MOISTURE CONTENT OF SHREDDED FLUE-CURED TOBACCO AS AFFECTED BY RELATIVE HUMIDITY, TEMPERATURE AND TIME[1]

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Flue-cured tobacco was stored at 20 and 30 C and approximately 75, 80, 85, 87, and 95% relative humidity. Samples from these storage treatments, taken weekly for 4 weeks, were dried by freeze-drying and by oven-drying at 40 C/7 days, 100 C/16 hours, and 130 C/3 hours. Relative humidity of storage, length of storage, and drying method had significant effects upon weight losses during drying. As relative humidity, storage time, or drying temperature increased, weight loss increased. No significant conditions are suitable for controlling tobacco moisture effects were found for storage temperature. The 10 storage content in the range flue-cured tobacco is usually marketed. Of the methods tested oven drying at 100 C/16 hours was selected for future tobacco moisture content determinations. Equilibrium moisture contents were not reached after four weeks' storage under the 10 conditions tested.

INTRODUCTION

Moisture content is important in the handling, storage, marketing, manufacture, and preservation of flue-cured tobacco. The amount of moisture considered "proper" by the farmer during marketing differs considerably from that considered "proper" by the buyer for storage and manufacture. Most tobacco handlers have arrived empirically at safe moisture contents and can estimate whether or not a given pile of tobacco contains enough moisture to permit rotting by fungi. At present, tobacco grades do not reflect moisture content unless the U. S. Government grader believes the tobacco is too moist for safe storage for a few days. In this situation the tobacco is graded "W" or "No-G."[2] Otherwise, it is advantageous to the farmer to market tobacco as moist as possible without risking apparent deterioration. Moisture content directly affects gross return to the grower but, above safe levels, also increases the danger of deterioration. For this reason, tobacco is redried immediately after purchase to about 11% moisture (wet-weight basis); tobacco at or below 12% moisture is safe from deterioration by fungi for prolonged periods.

After farm curing and until purchased by the consumer as a finished product, tobacco undergoes several changes in moisture content. In 1967, a survey of 100 samples of tobacco from 12 tobacco markets in two tobacco belts showed moisture content ranged from 12.6 to 30.2% (8), and although not included in that report, the mean was 19.6%. For aging, tobacco is dried to 10-11% and stored for one to three years. During the manufacture of blended cigarettes in the United States, tobacco is remoistened to 16-20% for cutting and redried to 12-13% for machine manufacture into cigarettes. Most American cigarettes are marketed with 12-13% moisture.

Despite the importance of moisture content, we found neither a published method of moisture determination accepted as standard by the companies handling flue-cured tobacco, nor any published data comparing moisture contents determined by different methods. Several investigators (2, 6, 7, 10) discuss removing tobacco moisture, but only published references to the British method (heating at 100 C/16 hrs.) can be found (5).

Moisture contents for tobacco stored in environments at various relative humidities (RH) have been determined for burley (6, 10) and flue-cured (2), but the long-term rate of moisture absorption and whether or not an equilibrium moisture content was reached were not reported. Jeffreys (6) and Young *et al* (10) reported difficulty in determining equilibrium moisture content of tobacco above 77 to 80% RH due to mold growth, and Young, *et al* (10) indicated a need for information on equilibrium moisture contents above this range.

The influence of moisture content on the growth of fungi responsible for deterioration of stored cereal grains (1), forest seeds (G. A. Fakir, Ph.D. Thesis, North Carolina State University, 1969) and textiles (3) is well established. However, before this relationship can be investigated for tobacco, a method for determining moisture content must be selected.

The objectives of this study were to determine storage conditions that will provide a range of tobacco moisture contents in order to study the effects of moisture content on fungi growing in tobacco and to compare four procedures for determining moisture content.

