MICROFLORA OF FLUE-CURED TOBACCO BEFORE AND AFTER REDRYING[1]

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Mold- and nonmold-damaged flue-cured tobacco laminae and veins from auction warehouses were evaluated for microflora before, during, and after redrying. The amounts of the three predominant fungi, **Aspergillus repens, A. ruber,** and **A. niger,** in nonmold-damaged tobacco, measured as colonies per gram, ranged from 0 to 14,800. **A. repens** propagules in mold-damaged tobacco were as high as 3.2 million colonies per gram. Tobacco from areas adjacent to mold damage, but not visibly mold damaged, contained 64,600, 15,900, and 26,200 colonies per gram of **A. repens, A. ruber,** and **A. niger,** respectively. Fungi in mold- and nonmold-damaged tobacco were not eliminated by redrying and were similar in numbers and kinds to those found in other studies with flue-cured tobacco.

INTRODUCTION

Prior to 1965, fungi and bacteria associated with tobacco were studied mainly as organisms responsible for post-harvest rots, producers of off-flavors, and promoters of fermentation of certain types of cigar-leaf tobacco (7, 18). Since then, more detailed reports have been made on fungi (4, 5, 9, 12, 14, 15, 17), bacteria (10), and actinomycetes (8) associated with flue-cured tobacco during the various stages of plant growth, marketing, storage, in cigarettes and in cigarette smoke.

All flue-cured tobacco (*Nicotiana tobacum* L.) purchased on the auction markets is redried prior to long term storage. Intact leaves or threshed-leaf components are gradually heated in redrying machines to about 121 C (250 F) and conditioned to a final moisture content (MC) of 10-15% (wet basis) to reduce breakage during handling and packaging, and to prevent spoilage by fungi. Companies storing and shipping large quantities of redried tobacco, need to know if the stored product contains viable fungal propagules. If fungi survive redrying, the potential for mold damage remains.

This study was done to determine the numbers and kinds of microorganisms associated with tobacco at various times and locations during the redrying process and to determine if redried tobacco contained microflora.

MATERIALS AND METHODS

Tobacco sources. Tobacco used in the first study originated from auction warehouses in central and west central North Carolina and was received at a redrying plant in Wilson, N. C. in November, 1972. In the redrying process, tobacco is successively passed through two ordering cylinders where steam and water sprays, combined with tumbling action, opens, warms, and softens the leaf prior to threshing. Recirculating air in the cylinders is maintained at 40-60C (120-140 F) and 100% relative humidity and the tobacco remains in each cylinder about 3 minutes. Laminae and veins are threshed and separated by toothed rotors passing against stationary teeth, bars and sieves. Veins with laminae attached are further threshed until laminae and veins are free from each other. After threshing and separation by moving air, laminae are fed into horizontal silos for temporary storage prior to redrying; veins travel directly to a redryer for drying and packing. Veins pass through the drier in a 10-cm layer (carpet) and are dried, cooled and remoistened to about 8% MC. Vein redrying lasts about 15 minutes with a maximum air temperature of 121 C (250°F). Laminae are similarly processed but the process lasts 6 minutes and final MC is 10-12%. In both redrying lines, air temperatures are controlled automatically. After redrying, veins and laminae are packed in fiberboard boxes with polyethylene liners.

At various locations in the process, air used for separating laminae and veins, or conveying the product, is filtered through fabric sleeves to remove fine tobacco particles called offals.

Samples of 50-100 g of tobacco laminae or veins were removed from 10 locations in the processing line once a week for 4 weeks. Samples were enclosed in sterile plastic bags, and brought to the laboratory in an ice chest. Duplicate samples were taken at four intervals at each of the 10 locations, except for the packaging room where samples were taken only on the last two intervals.

Tobacco samples were taken: 1) as tobacco entered the redrying line; 2) after the first addition of water vapor (first ordering cylinder); 3) after the second ordering cylinder; 4)

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[1]*Cooperative investigations of the United States Department of Agriculture, The North Carolina State University Agricultural Experimentation Station, and Imperial Tobacco Ltd. Paper No* 1863 *of The Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, N.C. The use of trade names in the publication does not imply endorsement by the U.S. Department of Agriculture or the North Carolina Agricultural Experiment Station of the products names, nor criticism of similar ones not mentioned.*

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at the exit of the final threshing line; 5) at the exit from the blending silo just before entering the redryer; 6) from within the redryer after final heating (cooling room); 7) at the delivery end of the redryer following addition of the desired amount of moisture; 8) from the final package prepared for storage or shipment; 9) from cloth sleeves used to filter air used for the separation of threshed leaf; and 10) from cloth sleeves used to filter air from the drying room.

