do	Passed through the natural sweat.

α One sample of 1893 crop, grown at Canton, Conn., showed no trace of oxidase or peroxidase.

Tobacco, cured and sweated—Continued.

MISCELLANEOUS SAMPLES.

Date.	Sample.	Oxidase.	Peroxidase.	Remarks.
1899.				
Mar. 4	Maryland tobacco, Jewell	None		1898 crop.
4	Virginia	do		Flue cured; 1896 crop.
Sept. 25	Wisconsin	do		1897 crop.
25	Lititz, Pa., broadleaf	do		1893 crop.
25	Lititz, Pa., Habanaseed	do	Trace	1897 crop.
25	Hatfield, Mass., broad- leaf.	Moderate	Much	1898 crop, single-leaf cured.
Nov. 1	Ohio Zimmer Spanish ...	None	None	Stalk cured, crop of 1898, from Ohio.
	Ohio Little Dutch	do	Much	From Ohio.
Aug. 18	Hatfield, Mass., Habana seed long wrappers.	Much	do	Single-leaf cured and sweated in cases at 100° F. 1898.
18	Hatfield, Mass., suckers.	Moderate	do	Do.
18	Hatfield, Mass., top of suckers.	do	Moderate	Do.
18	Hatfield, Mass., ground leaves.	Little	do	Do.
Dec. —	Remedios, Cuba	None	Much	Crop of 1899.
	Pardido, Cuba	do	Slight trace	Do.
	Haiti	Trace	Much	Do.
	Puerto Rico	None	Moderate	Poor grade, 1899.
	Puerto Rico	do	do	Best grade.

We learn from these tables that the flue-curing process is unfavorable for the preservation of the oxidizing enzymes, and that sweated tobacco has frequently lost its entire content of oxidase, while the peroxidase is more resistant under the conditions of the sweating process. From the absence of oxidase in fermented leaves no conclusion can, of course, be drawn as to its amount before the sweating was begun. The more intensely the sweating process is carried on, the more of these oxidizing enzymes will be destroyed.

TEST FOR CATALASE IN VARIOUS TOBACCOS.

With regard to catalase, the third oxidizing enzyme of tobacco mentioned above (page 22), some experiments were made on various samples of cured and sweated tobacco to determine the amount of oxygen developed by it from hydrogen peroxide in from fifteen to forty minutes. From the energy of this action inference may be drawn as to the relatively larger or smaller quantity of the enzyme in different samples. Tobaccos that had been fire-cured, as the plug tobaccos, contain in most cases neither oxidase, peroxidase, nor catalase. Such samples examined were bright yellow Virginia, black African, and English olive-green leaf. A sample of Burley tobacco showed, however, slight traces of peroxidase and catalase. Since the temperature in this curing method often rises as high as 82° C. (180° F.) these observations do not surprise us. Also a flue-cured sample of yellow tobacco from North Carolina did not show traces of the three enzymes. Such tobaccos are not intended for cigars and are not submitted to the sweating process, hence the absence of the oxidizing enzymes is here not of any conse-

quence. But there are also conditions in the usual treatment of cigar tobacco which may injure the catalase. Thus no trace of it was found in one sample of sweated tobacco from Connecticut of 1897. In most cases, however, catalase is retained in the sweated cigar tobacco for a much longer time than either oxidase or peroxidase.

The comparative tests for α and β catalase were carried out in the following way: The air-dried samples were very finely powdered after removal of the midrib. After remaining twenty-four hours in an exsiccator 2 grams were extracted for five hours with 40 c. c. of water at the ordinary temperature with frequent shaking, and then filtered through filter paper. Twenty cubic centimeters of the filtrate were mixed with 5 c. c. of neutralized commercial hydrogen peroxide. The development of oxygen by the soluble or β -catalase commences a few seconds after mixing. Then the extracted tobacco powder was, after washing, suspended in 30 c. c. of water and 10 c. c. of hydrogen peroxide added. The oxygen here developed was due to the action of the insoluble or α catalase. The proportion of the developed oxygen gradually decreased in all cases after a short time, since the hydrogen peroxide in the concentration applied oxidized and killed the enzym rather soon. The reaction was promoted by moderately shaking the flask.

In those cases in which after a rapid development of oxygen gas an early stop was noticed a second dose of hydrogen peroxide was added, which was repeated as often as found necessary until the reaction had been carried on for a given time.

It will be noted from the method of testing just described that the data relating to β -catalase are obtained from 1 gram of tobacco; those relating to α -catalase from 2 grams.

Thus far no data are known that would enable us to calculate the absolute weight of catalase from a certain volume of oxygen developed in a given time by the decomposition of hydrogen peroxide. But quite generally we might make the following distinction as to relative quantities. The amount of α -catalase is small when under the above-described conditions less than 50 c. c. oxygen are developed in fifteen minutes by 2 grams of tobacco; it is moderate when between 50 and 200 c. c. are developed, and high when the volume rises above 200 c. c.

As to β -catalase the amount may be called small when less than 20 c. c. of oxygen are developed in fifteen minutes by 1 gram of tobacco; moderate, at 20 to 100 c. c., and high, when the volume rises above 100.

Sample.	Oxygen developed.			Oxidase.	Peroxidase.	Remarks.
	Time of development.	By α -catalase.	By β -catalase.			
	<i>Minutes.</i>	<i>c. c.</i>	<i>c. c.</i>			
Connecticut broad-leaf.	5	22	1.0			Cured, 1899, but not sweated.
	10	34	1.8	Moderate	Much	
	15	42	2.0			
	30	66	2.0			
Florida, 1897.	5	32	13.0		Some	Sweated in bulk.
	10	45	19.4	None		
	15	76	26.5			
	20	84	29.0			
Maryland smoker, 1898.	40		36.8			Light-colored, thin leaf, sweated slightly in bulk.
	5	12	8.2			
	10	22	11.8			
	15	28	13.2	None	None	
Ohio Zimmer Spanish	30	36				Crop of 1897, sweated in bulk.
	5	108	17.1			
	10	174	32.0	None	Trace	
	15		40.5			
Wisconsin, crop of 1897, "Gummy" leaves.	20		45.2			Natural sweat.
	5	28	1.0	None	None	
	10	40	1.7			
	15	46	1.9			
Pennsylvania	20	49				Passed through the "natural" sweat, 1898 crop.
	5	32	4.2			
	10	50		None	Moderate	
	15	67				
Remedios, Cuba.	20	76	16.3			Sweated, 1899.
	30	86				
	40	89				
	5	27	3.2	None	Much	
Pardido district, Cuba	10	44	6.1			Sweated, 1898.
	15	60	7.5			
	20	71	8.0			
	5	14	8.2			
Puerto Rico.	10	20	11.0	None	Trace	Sweated, 1899.
	15	24	14.0			
	20	27	15.1			
	40	36				
	5	16	3.5			Sweated, 1899.
	10	20	4.4			
	15	25	5.1	None	Little	

It was thought that it would also be of interest to test cigars of commerce for oxidizing enzymes. Of the samples procured, which were probably of very different ages, not one contained oxidase or peroxidase, but catalase was present in all, although the amount differed considerably to judge from the energy with which hydrogen peroxide was decomposed. As to cigarette tobacco, five different brands of cigarettes were examined, viz, Old Dominion, Sweet Caporal, Gold Leaf, Chesterfield, and Egyptian Prettiest, but only in the last named was any catalase found.