MATERIALS AND METHODS

Tobacco source. Field-grown tobacco with relatively little brown-spot disease (caused by *Alternaria tenuis* Nees), was harvested and flue-cured in the usual manner (2) at the Border Belt Tobacco Research Station, Whiteville, N.C. Cured leaves were selected at random from all stalk positions, compressed, and shredded into one millimeter wide ribbons of varying lengths with a Himoff Tobacco Cutting Machine, Model B-35. Ribbons were stored at 15.8 to 17.4% moisture at room temperature (23-28 C) until used for the experiments.

Relative humidities. RH were maintained with saturated salt solutions, with an excess of salt, in closed 9.5-liter desiccators. Saturated solutions of the fol-

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[2] *Rule 23 and 24, Official standard grades for flue-cured tobacco. 1965. U. S. Department of Agriculture Consumer and Marketing Service, Tobacco Division, Washington, D. C*

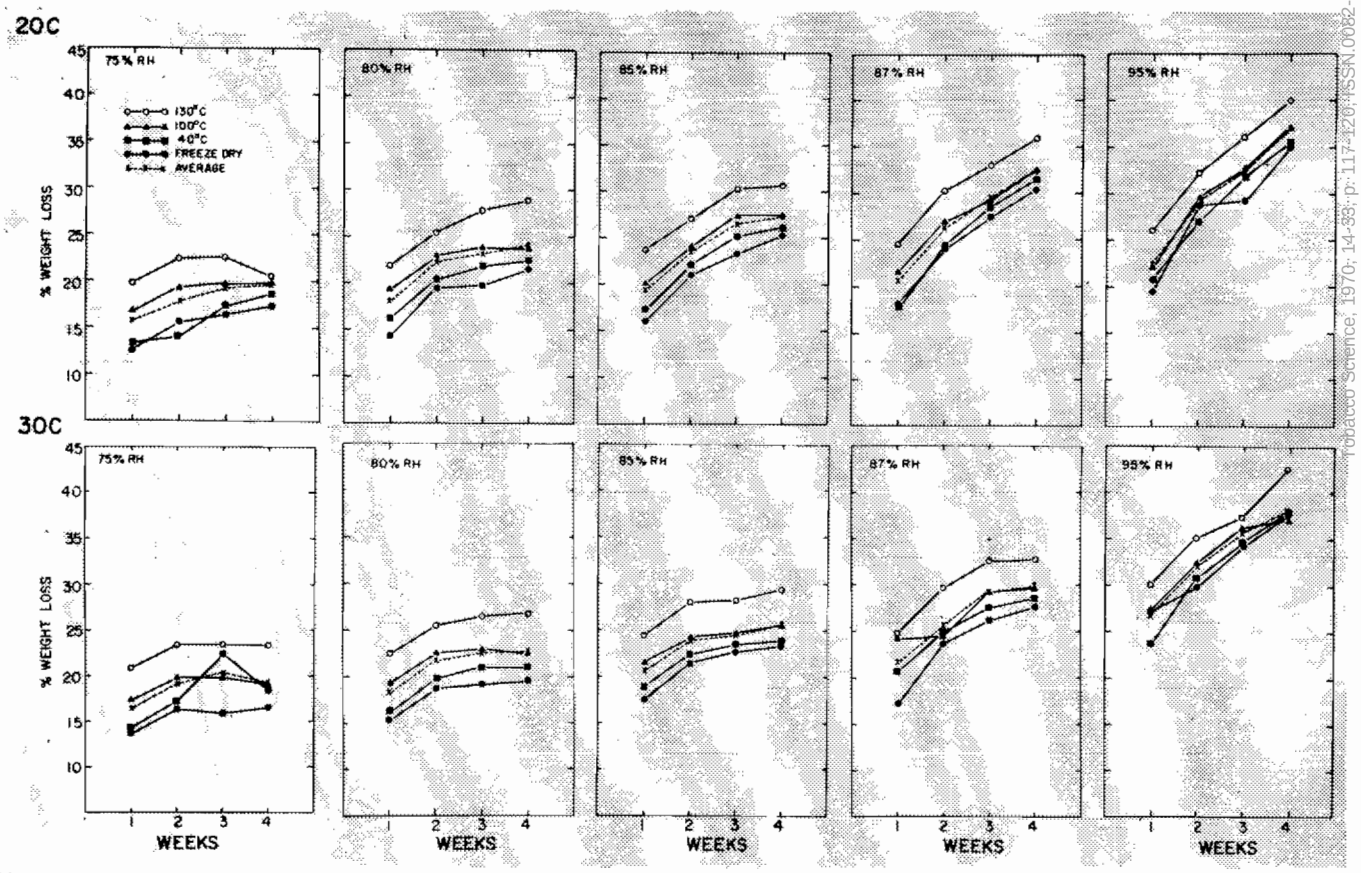

Figure 1—Percent weight loss (wet-weight basis) of flue-cured tobacco stored at five relative humidities and two temperatures determined by four methods at four weekly intervals. Each observation is the average of three replications.

lowing salts maintain the indicated relative humidities at 20 and 30 C, respectively: i) sodium chloride, 76.0 and 75.5% RH; ii) ammonium sulfate, 80.5 and 80.0% RH; iii) potassium chloride, 85.0 and 84.5% RH; iv) potassium sodium tartrate 87.0 and 87.0% RH; and v) lead nitrate, 97.0 and 95.0% RH (9).

Saturated salt solutions were used for humidity control in preference to either sulfuric acid or glycerol solutions because moisture exchanged between solutions and the tobacco during equilibration would change the concentration of these two regulating solutions. A saturated salt solution with an excess of salt does not change its concentration and therefore the equivalent RH above the solution remains constant.

Temperatures. The desiccators containing the five saturated salt solutions were placed in each of two LAB-LINE Controlled Environmental Rooms for temperature control at 20 and 30 C, ± 1 C.

Storage interval. MC was determined weekly for four weeks by each of four methods, for each of the five RHs, and two temperatures.