A total of 76 samples of laminae were collected and evaluated (9 locations x 2 samples x 4 dates + 1 location x 2 samples x 2 dates). Vein samples were taken from the redrier entry, the cooling room, and the delivery end of the redryer; a total of 24 samples of veins were analyzed (3 locations x 2 samples x 4 dates).

Another source evaluated was tobacco, compressed in 50 ft^3 hogsheads weighing approximately 430 kg (950 lb.), that had been mold damaged in temporary storage prior to redrying. Leaf samples were taken from 1) the mold-damaged area, 2) adjacent to the mold-damaged area where no mold was evident, and 3) 50-100 cm from the mold-damaged area. Samples of 100-150 g of laminae or veins from each area were sewn into 20 x 20 x 8 cm nylon mesh pillows and placed within the 10 cm thick carpet of tobacco laminae feeding through the redryer. Pillows were removed from the carpet before entering the redryer, in the cooling room, and at the delivery end of the redryer. The study was done 3 times for laminae and once for veins. A total of 27 samples of laminae (3 areas x 3 locations x 3 studies) and 9 samples of vein (3 areas x 3 locations) were analyzed for microflora. The results for the laminae are the mean of the 3 studies.

Microfloral analysis. In the laboratory, 10-g subsamples from both sources were analyzed for the numbers and kinds of viable fungal or bacterial propagules present on or in the tobacco by a method similar to that described by Christensen (2). This method is accepted as standard by the American Association of Cereal Chemists (1) for determining the number of kinds of fungi in flour. The method is based on serial dilutions (10^{-3} to 10^{-7}) of an original suspension containing 10 g of blended tobacco in 500 ml of a sterile solution of 0.15% agar. One ml of each dilution was placed in each of four sterile petri dishes and Czapek's (Difco) plus 6% NaCl agar (Cz+6) or weak tomato juice (Difco) agar (WTJ) that had been melted and cooled to 52°C were added to each of two dishes. The dishes were swirled to distribute the suspension, the agar allowed to harden, and the dishes incubated at room temperature (24-26°C). Colonies that developed were counted after 2, 4, and 6 days incubation. After final counts, on the sixth day, the dishes were incubated until the fungal colonies could be identified, usually after 10-30 days. Colony counts were determined by multiplying the dilution factor by the average number of colonies growing in the two petri dishes on the sixth day. Data used for the tables were based on the counts from paired dilution cultures that averaged 20-100 colonies per dish. Colony counts tabulated were based on the number of samples containing the microorganisms and not on the number of samples taken (see footnote a and b, **Table 1**). Fungal counts were made in Cz+6; bacterial counts were made in WTJ. Colonies of *Aspergillus* were identified as to species according to Raper and Fennell (11). Other fungi were not identified to species. Specific bacteria were not identified, but the number of colonies that developed were recorded.

Moisture content. Subsamples of tobacco from each source were heated in a ventilated oven at 100°C for 16 hr (6) and moisture content is expressed on a wet-weight basis.

RESULTS

Fungi isolated from both sources of flue-cured tobacco before, during, and after redrying were predominately species of *Aspergillus, Penicillium,* and *Rhizopus.* The more frequently occurring species were *A. repens* de Bary, *A. ruber* (K. S. & B.) Thom and Church, and *A. niger,* Van Tiegham (**Tables 1-4**).

The occurrence of different species of fungi in tobacco laminae and veins were not similar for all 10 locations (**Tables 1 & 2**). For Example, *A. repens* was isolated from 37 of the 60 samples of laminae from 8 plant locations, whereas *A. tamarii* Kita was found in only 2 of these samples. The number of propagules (presumably spores) of a fungal species also varied at different locations. The propagules of *A. repens* in the laminae were higher at the silo exit than at the input to the processing line or at the delivery end of the redryer. Propagule numbers of *A. niger* were largest at the threshing line and smallest at the exit for ordering cylinder 2. Propagules of other storage fungi were about equal at the other locations.