Oxygen developed in fifteen minutes.

Cigar samples.	Price.	By α -catalase.	By β -catalase.
		<i>c. c.</i>	<i>c. c.</i>
Arena	3 for 5 cents	91	5.5
Havanets	2 for 5 cents	520	41.0
Marquis L.	5 cents	71	13.2
Lucke Rolled		41	18.0
Union Glory	5 cents	85	8.0
La Valiar	10 cents	26	3.0
Herbert Spencer	do	273	29.5
General Arthur	do	304	13.3
No name	2 cents a piece.	61	8.5
Do	5 cents a piece.	102	17.5
Do	do	108	3.2
Do	do	32	3.8
Do	do	241	55.0
Egyptian cigarettes		36	2.0

It will be noticed from this table that there is no direct relation between the commercial value of a tobacco sample and its catalase content. On the one hand, however, it may be said that a comparatively high content of soluble or β -catalase would be indicative of this tobacco having been subjected to a sweating at a higher temperature, since the ammonia developed renders some catalase soluble. Further, since a thorough sweat is essential for the development of aroma, a high content of soluble or β -catalase would indicate an improved aroma, other things being equal. On the other hand, it might be inferred that a sample containing very little soluble, but comparatively much insoluble catalase (see No. 11 of the above table), has passed only through the natural sweat and could be improved by a subjection to sweating in bulk after petuning with a very diluted solution of ammonium carbonate.

In order to reach a reliable estimate of the extent of the transformation of the insoluble into the soluble catalase by the sweat only leaves of equal age and very similar to each other should serve for comparison. A cured leaf can not be compared with a sweated leaf of the same crop when the former happens to be a lower or sand leaf and the latter an upper leaf. Neither can thin, strawy leaves be compared with gummy leaves, since the differences between these are very great in regard to their enzym content. Thus, a not sweated, very thin, probably a sand leaf, yielded in fifteen minutes by the above test 7.7 c. c. oxygen for the soluble enzym and 69 c. c. for the insoluble, while a sweated, gummy leaf of the same crop yielded 147 c. c. and 360 c. c. oxygen, respectively. There can be no doubt, however, that the above tests, judiciously carried out, may prove of practical value in certain cases

In order to obtain comparable results, and to eliminate the factor of individual differences, upper leaves which can be distinguished by their heavier body were picked out of a lot of cured leaves and of a lot of leaves sweated in bulk, of the same crop.¹ After moistening them a little they were left over night until they became pliable, whereupon they were spread out and the adhering sand carefully brushed off. The central portions of 12 leaves were then deprived of the midrib, and after drying at 40° C. (104° F.)² finely pulverized. Two grams each of this powder were weighed out, and the tests carried out as above mentioned. The result was as follows:

Oxygen developed in thirty minutes.

	By α -catalase.	By β -catalase.
	c. c.	c. c.
Cured but not sweated, filler leaves	158	16.5
Cured and sweated, filler leaves	634	236.0
Proportions of the volumes of oxygen	1:4	1:14.3

¹ This tobacco, raised in 1899, came from the Experimental Farm of the Conn. Expt. Sta. in Poquonock, Conn. The curing was there supported by a careful application of artificial heat, and supervised by Dr. E. H. Jenkins.

² Tobacco kept at that temperature in a drying box does not become completely dry—still retains 4 to 5 per cent moisture.

We notice from these figures not only an enormous increase of β -catalase, but also a considerable increase of α -catalase. It had been observed twice before by the writer that the α -catalase also was increased in the sweated sample as compared with the cured sample. He ascribed this circumstance to accidental differences in the leaf character. But this supposition has to be abandoned in view of the above figures obtained from average samples. The only explanation the writer can offer that seems probable is, that there exists in the case of catalase, as with many other enzymes, a zymogen in the leaves, which, under the conditions brought about in the sweating process, is converted into the α -catalase enzyme itself.¹

The relative intensity of the fermentation, or sweating, has also a decided influence upon the amount of β -catalase. Thus, upper leaves moderately sweated for wrapper purposes contain less than upper leaves heavily sweated for filler purposes. The amount of oxygen developed in thirty minutes by β -catalase was observed with the sample of the former to be 186 c. c., of the latter, however, 236 c. c. (both of the same crop). The difference in the amount of oxygen developed in thirty minutes in both cases by α -catalase was but small, 619 and 634 c. c. oxygen, respectively.

In order to decide whether the petuning liquid² which was applied for the filler leaves, but not for the wrapper leaves, had contributed to the increase of the amount of catalase, 20 c. c. of that liquid were, after neutralization, mixed with 20 c. c. of water and 5 c. c. commercial hydrogen peroxide. The total amount of oxygen developed in thirty minutes was but 26 c. c. Since the amount of the petuning liquid applied was only 0.66 per cent of the weight of the tobacco, not more than 0.02 c. c. of oxygen gas would have been developed in thirty minutes by the amount of petune contained in 2 grams of tobacco applied for the test on catalase, an amount so small that it can be neglected.

DEVELOPMENT OF THE "GRAIN" OF THE TOBACCO LEAF.

The "grain" means to the tobacco manufacturer small points plainly visible on the extended leaves after the curing or after the sweating process. These points, often situated at the base of the hairs, appear under the magnifying glass as small globules of yellowish color, showing sometimes a radiated structure. Boiling with water or with dilute acetic acid does not remove them, but treatment with dilute hydrochloric acid does so at once. This makes it very probable that the chief mass of these globules consisted of calcium oxalate, a view confirmed by the microscopical observation of gypsum needles forming under the

¹ The writer holds as probable that while α -catalase may be generated from a zymogen, β -catalase is produced from α -catalase only, and not from a separate zymogen.

² This liquid was prepared from licorice and various condiments, with addition of some rum, according to the prescription given by Marcus L. Floyd in Report No. 62, U. S. Department of Agriculture, p. 19, Cultivation of Cigar Leaf Tobacco in Florida.

influence of concentrated sulphuric acid upon the "grain." Although there is some calcium oxalate present in the living leaf, the oxalic acid of those globules is produced by post-mortem oxidation, since they do not exist in the living leaf, the natural inference being that it is the oxidizing enzymes that produce them by inducing oxidation of various compounds, such as malic or citric acid, tannin, etc. The more oxidizable material, and the more oxidation is going on in the tobacco leaf, the more will the so-called grain develop in the curing and sweating process; hence it will in many cases, by no means in all, confirm the idea of some tobacco manufacturers that a well-developed grain is a good sign for the quality of the tobacco.

The following observations by the writer may be mentioned: A fresh tobacco leaf was divided into two portions. One portion was hung up in a covered beaker containing some water, the other was first heated to 60° C. (140° F.) in a closed vessel before being thus treated. By the sudden killing of the cells by heat the cell sap left them at once and spread uniformly over the surface of the leaf, while in the former case the gradual drying and the later death diminish the migration of soluble matter to the surface. After eighteen days the "grain," in the form of numerous globules of calcium oxalate, was formed in the normally curing leaf, but not a trace of it was seen in the leaf killed immediately after collecting it. This shows that these globular masses were formed in the curing process. In the fresh leaves deposits of calcium oxalate also occur as mentioned, forming crystalline sandy deposits, especially in mesophyll cells. Generally these seem too small to account for the appearance of the "grain" in cured and sweated leaves.