Drying methods. Subsamples of tobacco were dried by i) freeze-drying (VirTis 10-146 MR-BA Freeze-Mobile, equipped with a VirTis 10-MR-SA Tray Drying Chamber; freezing platen temp −45 C, overnight drying to platen temp 18 C, at 0.2-0.5 mm of Hg), ii) heating in an embedding oven (Lab-Line Instruments) at 40 C for seven days, iii) heating in a ventilated oven at 100 C for 16 hrs (5), and iv) heating in a forced-air oven (Freas, model 625, with air control at five) at 130 C for three hrs. Since weight loss during drying is not necessarily due entirely to loss of moisture, MC is used in this study to express percent weight loss and is calculated on a wet-weight basis.

Test unit. Ten-gram samples of tobacco were placed in tared stainless steel cans six centimeter in dia, 3.4 cm deep, and fitted with lids perforated with twelve four millimeter dia. holes.

Sixteen cans were placed in each desiccator, 12 in one layer and the remaining four on the rims of cans in the first layer, but not directly over another can. During equilibration, the lids were removed and placed under the cans. For MC determinations, the perforated lids covered the cans heated at 100 C/16 hrs, but remained under the cans in the other three methods. Each week, a can from each desiccator was selected at random for drying by each of the four methods. Following removal of the first four cans from each desiccator, the remaining cans were arranged in one layer.

Microorganisms. To prevent microorganisms from developing in the stored tobacco at high RH, two ml of propylene oxide was added for each liter of volume in each desiccator at the start of the tests. After the final weight loss was determined (4th week), 10 subsamples (five RH X two temperatures) for the first replication of the 40 C/7-days method were analyzed for micro-organisms by methods previously reported (8), except 10 g of tobacco were used instead of five g. If micro-organisms were present, heating at 40 C should not kill spores or the vegetative stage. The suspension less dilute than 1:1000 was not cultured, and if a colony was not found in the dilution of 1:000, the results are expressed as $<$1,000. The culture medium (25 g Difco tomato juice agar medium, 10 g Difco agar, distilled water to make one liter) used for the plate counts was found in preliminary studies to allow the growth of yeasts, bacteria, and filamentious fungi.

