Fungi Isolated from Flue-Cured Tobacco Sold in Southeast United States, 1968–1970

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Flue-cured tobacco leaves, from low- and middle-stalk positions, offered for sale in each of two markets, within each of five tobacco types, were evaluated for moisture content (MC) and filamentous fungi during August through October in 1968, 1969, and 1970. Alternaria alternata, Penicillium cyclopium, Aspergillus niger, Aspergillus repens, and Aspergillus flavus were most frequently isolated from cultured tissue. Other filamentous fungi that grew from the tissue included species from four genera of field fungi and seven species of storage fungi. Although the MC ranged from 11.0 to 22.5%, it averaged 16.4, 16.8, and 15.9% for samples taken in 1968, 1969, and 1970, respectively. Average populations of fungi per sample over the three years ranged from 0 to 1,528,500 colonies/g of tobacco.

Several species of storage fungi (mainly species of Aspergillus and Penicillium) commonly are isolated from marketed, damaged, and nondamaged flue-cured tobacco, but rarely are isolated from tobacco leaves immediately before or after curing (8–10) or from tobacco inoculated in the field with these fungi (7). As with cereal grains (1, 2), tobacco is invaded by "field" fungi before and "storage" fungi after harvest.

In an earlier report (8) differences in moisture content (MC) and in the numbers and kinds of fungi isolated from marketed tobacco were reported for samples from the Middle and Old Belt Markets in North Carolina. It was speculated then that the 3.6% difference in tobacco MC was responsible for the different species of fungi observed growing from cultured tobacco. To test this, a broader study was initiated to compare systematically the fungi and MC associated with two grades of tobacco, sold in each of two markets, within each of the five tobacco types, in each of three consecutive years.

There are five types of flue-cured tobacco having certain common characteristics and closely related grades. The five types are described elsewhere (5, 6). Briefly, type 11 is tobacco grown in northcentral North Carolina (N.C.) and southern Virginia; type 11a is grown in central N.C.; type 12 is grown in eastern N.C.; type 13 is grown in southeastern N.C. and northeastern South Carolina (S.C.); and type 14 is grown in southeastern Georgia and northern Florida. There are 94 markets in the flue-cured tobacco-producing areas (6). Markets sampled were in Danville and South Boston, Va. (type 11); Durham and Warrenton, N.C. (type 11a); Kinston and Rocky Mount, N.C. (type 12); Lumberton, N.C., and Conway, S.C. (type 13); and Valdosta and Waycross, Ga. (type 14) (Fig. 1). These samples were taken from August to October, 1968, 1969, and 1970.

A sample consisted of 5 to 15 leaves pulled from the center of each of five piles (weighing about 175 lb [80 kg]) of tobacco graded in either group B (leaf) or X (lug). B-grades grow in the middle of a 20-leaf plant. X-grades grow 2 or 3 leaves from the bottom of the plant. Each sample was put into a separate plastic bag, sealed, and transported to the laboratory for analysis. Samples not immediately evaluated were stored at 3 to 4 C until tested. For each type both warehouses were visited and samples from both grades were collected the same day.

The fungi associated with the leaves in 1968 were determined by two methods. In one, 25-leaf discs (9-mm diameter) per sample were cultured on Czapeks agar with 6% NaCl according to a procedure previously described (7). The Aspergillus colonies were identified as to species according to Raper and Fennell (4). Fungi not sporulating were recorded and tabu-
lated as unknown filamentous fungi. The percentage of the 25 discs which yielded a given fungus was determined for each sample. The overall percentage of discs from which a given fungus grew was computed on the basis of the total number of discs cultured from all samples which yielded the fungus.

Fungal populations in each sample for 1968, 1969, and 1970 were determined by chopping 10 g of tobacco in 500 ml of 0.15% agar in a food blender and making subsequent cultures from the dilutions. Results are expressed as the geometric average number of colonies per gram for all samples which yielded the indicated fungus. This technique has been used to determine the numbers and kinds of fungi associated with tobacco (9, 11) and is a slight modification of procedures used to determine the fungi associated with stored cereal grains (1). The geometric average was used rather than the arithmetic average because it reduces the distortion from averaging colony counts in the millions with those in the thousands.

Percentage MC (wet-weight basis) was determined by drying 6.47 g at 100 C for 16 hr (3).

Because comparison of the data showed similar MC and fungal populations for tobacco samples from different stalk positions, among markets, types, and years, all data were combined for this report.

The fungi growing from cultured leaf discs in 1968 are presented in Table 1. *Alternaria alternata* (Fr.) Keissl (A. tenuis Nees), *Pencillium cyclopium* Westling, *Aspergillus niger* Van Tiegham, *Aspergillus repens* de Bary, and *Aspergillus flavus* Link occurred most frequently. Although some species grew from 100% of the cultured discs of individual samples, the overall percentage was 42.3% or below. Not all samples were invaded by fungi; the same fungi did not grow from all samples; some samples were invaded by only one fungal species. Bacteria and yeasts did not grow well on this medium and their frequency was not recorded.