The nature of the "grain" was also investigated by Dr. W. C. Sturges, who arrived at the same conclusion as the writer and at about the same time. That author had the kindness to communicate the following observations:

The crystalline deposits are insoluble in water, but are completely and readily soluble in dilute hydrochloric acid. On heating, these deposits swell to three or four times their original volume. Examination under the microscope of these heated masses in water, with the addition of hydrochloric acid, show that they are at once dissolved with copious evolution of gas. These observations, coupled with what we know of the occurrence of calcium oxalate in leaf tissues, lead me to conclude that the grain of certain grades of tobacco is caused by the deposition in limited areas of crystalline aggregations of this salt.

REMARKS ON THE DEVELOPMENT OF THE BROWN COLOR.

The slow starvation process of the tobacco leaf in the curing barn is connected with the yellowing of the leaves, followed by the characteristic brown. The yellow color is chiefly due to the etiolating of the chlorophyll granules because of the absence of bright daylight, and sets in while the cells are still alive, while the brown color sets in after their death. The changes of the green to yellow and of the yellow to brown start and proceed generally in the same order, commencing at the margin and about midway between the lateral veins. Along

the thicker part of the veins the tissue remains green longest. Gradually a stage is reached in which only the midrib and basal parts of the lateral ribs still have their normal color, being fringed by but narrow strips of yellow mesophyll, the chief mass of the leaf having turned brown.¹ Thus, the layman is inclined to infer that the substance which turns yellow is also the same that turns brown later on. This, however, is not correct. The yellow color is due to a chemical change of the chlorophyll green, while the brown color is due to the oxidation of several different compounds contained in the cell sap. The brown color shows also in the veins of the leaf, but not the yellow, since there is too little chlorophyll in the veins. It sometimes happens, however, that leaves in the barn die quickly by drying up instead of slowly by starvation, thus not allowing sufficient time for development of the yellow color, and in this case the brown color develops directly upon the still green leaf. Such leaves show a distinctly green coloration when held between the eye and the bright daylight, and on treatment with strong alcohol they yield a green solution of chlorophyll, showing that the green is not destroyed, but merely concealed by the brown. The chlorophyll, therefore, is not the source of the brown coloration. Furthermore, when a fresh leaf is dried at a moderate temperature in an air bath it will no longer turn yellow when exposed for a considerable time to a moist atmosphere, showing that the change from green to yellow in the curing barn is still a process of life, although a pathologic one.

As to the brown color, it can easily be shown that it is produced by the action of oxidase and not by the action of peroxidase, since after "killing" the oxidase the brown color fails to develop. Freshly expressed juice of the midrib was heated for four minutes to 75° C. (167° F.) and left with a little chloroform in a flask plugged with cotton. After ten days this liquid was still colorless, although containing peroxidase, while the control liquid became light brown within one day.² The juice of the lamina turns dark brown much sooner.

WHY DOES THE OXIDASE NOT PRODUCE THE BROWN COLOR WHILE THE LEAF IS ALIVE?

The oxidizing enzymes are evidently contained in the plasmatic living part of the cell, and not in the cell sap which fills the vacuoles. On the other hand, the matters easily oxidized by them and representing products of metabolism or by-products of certain synthetical operations are mostly contained in the cell sap. These matters, often of a chromo-

¹ The brown color darkens still more during the sweating process. When the expressed juice of green tobacco leaves, after filtering, is exposed to air it gradually forms an insoluble deposit, the greater part of which consists of a phlobaphen-like, dark-brown substance, and the smaller part of a colorless flocculent compound. The former is easily soluble in dilute ammonia; the latter is not.

² It may be mentioned here that, according to A. Mayer, a dark-colored tobacco is obtained by a rich nitrogenous manuring, and that, according to Kosutany, barn-yard manure with ammonium salts yields a product of a reddish brown tint, while nitrate manuring yields a more greenish-brown one.

genic character, are, of course, produced in the plasmatic parts, but secreted rapidly into the cell sap. When the cells die the soluble substances locally separated in the cytoplasm and the cell sap intimately mix, since the coagulating protoplasm, becoming easily permeable, can no longer retain any soluble matters—as, for example, oxidase. Thus after death the direct action of the oxidase on the oxidizable matter of the cell sap sets in with a production of colored bodies, as is the case in the tobacco leaves. This action can also be noticed very well in the juice pressed out from the fresh leaves, since this juice, containing cell sap as well as soluble matters previously contained in the protoplasm, turns brown rapidly on coming into contact with air. The darkening of the juices of potatoes, beets, and other plants depends upon the same principle.

CHROMOGENS OF TOBACCO.

The nature of the chromogenic compounds has not yet been fully revealed. It was pointed out in Report No. 59, p. 12, that several different compounds have to be assumed as contributing to the color in the tobacco leaf, and that tannin as well as nicotine contribute only a part of it. It is true that the darker tobaccos are generally considered as being the stronger—that is, contain the most nicotine—but, on the other hand, it has to be kept in mind that the veins, which contain less nicotine than the lamina, are generally much darker colored, a fact hardly to be explained merely by the thicker layer of cells. The fact, however, that the juice of the lamina turns dark more quickly and intensely than the juice of the rib makes it probable that the chromogen of the ribs is not easily soluble and differs from that of the lamina. In the lamina it is the mesophyll and not the epidermis that contains the chief quantity of the chromogen.

The main chromogens of the leaves are insoluble in alcohol, as is the brown product itself. A piece of young, green tobacco leaf was left in alcohol for one day, the chlorophyll being extracted and the leaf becoming almost colorless. Upon moistening with water it turned brown within two days, the color being darkest in the ribs and veins. The main chromogen, therefore, had not been removed by the alcohol treatment.

There is but very little chromogen in the pith, since it becomes only slightly brown after several days' exposure to the air; equally little is contained in the vascular bundles of the stalks, except in the youngest part. The study of the nature of the chromogens encounters considerable difficulties.¹

¹ After this was written, an article of Behrens appeared in the *Landw. Vers. Stat.*, vol. 52, in which he discusses the production of the color. He thinks that the brown substance is formed from a chromogen which is split off from a glucosid, and that this glucosid is formed during the first or starvation period of the leaves in the barn. This opinion can hardly be accepted when we observe the rapidity with which the juice of the fresh leaves turns brown upon exposure to the air, showing that the chromogen, or rather the "soluble" chromogen, is already present in the normal leaf.

SUNBURNED TOBACCO LEAVES.

When the tobacco plants are harvested it often occurs that the cut plants are left for several hours in the field, exposed to a scorching sun, and many of the leaves are killed either in part or in isolated spots. These prematurely killed portions remain green and never change color in the curing barn. There are, indeed, not infrequently found among the cured leaves of commerce some with green spots on them, due to this circumstance. The microscope reveals the fact that the fine veins of such spots are only of a faint brown color, compared with the well-cured portions.

It appears to be of some interest to decide whether it is only the effect of the intense light which causes such a change in the properties of the leaf, or whether a partial drying up in the sun forms one of the conditions. Possibly the light may kill the enzymes as soon as the cells themselves are killed by the heat. The gathered leaves warm up with the sun's rays considerably more than the leaves of plants standing in the field, where the transpiration current provides moisture which lowers the temperature by continuous evaporation.