Experimental design. The experiment was set up in a split-split plot with the whole plot arranged as a randomized complete block design, replicated three times, and the data were analyzed statistically by an analysis of variance. Significant and highly significant differences, as expressed in the text, are at the 0.05 and 0.01 confidence levels, respectively.

Table 1. Percent weight loss, determined by four methods, of tobacco stored at five relative humidities. Each value is an average for three replications at two storage temperatures and four weekly intervals

Method	Relative humidities 75	80	85	87	95	Average
Freeze dry	15.55	18.64	21.36	24.41	30.26	22.04
40C/ 7 days	16.75	19.87	22.42	25.55	30.28	22.97
100 C/ 16 hrs	18.91	22.31	24.40	27.35	31.86	24.93
130 C/ 3 hrs	22.00	25.65	27.76	30.48	34.92	28.16
Average	18.30	21.62	23.99	26.95	31.83	
Relative humidity averages					LSD 0.05	0.96
					0.01	1.32
Method averages					0.05	0.46
					0.01	0.60
CV					7%	

Table 2. Percent weight loss of tobacco determined by four methods at four weekly intervals. Each value is an average for three replications at five relative humidities and two storage temperatures

	Weeks stored 1	2	3	4
Freeze dry	17.15	21.94	23.48	25.61
40 C/ 7 days	17.93	22.38	25.35	26.24
100 C/ 16 hrs	20.98	24.65	26.61	27.49
130 C/ 3 hrs	23.89	27.85	29.72	31.19
Average	19.98	21.20	26.29	27.63
Storage time averages			LSD 0.05	0.46
			0.01	0.60

Table 3. Percent weight loss of tobacco stored at five relative humidities and two storage temperatures. Each value is an average for three replications by four methods at four sample intervals

Temperature	Relative humidities 75	80	85	87	95
20	17.83	21.80	24.18	27.27	30.28
30	18.78	21.35	23.79	26.62	33.38
Average	18.30	21.57	23.99	26.94	31.83

Table 4. Percent weight loss of tobacco stored at two temperatures and determined by four methods. Each value is an average for three replications at five relative humidities at four sample intervals

Temperature	Freeze dry	40 C/ 7 days	100 C/ 16 hrs	100 C/ 3 hrs
20	21.77	22.57	24.83	27.91
30	22.31	23.38	25.04	28.41
Average	22.04	22.98	24.94	28.16

Table 5. Bacterial colonies/gram of tobacco stored four weeks at five relative humidities and two temperatures. The colony count is based on averages of two plates

Temperature	Relative humidity 75	80	85	87	95
20	52,000	14,500	3,000	1,000	<1,000
30	<1,000	<1,000	1,000	2,000	1,000

Table 6. Percent weight loss (dry-weight basis) of tobacco stored at five relative humidities as determined by six methods

Relative humidity	Method Freeze[a] dry	40 C/[a] 7 days	100 C/[a] 16 hrs	130 C/[a] 7 days	Dixon[b] et al	Jeffrey[b]
75	15	20	22	27	23	22
80	23	25	27	35	28	28
85	27	29	32	39	33	35
87	33	34	39	43	36	47
95	43	43	46	54	(50)[c]	62

[a] *The first four columns are data from Table 1 converted to dry-weight basis.*
[b] *Dixon, et al and Jeffrey's data were taken from their plotted curve.*
[c] *An estimated value based on extending their plotted curve to 95% RH.*

RESULTS

Figure 1 shows weight losses (MC) determined by four methods for tobacco stored at two temperatures and five RH. Each observation is the average of three replications. Two results were expected: i) tobacco at high RH had high MC, and ii) as drying temperatures increased, MC increased. MC approached equilibrium only at 75 and 80% RH. At high RH, tobacco did not reach equilibrium MC after four weeks' storage, with the effect more evident at 20 C than at 30 C. The average MC, determined by the four methods, is very close to the MC determined by heating at 100 C/16 hrs.