Since fungal propagules at the different locations were determined for samples of tobacco received from different farms and not based on the same tobacco from one farm source sampled at each location, these differences may be due to differences prior to redrying. In such an instance, these differences in fungal propagules may not be entirely due to handling practices during redrying.

Table 1. Number and kinds of microorganisms in nonmoldy flue-cured tobacco laminae before and after redrying.

Plant location	Moisture content	Aspergillus repens	ruber	niger	flavus	ochraceus	tamarii	Penicillium spp.	Rhizopus sp.	Unknown bacteria
Input to line	17.8	1,200[a](5)[b]	500(1)	2,100(5)	500(1)	500(1)	0	1,100(2)	500(2)	340,000(8)
Cylinder ordering exit 1	19.2	2,700(4)	500(1)	1,700(6)	0	500(3)	0	0	1,900(4)	220,000(8)
Cylinder ordering exit 2	20.6	2,000(2)	500(1)	1,200(2)	500(1)	0	500(1)	500(1)	6,400(2)	98,000(8)
Threshing line exit	20.5	1,400(5)	2,900(2)	6,900(3)	500(2)	0	0	0	4,500(1)	00,000(8)
Silo exit	18.4	7,000(8)	1,900(6)	5,900(8)	500(1)	1,300(3)	0	2,200(3)	1,000(1)	140,000(8)
Redryer cooling room	7.5	2,330(8)	900(6)	2,600(8)	500(1)	0	1,000(1)	0	800(3)	160,000(8)
Redryer delivery end	11.4	1,160(3)	800(6)	1,700(7)	0	0	0	0	1,000(2)	130,000(8)
Packaging for shipment	—	700(2)[c]	1,000(1)	1,500(4)	0	0	0	0	500(1)	460,000(4)

[a] *Microbial counts are based on a dilution series of 10 g of tobacco per sample, expressed as colonies/g, and were determined by computing the geometric mean for the samples yielding the microorganism based on 4 runs, 2 samples per run (maximum number is 8). Exception, see footnote c.*
[b] *Numbers in parentheses indicate number of samples yielding the microorganisms.*
[c] *Microbial counts based on a dilution series of 10 g of tobacco per sample, expressed as colonies/g, geometric mean from 2 runs, 2 samples per run (maximum number is 4).*

Table 2. Number and kinds of microorganisms in nonmoldy tobacco veins and offals.

Plant location	Moisture content	Aspergillus repens	ruber	niger	flavus	ochraceus	tamarii	Penicillium spp.	Rhizopus sp.	Unknown bacteria
Veins										
Redryer feed end	18.3	3,600[a](7)[b]	1,500(4)	1,800(6)	500(1)	0	0	0	1,500(1)	110,000(8)
Redryer cooling room	6.8	0	0	0	0	0	0	0	0	27,000(8)
Redryer delivery end	7.5	0	0	0	0	0	0	0	0	99,000(8)
Offals										
Threshing room	3.0	7,400(8)	2,000(5)	11,000(8)	2,600(5)	2,700(8)	1,000(2)	4,000(8)	1,000(1)	5,500,000(8)
Drying room	5.0	0	0	0	0	0	0	0	700(3)	180,000(8)

[a] *See footnote a, Table 1.*
[b] *See footnote b, Table 1.*

Generally, however, fungal propagules recovered from the laminae were larger after threshing and preparation for redrying. After redrying, fungal propagules were similar to those in tobacco entering the redrying plant.

Bacterial counts in laminae and veins varied with the location; generally, bacterial counts were lower in samples taken after redrying.

Fungal and bacterial counts in tobacco offals (**Table 2**) collected from threshing room air were considerably larger than from redrying room air.

In the second source of tobacco, the predominant fungi associated with nonmold-damaged, adjacent to mold-damaged, and mold-damaged tobacco laminae and veins, before and after redrying, were *A. repens*, *A. ruber*, and *A. niger* (**Table 3** and **4**). The propagules of these fungi were decidedly reduced by redrying, the greatest reductions being for *A. repens*. Redrying did not eliminate viable fungal propagules from laminae or veins from tobacco mold-damaged and adjacent to the mold damage. In an apparently mold-free area, selected by visual examination and adjacent to mold-damaged tobacco, propagules of fungi ranged from 100 to 64,600 colonies per gram.