The populations of fungi (expressed as colonies per gram of tobacco) determined for samples collected in 1968, 1969, and 1970 are presented in Table 2. Although not shown in the table, 31 of 300 samples had populations that reached or exceeded 10,000 colonies/g; 23 of which were between 10,000 and 99,000 colonies/g; 3 of which were 105,000, 135,000, and 160,000 colonies/g; and 5 ranged from 3 million to 22 million colonies/g. Of the fungal species recorded, *Aspergillus versicolor*, *Penicillium cyclopium*, *Alternaria repens*, and *Bacillus subtilis* were the most common. Because *A. versicolor* is a parastic fungus which appears to be a common inhabitant of tobacco, it was determined whether this fungus grew in the tobacco samples on the basis of the number of colonies/g.

### Table 1. Fungi growing from tobacco discs cultured on Czapeks + 6% NaCl agar

| Fungus                | Average (%) | Range (%) |
|-----------------------|-------------|-----------|
| *Alternaria alternata*| 22.7* (58)* | 4 to 96   |
| *Aspergillus flavus*  | 15.8 (44)   | 4 to 100  |
| *Aspergillus niger*   | 19.4 (70)   | 4 to 96   |
| *Aspergillus repens*  | 12.3 (48)   | 4 to 76   |
| *Penicillium cyclopium*| 25.1 (63)  | 4 to 100  |
| Other filamentous fungi| 42.3 (24)  | 4 to 72   |
| Unknown filamentous fungi| 8.7 (35)  | 4 to 64   |

* The number is based on 25 discs from each of five samples of tobacco graded X and each of five samples graded B, from each of two markets, from each of the five tobacco types (total 100 samples). The average percentage of discs from which the indicated fungus grew was computed on the basis of the total number of discs cultured from all samples which yielded the fungus.

* Number in parenthesis is the number of samples yielding the fungus.

* Including field fungi species of *Cladosporium, Fusarium, Nigrospora, Syncphalastrum*, and storage fungi *Aspergillus amstelodami, A. candidus, A. ochraceus, A. parasticus, A. tamarii, A. versicolor*, and *A. ruber*. 
Table 2. Fungi per gram of tobacco in dilution cultures from 10-g samples

| Fungi                      | 1968        | 1969        | 1970        |
|----------------------------|-------------|-------------|-------------|
| Alternaria alternata       | 2,000 (32)  | 1,700 (39)  | 2,000 (30)  |
| Aspergillus flavus         | 1,100 (21)  | 4,300 (4)   | 1,400 (8)   |
| Aspergillus niger          | 1,900 (39)  | 1,700 (17)  | 1,000 (21)  |
| Aspergillus repens         | 700 (16)    | 2,300 (27)  | 1,300 (23)  |
| Aspergillus ruber          | 800 (4)     | 2,900 (13)  | 700 (5)     |
| Penicillium cyclopium      | 1,400 (28)  | 1,700 (29)  | 3,000 (19)  |
| Other filamentous fungi    | 600 (13)    | 4,400 (12)  | 800 (17)    |
| Unknown filamentous fungi  | 1,400 (3)   | 2,400 (24)  | 1,600 (16)  |

* The geometric average was determined from each of five samples of tobacco graded B and X, from each of two markets, in each of five tobacco belts (flue-cured types 11–14), in each of 3 years. The logₐ₁₀ value for the number of colonies per gram were added, the total was divided by the number of samples yielding the microorganism, and the logₐ₁₀ mean was converted to this number using the antilog₁₀.

* Number in parenthesis is the number of samples yielding the fungus.

* Including field fungi species of Cladosporium and storage fungi Aspergillus amstelodami, A. candidus, A. ochraceus, A. tamarii, and A. versicolor.

populations over 1 million, three were *P. cyclopium*, one was *A. repens*, and one was *Aspergillus ruber*. Many samples had mold counts so low that no colonies grew in the initial dilution cultures of 10⁻³ and this accounts for the failure to detect fungi in so many samples.

The average and range in MC of tobacco graded X was 16.1% and 11 to 20.8%, 16.5% and 13.5 to 22.2%, and 15.5% and 12.3 to 19.5% in 1968, 1969, and 1970, respectively. The average and range in MC of tobacco graded B was 16.8% and 13.6 to 22.5%, 17.1% and 14.9 to 21.6%, and 16.2% and 14.2 to 21% in 1968, 1969, and 1970, respectively.

The two grades of tobacco sold in these years in the five tobacco regions (types 11–14) had essentially the same ranges in the numbers and kinds of fungi and in MC. Further study of the fungi associated with marketed tobacco does not seem justified because the species of fungi associated with such widely varying samples were similar. Moreover, studies with stored tobacco (11) and other crops (2) have shown that the storage environment (temperature and relative humidity) determines to a large extent which fungi will grow and at what rate.

Further studies on the factors that influence growth of storage fungi seem justified and are in progress.

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