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Evaluations of Cigarettes Made with Mold-Damaged and Nondamaged Flue-cured Tobacco*

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INTRODUCTION

Surveys for the microorganisms associated with flue-cured tobacco (*Nicotiana tabacum* L.) show almost a constant association between the species of *Aspergillus* and tobacco following curing, and during marketing and storage (3, 12, 13). In addition, these fungi are mainly responsible for post-harvest rots and deterioration of tobacco (4, 11). Because of this substrate-saprophyte association we evaluated the taste and examined the smoke delivery of cigarettes made with tobacco damaged by these fungi. To do this, we evaluated tobaccos damaged in controlled conditions (referred to hereafter as "experimental storage") and damaged in commercial handling and storage.

MATERIALS AND METHODS

Tobacco Sources

Flue-cured tobacco, variety NC-2326, was grown under the usual cultural practices at the Upper Coastal Plain Research Station, Rocky Mount, N. C., during 1972. Leaves from the second priming (leaves from nodes 4–6) were harvested by hand and cured in the usual manner. After curing, the mid-rib was separated from the laminae by hand and discarded. Fifty pounds of laminae were stored at 80% relative humidity and 27° C in controlled environment chambers until mold growth appeared on the 19th day. A similar portion was stored dry at 24–26° C for the same period. On the 29th day, both tobaccos were steamed until they contained 16–18% moisture (wet-basis), then shredded at 30 cuts/inch and made into 85 mm long cigarettes on a commercial type cigarette making machine, using a regular type cigarette paper.

The second tobacco source was a composite of samples taken from 430 kg hogsheads of flue-cured tobacco received at a redrying plant in Wilson, N. C., directly from auction warehouse markets. When the tobacco was unpacked and mold-damaged tobacco was found by visual inspection it was removed, put into a plastic bag and stored at 2–3° C until 90 kg had accumulated. A similar amount of nondamaged tobacco from the same hogshead (when possible) was removed and stored. Care was taken to sample similar grades and take equal portions by weight. However, sample pairing was not as successful as hoped. Nicotine content and smoke delivery were more variable in the commercial tobaccos than in the experimental storage tobacco. Mid-rib tissue was not removed from commercial tobacco, but was included in the tobacco manufactured into cigarettes as previously described.

The cigarettes manufactured from both sources were stored at 2–4° C until tested.

Mold Evaluation

Population counts of saprophytic storage fungi (measured as colonies per gram of tobacco) before and after cigarette manufacture were determined by procedures previously reported (12). Briefly, colonies growing in petri dishes containing Czapek's agar (Difco) + 6% NaCl were counted in the serial dilutions of an original 10 g tobacco sample. The number of developing colonies was used as a quantitative measure of fungal contamination. This procedure is similar to that used to determine microbial contamination of flour and has been adopted as standard by the *American Association of Cereal Chemists* (1). The data are presented as geometric means of 5 to 20 samples taken from each tobacco source. Species of *Aspergillus* were identified according to *Raper* and *Fennell* (7); species of *Alternaria* and *Cladosporium* were not identified.

Smoke Panel Evaluation

Smoke panels from commercial tobacco companies evaluated cigarettes made with tobacco from both sources. The panel size for a company ranged from 8 to 10 members; one company did not enumerate panel size. Cigarettes were coded and panel members were asked two questions: i) "Do the lots differ?" and ii) "Which lot do you prefer?". Their answers were recorded and tabulated. Some panelists offered additional flavor descriptions. Four companies parti-

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cipated in smoking cigarettes made with tobacco from the experimental source; 7 companies participated in smoking cigarettes made with tobacco from the commercial source.

Chemical Analysis

Mold-damaged and nondamaged tobacco cigarettes were conditioned at 74 ± 2° F and 60 ± 2% relative humidity. They were then weight-selected and smoked to a butt length of 30 mm according to *Federal Trade Commission* Specifications. Cambridge particulate matter, nicotine and water delivery were determined according to a protocol established in an earlier study (6).

Those compounds in mainstream smoke capable of passing through a Cambridge trap are classified as constituents of the vapor phase. Although this fraction of smoke is primarily composed of gases such as carbon dioxide, oxygen, nitrogen, argon, etc., there are also present a variety of organic species. The vapor phase analysis described below routinely monitors 43 of these organic compounds and quantitatively determines 33 selected ones. Typical examples of compounds are acetone, methanol, acetaldehyde, crotonaldehyde, acrolein, acetonitrile, furan, 2-methyl furan, 2,3-dimethyl furan, etc.

