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Fungi Isolated from Flue-Cured Tobacco Inoculated in the Field with Storage Fungi

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Flue-cured tobacco inoculated in the field with *A. amstelodami*, *A. flavus*, *A. ochraceus*, *A. repens*, *A. ruber*, and a species of *Penicillium* was rarely invaded by these fungi. Regardless of inoculum, the predominant fungi reisolated from green tissue were species of *Alternaria* and *Cladosporium*. After curing, *A. repens*, *A. niger*, and species of *Alternaria* and a species of *Penicillium* were the most commonly isolated fungi. The fungus used as inoculum was not the predominant fungus reisolated from green or cured tissue. Conditions during handling and storage prior to marketing probably determine when storage fungi become associated with the leaf and which species becomes predominant.

Several species of *Aspergillus* and *Penicillium* are commonly isolated from marketed, damaged, and nondamaged (4) flue-cured tobacco (*Nicotiana tabacum* L.) but are rarely isolated from green tobacco leaves immediately after flue-curing (5). The purpose of this study was to determine whether selected storage fungi could infect living tobacco leaves in the field and whether the fungi used to inoculate the growing plant survived flue-curing and were the predominant fungi isolated from the cured leaf after the usual handling of the tobacco in preparation for marketing.

Flue-cured tobacco variety 'Coker 319' was grown under normal field conditions at the Oxford Tobacco Research Station, Oxford, N.C., in the summer of 1967. The plants were topped and suckered, and the lower six leaves on each plant were harvested before the test began. Test plots contained 10 plants per row, and each plot was separated from other plots by nontreated rows of the same variety.

Test fungi were *Aspergillus ruber* (K. S. & B.) Thom & Church (MQF-2), *A. ochraceus* Wilhelm (MQF-5), *A. amstelodami* (Mangin) Thom & Church (MQF-3), a species of *Penicillium* (MQF-7) originally isolated from moldy (damaged) tobacco, and *A. repens* DeBary (MQF-1) and *A. flavus* Link (MQF-6) originally isolated from nonmoldy (nondamaged) marketed tobacco. Inoculum for each fungus was prepared by washing previously inoculated oat seeds with distilled water containing 0.06 ml of Tween-80 per liter. The spores in the suspension were counted in a hemocytometer and diluted to 100,000 spores/ml with the Tween-80 solution. For each treatment, upper leaf surfaces on 10 test plants in each plot were sprayed with the spore suspension until run-off occurred on each leaf. Plants in two plots were sprayed with the Tween-80 solution for controls.

Plants were sprayed on 12 August 1967, and mature leaves were harvested on 16, 23, and 30 August and 6 September 1967. Immediately after harvest, about half of the leaves from each treatment were placed in an ice chest and transported to the laboratory in Raleigh; the remainder was flue-cured in the usual manner (3). In the laboratory, 50 discs (9 mm) from each treatment were cut from the green leaves, surface disinfected for 30 sec in 1% NaOCl, rinsed with sterile distilled water, and cultured on Czapek solution-agar (Difco) with 6% NaCl. Culture dishes were observed for microorganisms after 5, 9, and 12 days of incubation at room temperature. The fungi growing from the discs were identified, whenever possible, in the original petri dish culture. When this was not possible, the fungi were subcultured and stored for later identification. Species of *Aspergillus* were identified by the method of Raper and Fennell (1).

From the other portion of the harvested leaves that had been flue-cured, 50 discs (9 mm) from each treatment were cut, washed in running tap water for 30 min, and cultured on Czapek solution-agar (Difco) with 6% NaCl. These samples were not surface disinfected because earlier studies had demonstrated that treatment with 1% NaOCl eliminated fungi from cured leaf tissue. The petri dishes were observed and fungi were identified as previously described.

Results (Table 1) demonstrate that species of *Aspergillus* and *Penicillium* sprayed on tobacco leaves in the field are rarely reisolated from green tissue by using the procedures described in this report. The inability to reisolate these species supports the generally accepted theory that their primary nutritional role is saprophytic. The results also show that the species of storage fungi

TABLE 1. *Fungi isolated from 9-mm discs of tobacco before (BC) and after (AC) curing, inoculated in the field with five species of Aspergillus and a species of Penicillium, and harvested at four intervals, based on culturing 50 discs of tobacco for both samples*