A piece of ripe leaf was heated in a closed test tube in a water bath for five minutes to from 60 to 61° C., which caused the loss of the natural stiffness (turgor), the change being caused by the loss of the osmotic properties of the plasma sack when the leaf dies. Another piece of the same leaf was heated to 60° C. for five minutes, and then for five minutes longer to a temperature of from 70 to 71° C. Both of these pieces were hung up in a covered beaker containing some water, and compared with another piece of the same leaf not so treated. After eighteen days the pieces were moistened and spread on a glass plate, when it was easily recognized that the color of the piece heated to a temperature of from 60 to 61° C. was almost the same as that of the control piece, while the color of that heated finally to a temperature of from 70 to 71° C. had a far different look, only the larger veins having become brown,¹ while the lamina was of a weak yellowish green. This shows that the production of color is due to the action of the oxidase (not yet killed at 60° C.), and not to the peroxidase (not yet killed at 70° C.), since a special test proved that the piece heated for five minutes to a temperature of from 70 to 71° C. contained considerable peroxidase, but no longer any oxidase. Still the analogy to the sunburned leaves was not fully established by this test, since the sunburned leaves retain their full natural green, while with the samples mentioned the original green was somewhat changed. Tests in which the pieces of the leaf were not, however, treated in closed test tubes, but were left to dry openly in an air-bath at 58° C., gave a different result. In these the green was preserved almost in its natural condition, and

¹This browning was probably developed between 40 and 66°, after the death of the cell and before the death point of the oxidase was reached.

the subsequent exposure to the moist air led only to a coloration of the veins. The lamina remained green. The simultaneous effect of heat and of drying up must unite, therefore, to produce the phenomena observed in "sunburned" leaves.

"WHITE VEINS."

The phenomenon of "white veins" is observed in the curing barn after a long spell of dry and warm weather. The veins then lose the capacity of turning brown on the surface, and preserve a whitish or rather colorless appearance after the curing process is over. On holding such a leaf between the eye and the light, a sufficiently dark-brown coloration of these veins is plainly seen, hence it is only the surface which is devoid of coloring matter. There is hardly any other explanation than that by a too rapid drying the epidermis cells of the veins died too quickly and this premature death caused the entrance of air into and perhaps also below them,¹ leading to the optical illusion that coloring matter is absent in the veins, although the interior cells (those of the vascular bundle) contain it.² The microscope reveals colorless and air-containing tissue around the brown sieve-tissue masses in the "white veins," while in the normally colored veins the tissue surrounding the sieve tissue is more or less yellowish brown. The presence of "white veins" decreases the value of the wrapper leaf, while it has no influence upon the value of the fillers. On the contrary, such fillers may often be very valuable. To prevent the "white veins" there seems no other way possible than to keep the barn closed in prolonged hot, dry weather and to occasionally sprinkle water through the barn.

"SALTPETER" ON TOBACCO.

A phenomenon resembling to some extent that of "white veins," but in reality very different from it, is the efflorescence of a white saline mass upon the midrib and lateral ribs of the curing tobacco leaf. The tobacco planters and manufacturers call it "saltpeter," but sometimes there are only traces of saltpeter (nitrates) found in these efflorescences. They contain principally a mixture of potassium, sodium, calcium, and nicotine salts. The writer has not chanced to observe an efflorescence consisting only of saltpeter, although the possibility of such an occurrence can not be denied. The phenomenon is probably caused by a long spell of dry weather in the first stages of the curing.

Finally, it may be mentioned that sometimes a white mycelium of some fungus makes its appearance on the veins of the curing leaf,

¹ Such a phenomenon is also frequently noticed in August on the leaves of the peach tree, as Dr. Galloway has kindly pointed out to the writer.

² Mr. Floyd reported to the writer that occasionally white veins are also noticeable in plants while growing in the fields, and that in such cases the leaves are usually of a very dark green.

since the veins retain more moisture than the lamina and thus favor fungous growth more than the latter. This phenomenon is, however, very easily distinguished from that of the genuine "white veins."

METHODS OF SWEATING.

There are three methods of sweating practiced in the United States, viz:

- (1) The natural sweat in cases.
- (2) The forced sweat in cases.
- (3) The sweating in bulk.

The natural sweat is a slow process going on in cases. The low temperature in winter and spring in conjunction with the limited access of air is, however, an unfavorable condition. Besides, the absence of an energetic sweating process favors the growth of fungi upon the tobacco leaves when the tobacco has been packed too moist. The higher temperature used in the other two methods hastens the process of sweating and the development of aroma, and thus the tobacco can be put on the market a considerable time in advance of what could ordinarily have been done. The advantage of an early market is much desired by all packers.

The "forced sweat in cases" requires about the same length of time as the "sweating in bulk." A natural sweat for from six to ten months, followed by a sweating in bulk for from two to four weeks, is applied in Lancaster, Pa., for filler tobacco. Only when fear of must exists is new tobacco there at present subjected directly to the "forced sweat in cases," and this process is sometimes followed by a short "sweating in bulk" when the color for the fillers has not become dark enough.¹

An important difference between the "sweating in bulk" and the "sweating in cases" is the greater access of air in the former method. The amount of oxygen for the "sweating in cases" is restricted to that contained in the air of the cases² and in the air which enters through the sides and at the corners. By this lessening of the access of air the processes of oxidation are retarded and a considerable rise of temperature by the sweating itself is prevented. Hence, this method is carried on in rooms at from 37.7° to 43.3° C. (100–110° F.) without fear of undue spontaneous further rise in temperature in the tobacco

¹ Dark fillers and light wrappers are at present in demand. A thorough sweat, although leading to a fine aroma, will also produce a dark color, hence the sweat has to be limited when wrappers of a light color are to be produced. This is one of the reasons that the wrappers are moistened with less water than the fillers before sweating. The tip of the leaf is better adapted for wrappers than the base or "heel," as the tobacco manufacturer calls it, on account of its greater flexibility—produced by differences in the vascular bundles near the tip which consist mainly of spiral vessels, while near the base there are also pitted vessels.

² This amount is small, as the tobacco leaves are packed in cases with the aid of a press. No pressure is applied for the fermentation in bulk, in order not to diminish the necessarily limited access of oxygen.

itself. The cases are piled up in these rooms heated often merely by an iron stove, which holds a vessel of water to produce a moist temperature so as to prevent the tobacco from drying out too much.

With the "sweating in bulk" the condition differs.¹ The rooms generally have a temperature averaging from 21–28° C. (70–85° F.), and the only covering for the tobacco consists of blankets, which admit air freely between the sweating "hands" of tobacco. The oxidation processes can therefore be carried on with much more energy, leading to a considerable rise in temperature above that of the room, a rise amounting daily to from 5 to 8° C. (9 to 14° F.). A great advantage of the fermentation in bulk is that these bulks are repeatedly turned over and rebuilt, which enables a minute examination. A careful workman is thus able to observe whether the process is going on normally and can frequently remedy troubles. The forced sweat in cases, however, does not have this advantage. As to the ammonia produced by the sweating, there can be no doubt that it is a normal product of the destruction of the nitrogenous compounds by the oxidizing enzymes, and not a product of putrefaction, as held by some authors. The writer showed in Report No. 59 that bacteria of putrefaction can not thrive upon tobacco leaves in the sweating condition. Every normally sweated tobacco contains some ammonium salts.² It depends upon the amount of ammonia thus formed whether a tobacco of commerce shows a faintly acid, a neutral, or a faintly alkaline reaction.