The smoke sampling device used in this vapor phase analysis has been previously described in detail (2, 5). Six cigarettes are inserted into the smoking machine and a timer is started. The individual cigarettes are then ignited, one per puff, on puffs one through six. The smoke is puffed into an evacuated 210 ml sample volume and after a short-timed delay, the sample volume is reevacuated to make ready for the next puff. The sample, a mixture of smoke from six cigarettes of different butt lengths, is injected into a gas chromatograph (GC) on puff number eight. A GC separation is accomplished on an 18-foot × 1/16 inch stainless steel column plus a 1 foot precolumn each filled with 100/120 mesh Porapak Q®. After a 9-minute hold the column is heated from ambient to 220° C at 3.3°/min; the separated components are detected with a flame ionization detector and measured with a digital integrator.

The carbon monoxide/carbon dioxide analysis utilized a novel reaction column described by *Watanabe* and *Kobashi* (10). The GC column affords excellent separation without requiring a chromatograph with sophisticated subambient capability or temperature programming. For both convenience and sampling uniformity the major organic component and CO/CO_2 analyses are run simultaneously by the same sampling system. Nitric oxide and hydrogen cyanide are determined without modification according to published methods (8, 9).

RESULTS

Storage fungi associated with mold-damaged tobacco from experimental and commercial storage were *Aspergillus repens* de Bary, *A. ruber* (K. S. & B.) Thom and Church, *A. niger* Van Teghem, *A. ochraceus* Wilhelm and *A. flavus* Link (Table 1). *Alternaria* and *Cladosporium* species, considered as field fungi, were associated only with leaves of nondamaged tobaccos. Colony counts made from mold-damaged tobacco in the experimental tobacco source were larger than those made from mold-damaged tobacco in the commercial source. Apparently shredding and manufacturing reduced fungal propagules in mold-damaged tobaccos.

Smoke panelists distinguished cigarettes made with mold-damaged and nondamaged tobaccos from experimental or commercial storage (Table 2). In addition, they preferred cigarettes made with nondamaged tobaccos. Additional qualitative comments offered by the panels indicated cigarettes made with mold-damaged tobacco were low in sweetness, high in throat impact, peppery, bitter taste, mild, and characteristic of unaged tobacco.

Table 1. Quantitative determinations of the number and kind of fungi associated with damaged and nondamaged flue-cured tobacco from an experimental and commercial storage.

Tobaccos	No. of samples	Fungal colonies/g tobacco (× 1,000*)					
		Storage fungi				Field fungi	
		A. repens + *A. ruber*	*A. niger*	*A. ochraceus*	*A. flavus*	*Alternaria*	*Cladosporium*
Experimental storage							
Nondamaged leaf	20	1	19	0	1	2	0
Damaged leaf	5	24,784	1,388	56	0	0	0
Nondamaged shreds	5	2	7	0	0	0	1
Damaged shreds	5	12,128	1,016	37	0	0	0
Commercial storage							
Nondamaged leaf	10	20	10	0	0	2	1
Damaged leaf	10	1,036	1	2	1	0	0
Nondamaged cigarettes	10	26	0	0	0	0	0
Damaged cigarettes	10	338	0	0	0	0	0

* Geometric mean is based on a dilution series of 10 g of tobacco per sample.

Table 2. Smoke panel evaluation of cigarettes made from tobacco damaged and nondamaged in experimental and commercial storage.

Experimental* storage			
Questions asked	Panelists' reply		
	Yes		No
Do they differ?	44		14
	Nondamaged	Damaged	No choice
Which do you prefer?	31	13	14

Commercial** storage			
Questions asked	Panelists' reply		
	Yes		No
Do they differ?	52		3
	Nondamaged	Damaged	No choice
Which do you prefer?	43	9	3

* Summary of 4 responding companies.
** Summary of 7 responding companies.

Except for the nicotine content of the commercial storage tobacco and its resultant tar, there were no significant differences in total particulate matter or quantity of specific vapor phase components measured in the smoke from mold-damaged and nondamaged tobacco cigarettes (Table 3).

Viable fungal spores or propagules were associated with both sources of tobacco following cigarette manufacturing. We wondered if propagules could be trapped on a Cambridge filter pad and would grow from the pad in culture. To test this, Cambridge filter pads were autoclaved for 40 minutes at 121° C and 15 psi; filter holders were covered with 70% ethanol for 2 minutes and dried in an aseptic forced air chamber (Microvoid). A laboratory technician took 3 puffs from lighted and nonlighted cigarettes made with mold-damaged and nondamaged tobaccos from the experimental storage. Filter pads were removed with sterile forceps and placed on 7 different culture media (Table 4) with the surface closest the cigarette facing upward. Culture dishes were incubated at 24–26° C for 10 days and the fungal colonies that grew from the filter pads recorded and identified. *Aspergillus repens* and *A. ruber* were the only fungi that grew from cultured Cambridge pads and they were more prevalent in media supplemented with sucrose or NaCl. Bacteria grew from filtered air or smoke from cigarettes made with nondamaged tobacco, but species were not identified.