Analysis of variance showed a highly significant effect on MC for the RH of storage (**Table 1**), the method of MC determination (**Table 1**), and the length of storage (**Table 2**). **Table 3** shows the average MC of the tobacco stored at five RH for the two temperatures. **Table 4** shows the average MC of the stored tobacco by the four methods. No significant differences were found for temperature and replication, hence LSD's were not included in **Tables 3 & 4.**

Bacteria were the only viable microorganisms isolated from the tobacco incubated four weeks at the five RH and two temperatures (**Table 5**). The organism was tentatively classified as a species of *Bacillus*. At 20 C, bacterial populations decreased with an increase in RH, but at 30 C, bacterial populations were less than 1,000 colonies/gram for tobacco stored at 75% RH and remained near 1,000 colonies/gram at the higher RH. Viable yeasts and filamentous fungi did not grow in cultures made from the suspension more dilute than 1:1,000.

DISCUSSION

In general, the five RH conditioned tobacco to MC (averaged by four methods ranged from 18.3 to 31.8%, (**Table 1**) near the range that flue-cured tobacco encounters in usual handling and processing during marketing (12.6 to 30.2%). The RH reported here are suitable for studying tobacco at the high end of this MC range. As expected, tobacco stored in higher RH absorbed more moisture than tobacco in lower RH and hence had a higher MC. The 20 and 30 C storage temperatures had no significant effect on the MC maintained by the saturated salt solutions (**Table 3**). Although the temperatures used have a slight effect on the RH maintained by saturated salt solutions and equilibrium moisture contents of hygroscopic materials, these differences were not detected by this experiment. The cause of the higher MC at 95% RH at 30 C than at 20 C is not clear from these data.

The method used to remove moisture from tobacco had a definite influence on MC (**Table 1**). MC increased as higher drying temperatures of shorter duration were used. Either the high drying temperatures volatilized substances other than water and their loss accounts for the additional weight loss or moisture was not removed completely by the low drying temperatures.

Equilibrium MC was not reached during four weeks of storage; highly significant differences in MC oc-

cured after each of the four test periods **(Table 2).**

Fungi are a problem when tobacco is stored above 80% RH (6, 10). Propylene oxide prevented growth of fungi at the RH and temperatures used in this study. At 20 C, the action of this compound as a bactericide was enhanced at high RH, confirming the work of Himelfarb, *et al* (4). These data (**Table 5**) also suggest that propylene oxide may be more effective at 30 C than at 20 C and that higher temperature may negate the RH effect.

For future determinations of MC of tobacco we will use 100 C/16 hrs. Moisture determined by this method is the closest to the average of the four methods at the RH, temperatures, and intervals tested. Although using different drying temperatures would probably give a different average, the range tested here generally represents the range of temperatures used to dry seeds (11) and hygroscopic materials. In addition, when the wet-weight MC determined by 100 C/16 hours are converted to dry-weight MC, they are more comparable to those of Jeffrey (6) and Dixon, *et al* (2), for all but the samples stored at 87 and 95% RH (**Table 6**), than the MC determined by the other methods.

The MC for the high RH, particularly 95% RH, are slightly lower than those found by Jeffrey (6) and Dixon, *et al* (2). Different drying temperatures or different storage periods, not specified in these reports could account for this lack of agreement. Another possible explanation for this is that metabolic water may have accumulated from microbial respiration. Propylene oxide prevented mold growth in the present study, whereas molds were apparently not controlled in either of the other studies. In current experiments, tobacco inoculated with *Aspergillus repens* De Bary and stored under similar conditions resulted in higher MC than reported here for fungus-free tobacco.

Effects of MC on the rate of growth of storage fungi in flue-cured tobacco under various environmental conditions are being investigated.

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