As to the question which especial compounds are attacked in the sweating process by the oxidizing enzymes in the leaves, it is probable that all soluble organic compounds present in the leaf are attacked, but with a different degree of intensity. Although certain compounds, such as nicotine, may be attacked with difficulty, a noticeable amount may nevertheless become oxidized in the course of time, since the sweat in the bulk or the forced sweat in cases lasts about six weeks. The oxidation need not lead directly to the production of carbonic acid with every compound, as there may be formed intermediate oxidation products, which undergo further oxidation but slowly. By allowing the temperature to rise to about 65° C., which is 5 degrees higher than the generally adopted maximum, the oxidation may even extend so far that the aroma first produced is again destroyed and that the extractive matters are diminished until a very large percentage of the tobacco consists of mere cellulose. Tobacco manufacturers call such an inferior product "papery." Nevertheless the temperature of the fermenting wrappers in Sumatra is sometimes

¹ Report No. 60, U. S. Department of Agriculture, Temperature Changes in the Fermenting Piles of Cigar Leaf Tobacco, by Milton Whitney and Thomas H. Means. This report contains a full description of the "sweating in bulk" as practiced in Florida.

² Thoms obtained 38 grains of ammonium sulphate from 15 kilograms sweated tobacco on distillation with steam; but only a part of the ammonia is obtained in this way. Various analyses give from 0.05 to 0.5 per cent ammonia in tobacco.

allowed to reach 65° C. (109° F.), as Van Bemmelen reports.¹ When this temperature is kept up for some time or is allowed to rise some degrees higher, the tobacco loses its power of heating up again when the heap is repacked. The oxidase and catalase are now killed. Such tobacco is called "cooked." It is knotty and curled and the fiber has lost much of its elasticity.²

NOTES ON THE BACTERIAL HYPOTHESIS OF SWEATING.

Although it was shown in Report No. 59 that Suchsland's bacterial hypothesis of the sweating of the tobacco is unfounded and that the true cause of the oxidation in the sweating process must be attributed to the presence of oxidizing enzymes in the tobacco leaf itself, nevertheless some persons still adhere to the bacterial hypothesis. But it is a simple matter for anyone to convince himself by direct microscopical examination of scrapings from moistened sweating wrapper leaves that the numerous bacteria which would be expected, by the hypothesis, are not there. Even if we assume that the supposed tobacco bacteria are characterized by extraordinary capacities of oxidation and fermentation, and that therefore a restricted number might account for a great deal of work, several millions at least should exist for every square inch. Further, it is evident that the conditions on the tobacco leaves are most unfavorable for bacterial growth. It was pointed out by the writer that the water content of a fermenting wrapper leaf is utterly insufficient to bring the nutritive compounds from the interior of the cells to the surface, the moisture being barely sufficient for the proper imbibition of membranes and cell contents. The easily soluble matters, such as oxidizing enzymes, will no doubt dissolve, but this solution will be retained in the chief mass of the cell contents.

Let us assume that all the water of a sweating leaf would be available for the cell contents—none being retained by imbibition of the cell membranes—and, further, that one-half of the cell contents is easily soluble in water, although in reality two-thirds are easily soluble in their own weight in water. A fermenting wrapper leaf with a water content of 20 per cent³ would then contain a solution of organic matter of a strength of 50 per cent, since ripe wrapper leaves of good quality and capable of fermentation contain in the dry substance an average of 40 per cent of matter soluble in water.⁴ In such a solution of 50 per cent of organic substances bacteria can not multiply. Even mold

¹ Landw. Vers. Stat. vol. 37, p. 383.

² This is the experience of tobacco manufacturers of Florida, and has been mentioned to the writer by Mr. M. L. Floyd.

³ The tenacity and cohesion of wrapper leaves are generally seriously injured as soon as the water content is increased above 27 per cent while the sweating is going on.

⁴ Of course the percentage of soluble matters varies considerably in different grades of tobacco.

fungi find it rather difficult to live under those conditions.¹ In reality the conditions are still much less favorable for bacteria than we have assumed in this calculation. The high temperature is also an obstacle to most bacteria. Fermenting wrapper leaves sometimes reach a temperature of 60° C. (140° F.), and still ferment again when the heap is repacked. It would be a very easy matter to isolate the active bacteria, if they should belong to the few thermophylic kinds that can be active in such a high temperature. But nine years have passed since the promulgation of the bacterial theory of tobacco fermentation, and thus far there is nowhere to be found a scientific description of the supposed tobacco bacterium.

The following experiment shows that bacteria from the very surface of the tobacco leaves can not thrive in a concentrated tobacco extract. Such an extract containing 30.2 per cent of solid matter was sterilized and a small piece (about 1 cm. square) of a freshly cured tobacco leaf² introduced, after which the flask was plugged with cotton. After 10 days neither a scum nor a turbidity indicating the presence of bacteria could be noticed, but some clot-like masses were observed. These masses proved to consist of a mycelium of a fungus, while even with the highest magnifying power no microbes could be observed. However, a dilute solution, prepared by diluting the former with ten times its volume of water, formed a bacterial scum within two days upon exposure to the air.

In a second trial, with a concentrated solution of 25 per cent of tobacco extract (contained in a test tube) exposed to the air for ten days to admit various kinds of microbes, there was neither a scum nor a bacterial turbidity produced. On microscopical examination, however, some few bacteria could be noticed. Now, if solutions of such concentrations are so very unfavorable for bacterial growth, how much more must this be the case for the much higher concentration to be found in the cells of the fermenting wrapper leaves?

Further experiments proved that even at a water content of 35 and 40 per cent present in fermenting fillers³ bacteria can not flourish upon the leaf surface at the temperature of the fermenting piles. In freshly cured heavy tobacco the water was determined and enough water added so that in two samples the percentage reached 35 and 40 per cent, respectively. These leaves were rolled up and placed in flasks, which were sealed with paraffin and kept for five days at a temperature of from 53° to 55° C., there being an abundance of air in the flasks. For

¹ According to Splendore (Revista Tecnica, Roma, 1899), *Oospora Nicotianæ* can not develop upon tobacco that has less than 26 per cent of water, and, according to Behrens (Landw. Vers. Stat., Aug., 1899), *Botrytis* requires at least 30 per cent of water in cured tobacco to be able to grow on it.

² This tobacco contained catalase, oxidase, and peroxidase and was subjected to "sweating" or "fermentation in bulk." It sweated normally.

³ Hands of filler tobacco are dipped with their base deeper into the water than hands of wrapper tobacco, thus securing a higher water content.

a short time the temperature reached 59°, but since even 60° is frequently observed in the fermenting tobacco piles this temperature should not be expected to kill the supposed tobacco bacteria. The microscopical examination of the leaves after five days proved the absence of a bacterial coating. Only here and there a microbe was found, but upon staining and counting in everything which had any resemblance to a spore or bacillus there were not more than 600 microbes to the square inch. The examination was made not only of scrapings of the moistened surface, but of the triturated parts of the leaf itself.

Recently a treatise by H. Vernhout has appeared in which a microbe closely related to *Bacillus subtilis* is described from fermenting tobacco leaves in Java.¹ The author ascribes to this the phenomena of tobacco fermentation. The proof, however, that this microbe can produce fermentation and can multiply on tobacco leaves with only 25 per cent water has not been adduced. His further remark that a sample of cured tobacco did not contain any oxidase or peroxidase does not require any further comment, since these enzymes may disappear as above mentioned under unfavorable conditions, while catalase, the third oxidizing enzyme, still may persist.