DISCUSSION

Smoke from cigarettes made with mold-damaged tobacco i) was found to be different by most cigarette taste panelists; ii) contained viable fungal propagules; and iii) had no significant differences in delivery of 39 organic and inorganic chemical compounds we assayed. These findings suggest that use of mold-damaged tobacco in cigarette manufacturing should be avoided because of the off-flavors present. Also to be considered is that fungal spores (specifically species of *Aspergillus, Alternaria,* and *Cladosporium*) are common allergens to the respiratory tract. The obvious solution to the problem is simply not to use mold-damaged tobacco in cigarette manufacture. However, this may be confounded by an inability to easily recognize mold invasion in areas adjacent to mold damage, as has been reported elsewhere (14). To be absolutely confident, tobacco used for cigarette manufacture should be routinely assayed for storage fungi.

SUMMARY

Flue-cured tobacco damaged by species of *Aspergillus* from commercial and experimental sources was shredded and made into cigarettes. Paired samples of nondamaged tobaccos served as controls. Subsamples of cigarettes were analyzed for viable fungal propagules/g, 39 organic and inorganic compounds in the smoke and smoke condensates, and taste preference. Principal fungi associated with leaves and shreds of mold-damaged tobacco were *Aspergillus repens, A. ruber,* and *A. niger.* Except for the original differences in the nicotine content and in the tar, there were no significant differences in total particulate matter nor in the amount of specific vapor phase components measured in the smoke from cigarettes made with mold-damaged and nondamaged tobaccos. Smoke panelists distinguished between cigarettes made with mold-damaged and nondamaged tobaccos and preferred cigarettes made with the latter. Viable fungus spores passed through the tobacco cylinder from lighted and nonlighted cigarettes. These data suggest the use of mold-damaged tobacco in cigarette manufacturing is to be avoided because of off-flavors and because the fungi isolated are common allergens to the respiratory tract of humans.

ZUSAMMENFASSUNG

Durch *Aspergillus*-Arten geschädigter „flue-cured"-Tabak aus Versuchsanbau und aus dem Handel wurde geschnitzelt und zu Cigaretten verarbeitet. Paarige Proben ungeschädigter Tabake dienten als Kontrollen. Untergruppen der Cigarettenproben wurden auf geschmackliche Vorzüge sowie auf das Vorkommen von lebensfähigen Pilzsporen (je g) und von 39 organischen und anorganischen Verbindungen im Rauch und Rauchkondensat untersucht. Die auf den Blättern und Blattschnitzeln der pilzgeschädigten Tabake hauptsächlich anzutreffenden Pilzarten waren *Aspergillus repens, A. ruber* und *A. niger.* Abgesehen von den ursprünglich vorhandenen Unterschieden im Gehalt des Rauches an Nikotin und Kondensat waren zwischen den Cigaretten aus pilzgeschädigten Tabaken und jenen aus ungeschädigten Tabaken weder bezüglich des Gesamtkondensates des Rauches noch bezüglich des Gehaltes an bestimmten Inhaltsstoffen der Gasphase signifikante Unterschiede festzustellen. Mitglieder einer Geschmacksjury unterschieden zwischen den Cigaretten aus pilzgeschädigten

Table 3. Analysis of smoke constituents from mold-damaged and nondamaged flue-cured tobacco cigarettes.