Fungi isolated	Time after inoculation (day)	Non-inoculated control (%)		Non-inoculated control (%)		Storage fungus inoculum											
						A. amstelodami (%)		*A. flavus* (%)		*A. ochraceus* (%)		*A. repens* (%)		*A. ruber* (%)		*Penicillium* sp. (%)	
		BC	AC	BC	AC	BC	AC	BC	AC	BC	AC	BC	AC	BC	AC	BC	AC
Alternaria	4	99	18	99	12	98	52	98	14	97	12	99	24	100	6	100	24
	11	99	100	96	41	100	88	94	20	98	78	98	54	97	60	100	74
	18	95	94	100	68	97	86	100	76	100	86	96	42	97	62	98	26
	25	99	86	100	100	98	94	97	90	96	92	100	100	99	98	99	96
Cladosporium	4	59	4	63	0	37	4	59	0	58	2	79	2	58	0	69	0
	11	81	0	98	0	79	2	85	4	88	4	90	0	94	4	93	2
	18	89	8	81	14	75	2	89	10	88	4	84	8	95	2	64	0
	25	69	6	67	2	76	2	78	2	58	4	84	4	75	0	80	2
A. amstelodami	4	0	44	0	4	7	26	0	32	2	42	0	26	0	16	0	6
	11	0	0	0	0	0	4	0	2	0	2	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	25	0	6	0	4	0	0	0	4	0	4	0	0	0	0	0	0
A. flavus	4	0	0	1	0	0	0	13	12	2	0	0	0	0	0	0	0
	11	0	0	0	0	1	2	10	8	0	0	0	2	0	0	1	0
	18	0	4	0	10	0	0	0	6	0	0	0	0	0	8	3	8
	25	0	2	0	0	0	0	0	12	0	4	0	0	0	2	0	2
A. ochraceus	4	0	4	0	0	1	0	0	0	33	0	0	0	0	0	0	2
	11	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	2	0	0	0	0	0	0	0	0	0	4	0	0	0	0
	25	0	2	0	0	0	0	0	8	0	0	0	4	0	2	0	0
A. repens	4	0	52	0	52	6	72	1	66	3	42	2	52	0	52	1	60
	11	0	30	0	74	0	84	0	90	0	98	0	82	0	74	0	72
	18	0	10	0	6	0	18	0	0	0	22	0	6	0	6	0	6
	25	0	12	0	6	0	10	0	6	0	20	0	4	0	18	0	2
A. ruber	4	0	8	0	8	18	0	0	10	0	0	0	34	0	6	0	10
	11	0	4	0	8	0	0	0	2	0	2	0	2	0	8	0	2
	18	0	2	1	4	0	0	0	2	0	2	0	2	0	4	0	0
	25	0	0	0	0	1	0	0	0	1	2	2	0	0	8	0	2
Penicillium sp.	4	2	4	2	2	1	0	3	12	0	0	1	2	0	8	19	32
	11	0	4	18	18	4	6	6	4	0	8	0	6	0	14	5	10
	18	0	96	0	82	12	44	0	60	0	78	4	90	2	100	0	100
	25	0	10	0	4	1	0	3	6	1	16	0	14	1	10	4	16
A. niger	4	0	40	0	54	0	26	0	26	0	10	0	26	0	30	0	42
	11	0	4	3	42	3	14	0	18	0	44	0	64	0	26	9	26
	18	0	12	0	68	0	16	0	10	1	12	0	18	0	8	1	18
	25	0	18	0	6	0	4	0	2	0	8	0	0	0	8	0	0
Other fungi[a]	4	3	2	1	2	0	6	0	12	1	0	0	2	0	0	0	0
	11	1	0	11	2	1	4	5	0	2	8	0	0	1	4	2	0
	18	1	2	1	0	2	2	1	0	2	2	2	4	0	0	1	0
	25	1	26	0	24	1	10	0	6	0	4	3	16	0	10	0	14
Unknown fungi	4	63	10	23	0	30	6	64	0	12	6	20	4	19	4	11	6
	11	54	0	31	0	56	4	60	6	23	2	29	2	58	6	8	4
	18	55	0	17	4	42	4	10	10	50	4	6	6	10	0	3	6
	25	20	0	13	0	11	2	39	8	14	4	28	0	30	6	24	0

[a] *Aspergillus tamarii, Epicoccum nigrum,* species of *Botrytis, Fusarium, Mucor, Nigrospora,* and *Syncephalastrum.*

used to inoculate tobacco plants in the field are not necessarily the fungi most frequently isolated after flue-curing and handling. After curing, *A. repens* grew from 0 to 98% of the discs and was absent in only one sample; *A. niger* Van Tiegham grew from 0 to 68% of the discs and was absent in two samples; and a species of *Penicillium* grew from 0 to 100% of the discs and was absent in three samples. Invasion by these three fungi was not associated with field infection.

In addition to species of *Aspergillus* and *Penicillium*, other fungi frequently were isolated from green and cured tobacco. A species of *Alternaria* (probably *A. tenuis*) grew from 94 to 100% of the green-leaf discs and from 6 to 100% of cured-tobacco discs. A species of *Cladosporium* was the second most common fungus growing from green tissue, but curing reduced its frequency of isolation to 0 to 14% and in nine samples it was eliminated.

This work indicates that storage fungi rarely infect tobacco in the field and supports a similar conclusion of Tuite and Christensen (2) regarding storage fungi on wheat. The study also indicates that exposure to specific storage fungi in the field has little effect in determining which storage fungi become associated with a particular tobacco sample.

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