SWEATING MUSTY TOBACCO.

The complaints about "musty tobacco," "white mold," and "black rot" in tobacco packed in cases are quite frequent. Moist weather toward the end of the curing, and especially while stripping and packing are going on, favors these injuries. Apparently quite healthy tobacco will often, after having passed through the natural or cold sweat, have a moldy odor² or a cover of a white film of fungus mycelium, or even patches where the leaves are transformed into a powder by the so-called black rot.³ Dark heavy tobacco is said to be most damaged by "white mold." Because of this damage the value of the tobacco is decreased to about one-sixth of the original. A tobacco manufacturer assured the writer that out of 1,600 cases of tobacco of a particular crop he found 1,300 cases more or less damaged in this way, and he estimated the damage annually caused in this country at about \$1,000,000. Repacking after removing the worst portions affords but a poor help and but for a short time. In the case of black rot further serious damage can be prevented more easily by repacking, since this fungus does not continue to spread after cold weather sets in in autumn. The chief remedy

¹ Onderzoek over Bakterien bij de Fermentatie der Tabak, Batavia, 1899.

² The moldy odor is caused by the growth of the common mold fungus, *Penicillium glaucum*.

³ In a case of black rot examined by Mr. E. A. Bessey, in which the cohesion of the parts of the leaf was destroyed and patches of it transformed into a pulverulent mass, the fungus *Sterigmatocystis nigra* (*Aspergillus niger*) was found.

applied at present is a "forced sweat," in which the musty odor disappears and the mycelia of the fungi are killed; but when this process is not thoroughly carried on the spores may be uninjured, and thus it happens that the musty odor may come back again when these spores develop.

This forced sweat is carried on in the presence of much water—45 or 50 per cent—and is therefore suitable only for fillers, since under such conditions the wrapper leaf would deteriorate considerably. The writer has observed that with a room temperature of 26.6° C. (80° F.) and a water content in the tobacco of 47.6 per cent the temperature of a large box filled with such moistened musty tobacco was markedly increased within four hours, and in two days reached a temperature of 40.5° C. (105° F.). From three to four days are allowed for this sweat in summer, and from one to two weeks in winter.¹ Tobacco that has passed through a "forced sweat" will not again heat up sufficiently, while after a cold or natural sweat it will heat up readily.

Some suggestions might be made as to how to prevent, as far as possible, the deterioration of cured tobacco by fungi. Whenever the stripping cellars are underground or immediately under the curing barn, the moisture of the air in the cellar often increases to such an extent that the stripped tobacco absorbs too much moisture before it is packed. When the water content of tobacco rises above 30 per cent there is great danger of the development of fungus spores which may be present on the tobacco leaves. Besides, in such damp localities various fungi develop on the floor and walls where organic impurities have gained lodgment, and hence the opportunities for the infection of the tobacco in the stripping process are much enhanced. Some farmers have recognized this dangerous source of infection and fumigate the stripping cellars with burning sulphur before commencing the work. The mycelia of fungi can easily be destroyed by this process, but the more resistant spores may escape destruction. Another means of cleaning the cellars is to whitewash them several times with freshly slaked lime and dry them out well with artificial heat before the stripping commences. It would be still better to have the stripping localities above ground and have them kept warm while the stripping is going on.

An essential step of progress would be made by giving up altogether the natural or cold sweat and by replacing it with the "sweating in bulk," to be carried out soon after the curing process is finished. The fungus spores, the germination of which is hastened by heat, are gradually killed in that state when the temperature rises above 50° C., and thus the fungus development injurious to the tobacco is easily avoided.

A few words may be said in this connection about measures for the prevention of stem rot. "Stem rot," a disease sometimes developing and spreading in the curing barn, has been investigated by Dr. W. C.

¹Such resweated tobacco is generally not packed again, but serves directly for the manufacture of cigars.

Sturgis, who named the fungus *Botrytis longibranchiata*.¹ He recommends that all the diseased stems and leaves be collected and burned at once, before the fungus is further developed and before its spores can be scattered about by any current of air. He further suggests sprinkling the floor of the barn with a mixture of equal parts of air-slaked lime and sulphur, and the fumigation of the tightly closed barn with burning sulphur a fortnight before the tobacco is harvested, and again after the removal of the cured tobacco from the barn. It may be added here that washing the floor with thick milk of lime which has been recently slaked in the usual way has also been found beneficial.

AROMA OF THE TOBACCO.

The odor developed in smoking a cigar may be partly due to the mere volatilization of products of the sweat and partly to the destruction of certain compounds. The heat of the glowing surface reaching the neighboring sections which are not in full contact with air causes there volatilization and various decompositions by dry distillation. Products of pleasant and of unpleasant odor are components of the smoke, and it is the chief aim of the progressive dealers in the tobacco trade to reduce the amount of the latter components to a minimum. In the smoke of tobacco have been found besides carbonic acid and water, nicotine, ammonia, carbonic oxid, occasionally nicotianine and traces of hydrogen cyanide, and hydrogen sulphide. Recently pyridine, trimethylamine, and butyric acid have been found in it by Thoms.² The pyridine is produced by the decomposition of nicotine, while the hydrogen cyanide, hydrogen sulphide, and butyric acid are probably derived from protein compounds.

The soil, climate, and weather on the one hand and careful treatment in the curing and sweating of the tobacco on the other have great influence in the production of a cigar leaf having the proper aroma in smoking. This flavor must be distinguished from the odor which the sweated tobacco leaves show directly without being heated or smoked. The writer proposes to designate the former alone as aroma, while the latter will be called simply odor. The use of this distinction is made in the following lines.

The odor of the sweated tobacco leaves doubtless stands in a certain relation to the aroma generated in smoking, generally a pleasant odor justifying the inference of a fine aroma; but this is by no means always so, and cases occur where a sweated tobacco of pleasant odor produces a disagreeable odor on being smoked. The aromatic substances may here escape perception because of the presence of other and undesirable compounds. The substances which impart the odor are easily soluble in water, since tobacco extracted with water and dried again has no longer

¹ Conn. Agr. Expt. Station, Annual Report, 1891, p. 185.

² Chem. Zeitg., 1899, No. 80.

its characteristic odor. "Nicotine free" cigars made of such tobacco are also devoid of any trace of aroma. Treatment with much alcohol also removes the odor more or less, and this alcoholic extract on evaporation at the ordinary temperature leaves a residue recalling the odor of the tobacco. This residue upon treatment with ether can be separated into a soluble and an insoluble part, but neither of these has any odor, showing that the substances which caused the odor are easily volatilized or chemically changed. Indeed, they are easily changeable, as can be observed when sweated tobacco is distilled with water. Five hundred and fifty grams of Florida tobacco of a superior odor were mixed with eight times its weight of water and nearly 300 c. c. were distilled off. On the surface of the slightly turbid distillate, which had a weak but rather disagreeable odor, resembling somewhat that of rancid oils, minute oil drops were seen. Not only was the distillate devoid of any specific odor, but also the contents left in the flask had lost the original odor. The odor is also easily removed by dry heat. Two hours exposure to air at a temperature of 100° C. (212° F.) suffices to deprive tobacco of most agreeable odor of this desirable quality.