The moisture content of the laminae increased during threshing and was reduced by redrying for packaging. These moisture content changes follow the general pattern of a nominal description of the moisture content changes during redrying.

Table 3. Number and kinds of microorganisms and moisture content in tobacco laminae before, during, and after redrying.

Tobacco samples	Redryer	Moisture content	Aspergillus repens	Aspergillus ruber	Aspergillus niger	Aspergillus ochraceus	Aspergillus tamarii	Penicillium sp.	Unknown bacteria
		%							
Nonmold damaged	Feed end	18.5	0	0	100[a]	0	0	0	1,137,500
	Cooling room	10.0	0	0	0	0	0	0	1,209,600
	Delivery end	7.4	0	0	0	0	0	0	179,400
Adjacent to mold damaged	Feed end	19.9	64,600	15,900	26,200	1,600	100	200	28,000
	Cooling room	10.4	29,700	6,400	14,600	0	200	0	39,500
	Delivery end	12.4	0	1,200	5,000	0	0	0	8,500
Mold damaged	Feed end	19.7	3,202,300	1,700	0	0	0	0	667,200
	Cooling room	8.8	111,300	18,200	0	0	0	0	28,800
	Delivery end	12.0	36,300	15,900	0	0	0	0	43,100

[a] *Microbial counts are based on a dilution series of 10 g of tobacco per sample, randomly taken from a 25-30 g pillow, expressed as colonies/g and determined by computing the geometric mean from 3 replications.*

Table 4. Number and kinds of microorganisms in tobacco veins before, during and after redrying.

Tobacco samples	Redryer	Moisture content %	Aspergillus repens	Aspergillus ruber	Aspergillus niger	Unknown bacteria
Nonmold damaged	Feed end	18.7	0	0	0	1,000,000[a]
	Cooling room	7.0	0	0	0	12,500
	Delivery end	6.9	0	0	0	30,000
Adjacent to mold damage	Feed end	19.7	45,000	13,000	5,500	17,000
	Cooling room	7.4	0	0	0	5,000
	Delivery end	8.3	0	0	0	7,500
Mold damaged	Feed end	23.0	120,000	1,000	0	6,000
	Cooling room	6.3	1,000	0	0	750,000
	Delivery end	6.0	1,000	3,000	0	4,500

[a] *Microbial counts based on a dilution series of 10 g of tobacco per sample, randomly taken from a 25-30 g pillow expressed as colonies/g and determined by computing the geometric mean from 3 replications.*

DISCUSSION

Microbial analysis and the efficacy of the redrying process in microbial decontamination of tobacco are based on the measure of the number of colonies of microorganisms per gram of tobacco. This procedure has been used previously (15, 16) to measure the numbers and kinds of fungi associated with tobacco. Fungi associated with moldy, nonmoldy, prized and redried flue-cured tobacco are similar in kind and number to the fungi isolated from marketed, stored, and damaged flue-cured tobacco previously reported (13, 14, 15) and also similar to the fungi associated with storage and deterioration of other agricultural products (3). The relationship of these fungi to tobacco was not unusual. Christensen and Kaufmann (3) demonstrated the relationship of substrate MC to fungal colonization in stored grains.

Since redrying did not eliminate storage fungi from nonmold-damaged tobacco leaf or from mold-damaged laminae and veins, keeping processed tobacco dry continues to be an important way to prevent mold damage in storage.

In the area adjacent to the visibly mold-damaged, 5 species of *Aspergillus* and a species of *Penicillium* were obtained. This suggests that simple visual examination is not a reliable indicator of fungal contamination of tobacco. Also, it may be unwise to assume that tobacco adjacent to a mold-damaged area is free of, or contains low populations of, fungi.

Large populations of storage fungi in tobacco offals collected in dust bags from the threshing room suggests that more efficient filtration of air at this location might reduce fungal contamination during tobacco processing.

ACKNOWLEDGEMENTS

The authors thank Mrs. Clementine Zimmerman and Mr. R. F. Harrison for technical assistance.

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