	Experimental storage		Commercial storage	
	Mold-damaged	Nondamaged	Mold-damaged	Nondamaged
Cigarette characteristics				
Weight (g)	1.20 ± .05	1.20 ± .05	1.18 ± .02	1.23 ± .02
Puff count	11.3	11.0	11.2	12.1
Nicotine content (%)	3.5	3.4	3.4	2.2
Delivery				
Cambridge particulate matter (mg/cig.)	40.8	38.0	45.1	47.2
Nicotine (mg/cig.)	3.2	3.1	4.2	3.1
Water (mg/cig.)	4.5	3.7	4.6	4.8
Major organic components (μg/puff)				
Methane	58.3	58.6	69.0	68.1
Ethylene	16.2	15.8	18.4	15.8
Acetylene	2.5	2.4	2.8	1.6
Ethane	21.1	21.3	26.7	21.4
Propene	16.6	16.4	20.2	16.8
Propane	12.0	11.8	16.7	12.4
Methyl chloride	8.0	8.6	25.6	33.6
Propadiene	1.2	1.0	1.1	0.8
Methanol	36.8	36.2	48.8	44.6
Acetaldehyde	62.6	60.3	69.6	65.6
2-Methyl propane	1.2	1.2	1.8	1.3
C-4 Alkene	7.2	7.2	9.1	7.6
1,3-Butadiene	4.6	4.4	4.7	4.1
Butane + trans-2-butene	5.6	5.6	8.1	5.9
cis-2-butene	2.2	2.2	2.8	2.2
Ethanol	0.4	0.3	0.4	0.3
Acetonitrile	13.7	12.6	22.6	15.8
Furan	5.0	5.0	4.5	5.3
Acrolein	7.0	7.2	3.7	5.8
Propionaldehyde	6.2	6.0	5.2	5.0
Acetone	36.6	36.8	35.3	37.6
Propionitrile / Isobutyraldehyde	8.8	8.4	11.4	9.3
2-Methyl furan	7.6	8.0	6.8	10.1
2-Butanone	13.8	13.4	15.0	14.3
Isobutyronitrile	2.6	2.3	3.0	2.4
Crotonaldehyde	1.8	1.8	1.6	1.8
Benzene	7.6	7.7	7.6	7.7
N-Butyronitrile	1.0	1.0	3.1	2.6
Isovaleraldehyde	7.2	6.4	8.6	8.1
2,5-Dimethyl furan	15.2	16.8	12.4	21.6
2-Pentanone	5.0	5.4	4.3	5.0
Toluene	13.4	12.9	18.0	17.0
Carbon monoxide (%)	2.2	2.2	3.0	3.1
Carbon dioxide (%)	6.0	6.2	7.3	6.6
Nitric oxide (μg/cig.)	72 ± 6	67 ± 1	108 ± 5	92 ± 16
Hydrogen cyanide (μg/cig.)	242	234	520	490

Tabaken und denen aus ungeschädigten Tabaken und zogen die letzteren vor. Bei angezündeten und nichtangezündeten Cigaretten war gleichermaßen eine Passage der lebensfähigen Pilzsporen durch den Tabakstrang zu beobachten. In Anbetracht der Beeinträchtigung des Geschmackes und der Tatsache, daß die isolierten Pilze gewöhnlich als Allergene im Respirationstrakt des Menschen wirken, erscheint es angezeigt, die Verwendung von pilzgeschädigtem Tabak in der Cigarettenherstellung zu vermeiden.

RESUME

On a coupé du tabac «flue-cured» contaminé par des espèces d'*Aspergillus* de sources expérimentales et commerciales, et on en a fabriqué des cigarettes. A chaque échantillon étaient couplées des cigarettes témoin faites à base de tabac non contaminé. On a examiné la présence de spores viables de champignons (par g de tabac) ainsi que de 39 composés organiques et inorganiques dans la fumée et le condensat de sous-échantillons de ces ciga-

Table 4. Number of fungal colonies growing from cultured Cambridge filters used to smoke cigarettes made with mold-damaged and nondamaged experimentally stored tobacco.

Agar media*	Damaged		Nondamaged			
	Lighted	Nonlighted	Lighted		Nonlighted	
	A. repens or *A. ruber*	*A. repens* or *A. ruber*	*A. repens* or *A. ruber*	bacteria	*A. repens* or *A. ruber*	bacteria
Tomato juice**	1	4	0	+	0	+
Malt extract 2 %**	0	2	1	—	1	+
Malt extract 2 % + sucrose 40 % + yeast extract 2 %	2	7	0	—	0	—
Potato dextrose**	1	0	0	+	1	—
Czapek's	2	2	0	+	0	+
Czapek's + 6 % NaCl	4	7	0	—	1	—
Czapek's + 20 % sucrose	9	15	0	—	2	—

* All Difco Laboratories, Inc. ** Identity of Aspergillus species tentative on these media.

rettes, et l'on a comparé les goûts à l'aide d'un panel de fumeurs. Les principaux champignons associés aux feuilles et rognures des tabacs contaminés de mildiou sont *Aspergillus repens, A. ruber,* et *A. niger.* A l'exception des différences en teneur en nicotine et en condensat préexistantes, on n'a trouvé aucune différence significative entre la fumée des tabacs contaminés et non contaminés, en ce qui concerne la matière particulaire totale, et les teneurs en composés spécifiques de la phase vapeur. Le panel de fumeurs a distingué les cigarettes contaminées de celles non contaminées, et préférait le goût de ces dernières. Des spores viables de champignons traversent le boudin de tabac, qu'il soit allumé ou non. Ces données suggèrent d'éviter l'usage de tabac contaminé dans la fabrication des cigarettes, à cause des différences de goût et aussi parce que les champignons isolés sont des allergènes généraux des organes respiratoires humains.

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