Tobacco leaves, even from the best tobacco regions, are by no means distinguished by peculiar flavor in their fresh or cured state.¹ The curing exerts a beneficial effect upon the quality of the aroma only in so far as a part of the protein is destroyed by it. Cured tobacco, even from the best sources, has a mere straw odor. The fine odor and genuine aroma are generated by the sweating process. The better regulated the sweating process, the finer the aroma, other things being equal. Whenever the sweat is not proceeding well—ascribed sometimes to a high fat content, or perhaps caused by imperfect curing, or by injuries to the oxidizing enzymes in the curing barn—the product will have a poor aroma. These circumstances make it clear that the original resin in the leaves can not be the cause of the aroma, as some authors have surmised. Indeed, the resinous matter from fresh or cured leaves does not show any particular flavor when heated; neither has the ethereal oil contained in the hairs of the fresh leaves a beneficial effect on the quality of the tobacco.

It is well known that it is especially the so-called gummy leaves that sweat best and give the finest aroma. Such leaves are found among the upper and not among the lower or sand leaves, and are very rich in extractive matters. When such leaves are shrinking in the process of drying in the curing process, these soluble matters may sometimes be forced to the surface, to some extent, in the form of a concentrated solu-

¹ While the juice of fresh tobacco leaves assumes, after a short time, the odor of cucumbers, fresh leaves dried at 50° C., and afterwards moderately warmed with water, gradually develop the agreeable odor of dried malt; an interesting fact, since the germinating barley on the malting floor also at first develops the odor of cucumbers.

tion, hence such leaves feel sticky or gummy to the touch. By a thorough sweat this "gum is taken out," as the manufacturer says; in other words these extractive matters are more or less oxidized.¹ It is therefore of interest to determine the amount of soluble matter left after sweating. This amount varies considerably, from 30 to 54 per cent. The insoluble matter consists mainly of cellulose, lignin, protein, coloring matter, fat, resin, and ash. With some samples the following results were obtained:

	1.	2.	3.	4.
Soluble	37.1	33.8	37.5	44.3
Insoluble	62.9	66.2	62.5	55.7

Sample No. 2 showed saline efflorescences (so-called "saltpeter") on the veins. Sample No. 4 consisted of co-called "gummy leaves." The ash varied from 19.4 to 22.1 per cent in those samples.

It was formerly believed that the aroma was chiefly due to the so-called tobacco camphor or nicotianine—a crystalline volatile product which was obtained by distillation of sweated tobacco with water. Hermstadt tried, as early as 1821, to isolate this aromatic principle. On distilling various samples with six times the weight of water he obtained a distillate yielding a precipitate with acetate of lead, from which a crystallized compound of a strong tobacco flavor was obtained, which he called nicotianine. Later on this product was studied by Posselt and Kieman, and also by Barral. Two formulas have been calculated from Barral's results, namely, $C_{23}H_{32}N_2O_3$ and $C_{36}H_{48}N_3O_5$. Barral states that it yields nicotine on distillation with potassa. This product may possibly be formed from nicotine in the process of sweating, but it is by no means contained in every tobacco. A number of chemists have failed to observe it and the writer has tried in vain to obtain it from Florida tobacco. The aqueous distillate from the Florida tobacco contained, besides small oil drops, some ammonium carbonate, and the precipitate obtained with basic acetate of lead was nothing but lead carbonate.² Probably the nicotianine is only an occasional product in very heavy tobacco. Odor as well as aroma is evidently caused not by a single compound, but by a mixture of com-

¹The word "gum" is applied in other countries in the same sense as the word "life" is with tobacco in America, signifying a certain elasticity of the tobacco leaves. The word "gum" in this connection is derived from the erroneous idea that the elasticity is caused by a content of "gummi elasticum" or caoutchouc. Behrens has recently shown that the "life" of fermented tobacco leaves is due to a greater amount of hygroscopic moisture, caused by a higher percentage of malates of potassa and soda.

²The results obtained by the firm of Schimmel & Co., in Leipzig (recently published in *Chemiker-Zeitung*, 1899, No. 80), are in full accord with the writer's observations. No nicotianine, but considerable ammonia was present in the aqueous distillate of the sweated tobacco tested.

pounds, nicotine contributing in a certain measure to the formation of these.¹

It was observed by Jenkins, Behrens, Kissling, and others that a certain amount of nicotine (one-fourth to one-third) is destroyed by the process of sweating; hence it is natural to connect the formation of aromatic products with the disappearance of a certain portion of the nicotine. Cured tobacco rich in nicotine may, by a thorough sweat, become poor in nicotine and rich in aroma, while after an insufficient sweat it will be rich in nicotine but poor in aroma.

It is a well-known fact that a high content of nicotine in a cigar is often associated with a poor aroma. Strong cigars are not always the best. But all attempts to produce by partial oxidation of nicotine substances of an aroma similar to that of good cigars have thus far failed. By moderate oxidation, as brought on by silver oxide or hydrogen peroxide, nicotyrine and oxynicotine result, while by a more powerful oxidation nicotic acid is formed—products which have no relation to the odor and aroma of sweated tobacco. The writer has also applied platinum-black as an oxidizing agent. It was left to act for several weeks on a 4-per-cent aqueous solution of nicotine at the ordinary temperature as well as at about 50° C., but the desired effect was not observed, although the nicotine became much altered. Evidently the oxidation, caused by the oxidizing enzymes in the sweating heaps, takes a different direction.

Recent investigations of Thoms² have shown that the flavor of the tobacco smoke is chiefly due to an ethereal oil which is produced by dry distillation in the process of smoking from a product of the sweat. Twenty kilos of tobacco, smoked by an aspirator, yielded 75 grams of this oil, which was of a stupefying odor and produced headache and trembling of the limbs when inhaled in a concentrated state. It contained, among other things, 9 grams of a phenol of creosote-like odor.

The best method of avoiding the poisonous action of the tobacco smoke while enjoying its beneficial stimulating effects is found with the Turks, Arabs, and Persians. The use of a wash bottle (nafas) before the smoke reaches the mouth removes a great deal of the poisonous products. Géorgiades found that each 18 grams of tobacco (a pipe full) showed 0.947 gram nicotine, the nafas through which this quantity of tobacco was smoked showed 0.595 gram nicotine, while the washed smoke from same amount contained but 0.0225 gram.

INJURY TO THE AROMA.

The aroma may be injured by various causes, as, for example, by a high content of fat or of protein in the tobacco. Certain tobaccos which

¹ Opinions as to the formation of the aroma are much divided. Van Bemmelen (Landw. Vers. Stat. 37, 388) holds that the organic acids of the leaves have a certain influence.

² Chem.-Zeitg., 1899., No. 80.

have a fine odor at the ordinary temperature have but a poor aroma when smoked, since the aromatic substances may be disguised by the products of dry distillation of fat and protein when the cigar is smoked. A high fat content is indeed very injurious. Other compounds also, interfering seriously with the aroma, appear sometimes to be produced under certain climatic conditions. For example, the tobacco produced in Connecticut in the unusually wet season of 1897 was so inferior that the sales did not pay the outlay for fertilizers.¹ The buyers claimed that the cured tobacco had a "stink" about it and that it also showed a similar odor after sweating. Such injurious products seem not infrequently to be present. It is on this account that tobacco is subjected in certain factories to a strong current of air for twenty-four hours at the ordinary temperature, in order to take out the "rank" odor before it goes to the bench for cigar making. Indeed the air which has passed through such tobacco contains a very sharp substance which brings tears to the eyes.

The influence of climate upon the formation of certain products in the tobacco plant may also be inferred from an observation made by Schimmel. While 43 kilograms of tobacco from South America yielded on distillation with water mere traces of a volatile oil, 15 kilograms of German tobacco yielded 6 grams of a thick and dark-colored oil having an odor similar to that of camomile. This oil contained, according to Thoms, no terpens, but it contained among other things a small amount of a phenol. A further injury to the aroma is to be expected from the treatment of the tobacco in the field with paris green and other arsenious preparations to destroy caterpillars. Many tobacco growers avoid the use of such preparations altogether and do not begrudge the extra expense for the labor required in picking off the caterpillars by hand. Others apply Paris green only in the young stages of the plant—1 part well mixed with 100 parts of flour—but in certain districts, where caterpillars occasionally become very numerous, Paris green is applied on the ripening leaves in proportions of about one-fourth of a pound to 40 gallons of water. In one pound of sweated tobacco from such fields M. Peter found from a trace to 1.7 grains arsenious acid.² Such tobacco when smoked may be expected to have a disagreeable odor, since even the smallest quantity of arsenious acid produces, in contact with a glowing substance, a disagreeable odor resembling that of garlic. But more serious than the injury to the aroma is the injury to health when such poisoned tobacco is habitually smoked. An injury to the odor of tobacco may also be caused by the development of certain fungi on cigars kept in a too moist atmosphere. Even a putrid odor may thus be produced, as Splendore has observed in one case.³

¹ On the other hand too dry seasons are just as injurious, since the tobacco produced may refuse to sweat.

² This would correspond to 0.025 per cent arsenious acid or about 0.05 per cent paris green.

³ *Il tobacco*, p. 11, 1899.

Numerous experiments have been made to improve the aroma of poor tobacco. Oxidizing media have been applied to destroy supposed injurious by-products. The writer tested the action of a 1 per cent solution of potassium permanganate for five minutes, but the results were not encouraging, on account of the injury to certain other properties.

Other methods consist in the application of substances of powerful aroma. For example, we find among the ingredients for the preparation of petuning liquids anise seed, cloves, cinnamon, coffee, and tincture of valerian.¹ In Cuba tonka beans and vanilla beans are also used to improve the aroma, but with very rank tobaccos even such ingredients prove insufficient. The important question of how to improve poor products deserves careful attention and further detailed study.

NITRITE CONTAINED IN SWEATED TOBACCO.

Although the tobacco of commerce has been the object of frequent and numerous analyses, one compound occasionally occurring in it has thus far been entirely overlooked. It is the nitrous acid present in the form of nitrites. While nitrates frequently occur in various living plants and also in fresh tobacco leaves, nitrites do not. Indeed, these salts would act very poisonously on the living cells if they accumulated to even a small degree. The writer has observed that the poisonous character of free nitrous acid is so great that it kills algæ after one day, even at a dilution of 1 part to 100,000 parts, although its acid character is so weak that it is easily liberated from the nitrites by dilute organic acids, such, for example, as are contained in the cell sap of most plants; hence the salts of nitrous acid are also very strong poisons wherever an acid-cell sap in the plants can liberate nitrous acid from them.

When tobacco leaves rich in nitrates, however, are subjected to the sweating process after being cured a portion of the nitrates is reduced, whereby, as an intermediate step, nitrites are formed, ammonia representing the final product. Some sweated tobaccos are so rich in nitrite that a concentrated extract, even when prepared in the cold, gives a decided odor of nitrous acid upon the addition of sulphuric acid, and even the smallest quantity of the extract produces at once separation of iodine from potassium iodide on addition of a little sulphuric acid. When upon dilution the brown coloration of the tobacco extract is sufficiently diminished, the well-known test of Gries for nitrites is also easily obtained. The tobacco extract containing nitrites gives a blue reaction with tincture of guaiac, but this reaction of nitrous acid is also obtained after boiling the extract for a minute, while the similar blue reaction caused by oxidase is made impossible after heating the extract a short time to 67° C. (153° F.), or, if the extract is weakly alkaline,

¹ Report No. 62 (p. 17), U. S. Department of Agriculture. Marcus L. Floyd.

to 75° C. (167° F.). By this control test erroneous conclusions can be avoided.

An accumulation of nitrites in the sweating tobacco might in the course of some weeks attack the oxidizing enzymes at the temperature of the fermenting heaps, thus terminating this process sooner than desirable. Hence nitrates should not be applied in too large quantities in the manuring of tobacco fields, since they may give rise to the formation of nitrites in the sweating process. Bacterial action need not be assumed in this process.

AMOUNT OF HEAT PRODUCED BY SWEATING IN BULK.

Since the loss of organic substance from the cured leaves in the sweating in bulk amounts to about 5 per cent dry matter, and the cured leaves consist of nearly 40 per cent of carbon and 6 per cent of hydrogen, an approximate calculation can be made as to the amount of heat generated by the oxidization going on in the heap. For an accurate calculation it would be necessary to know the exact amount of carbonic acid and water formed by the oxidation, and further, how much of the carbonic acid was generated from previously present carboxyl groups, and how much water was generated from previously present hydroxyl groups; moreover, it should be known how much total ammonia is produced in the sweat. But, on the assumption that only one-half of the carbonic acid and one-half of the water produced are due to perfect combustion, and that the carbon and the hydrogen in these products show the same proportion as in the cured tobacco, we will obtain numbers which are certainly rather an underestimation than otherwise. The fermenting piles are made up of from 2,000 to 7,000 kilograms of tobacco. Let us assume a pile with 2,000 kilograms of dry matter. The 5 per cent loss after the sweat would then amount to 100 kilograms. According to our assumption this would involve a complete combustion of 20 kilograms of carbon and 3 kilograms of hydrogen. Since one gram of carbon produces 7,678 units of heat and one gram of hydrogen 33,808 units of heat, it follows that 20 kilograms of carbon would yield 153,560 major calories and 3 kilograms of hydrogen 101,400 major calories. This heat would suffice to heat up 254,960 liters of water for 1° C.

CONCLUSION.

Various problems relating to the manufacture of tobacco have been touched upon in this report, some of them within easy reach of solution, others of a very difficult nature. The prevention of fungous attacks in the barn or in the cases, the regulation of the temperature and humidity in the curing process, and the proper control of the sweat are points that can easily be settled. In many cases the replacement of the stalk-curing by the single-leaf curing process may prove a financial success. But there are other problems of a more

delicate and difficult nature, as the prevention of the mosaic or calico disease and the proper composition of the tobacco leaf while ripening. Upon this composition depends the development of a desirable aroma in the sweating process. Climate and weather are here such potent factors that human art can accomplish directly but little. Too cool and rainy weather may favor, for example, the production of fatty matter, which certainly exerts an unfavorable effect upon the aroma in smoking. There may be produced, however, still other products which are unfavorable to the aroma. Too dry weather may also interfere with the proper composition of the ripening tobacco leaves. By crossing and selection, however, varieties of tobacco may possibly be produced that even under unfavorable climatic conditions will not form much of the compounds which injure the aroma. In regard to the selection of the seed, it may be mentioned that even now some farmers go so far as to import their seed directly from Cuba each year.

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