Bacterial Barn Rot of Flue-Cured Tobacco in North Carolina

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ABSTRACT

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Barn rot has become an important postharvest disease of flue-cured tobacco in North Carolina and the southeastern United States. This coincides with and may be related to an increase in the use of mechanized harvesters and bulk curing of leaf. Strains of *Erwinia carotovora* and *E. chrysanthemi* isolated from barn rotted leaves were pathogenic to tobacco. Reactions were significantly different among 20 tobacco cultivars representing eight tobacco types inoculated with a pathogenic strain of *E. carotovora* var. *carotovora,* but all cultivars were susceptible. Both commercial and noncommercial flue-cured tobacco seed were infested with numerous bacteria. Pectolytic positive bacteria infested 3–21% of the seed in the samples tested. Infested tobacco seed may be an important source of inoculum.

Barn rot has become an important postharvest disease of flue-cured tobacco in North Carolina and the southeastern United States. In 1976, 1977, and 1978, losses from barn rot were 0.45, 0.15, and 0.20% representing 4.3, 1.2, and 2.1 million dollars, respectively, of the North Carolina flue-cured tobacco crop (14,15). Barn rot now ranks sixth among 18 important flue-cured tobacco diseases in North Carolina.

In North Carolina, barn rot occurs frequently when wet, mechanically primed leaves are packed tightly in bulk curing barns with poor air circulation. It also occurs in conventional stick barns, especially those with poor air circulation when they are overfilled with wet tobacco leaves. Barn rot usually begins in the petiole or lamina as a water-soaked, discolored area of tissue. As the disease progresses, the discolored area darkens until it becomes black, then decomposes and emits a strong odor of decay. The damage may vary from a few leaves to the entire content of a barn.

Several bacteria and fungi can cause barn rot. The most common bacteria are *Erwinia carotovora* var. *carotovora* (syn. *E. carotovora, E. aroideae*) and *Bacillus polymyxa* (6,7,11). The fungi are *Botrytis cinerea, Sclerotinia sclerotiorum, Rhizopus arrhizus, Pythium* spp., and *Alternaria alternata* (8). The symptoms in bulk-cured leaf have implicated soft-rot bacteria as the causal agents of barn rot in North Carolina.

The purposes of this work were to identify the pathogen(s) responsible for the recent increase in barn rot, to examine the susceptibility of 20 tobacco cultivars, and to examine tobacco seed for the pathogen.

MATERIALS AND METHODS

Tobacco samples with barn rot were collected from flue-curing barns in 15 North Carolina counties during the 1975, 1976, and 1977 harvest seasons. Sections of diseased tissue were surface-disinfected in 20% Clorox for 2–5 min and treated in two ways. In the first treatment, leaf sections were washed in several changes of sterile distilled water, placed in a test tube with 10 ml of sterile water, and crushed with a sterile glass rod; a loopful of the suspension was then streaked on crystal violet pectate agar (CVP) (4), the plates were incubated at 25 C for 24–48 hr, and individual pectolytic colonies were transferred to yeast-dextrose-calcium carbonate agar (YDC) (16) or nutrient agar (NA). In the second treatment, surface-sterilized leaf tissue was plated on acidified potato-dextrose agar, incubated at 27 C, and examined daily for 5 days.

To test for pathogenicity, bacterial strains were grown on YDC or NA slants for 24 hr, the cells suspended in sterile distilled water and adjusted to a concentration of 10^6 to 10^7 colony forming units (CFU) per milliliter. Leaves, 10 cm × 20–25 cm, from greenhouse-grown plants of *Nicotiana tabacum* 'Hicks' were injured by puncturing them with needles, 10 holes/cm^2. Injured leaves were placed on wet paper towels and sprayed with a bacterial suspension by using a De Vilbiss hand-powered atomizer. Two layers of sprayed leaves separated by a double layer of wet paper towels were placed in clear plastic containers (25-cm diameter, 9 cm high). Containers were held at 27 C for 24–48 hr. Soft rot of leaves was indicative of pathogenicity.

Cell shape and gram reaction of bacteria were determined by the procedure of Preston and Morrell (12); motility and flagella were observed by the method of Mayfield and Innis (9), and colony morphology was examined on NA and YDC. Catalase, indole, and nitrate reduction and production of acid from lactose, maltose, or α-methylglucoside was determined in peptone water as described in Bergey's manual (2). Utilization of malonate was determined by the procedure of Edwards and Ewing (5), phosphatase was detected by Cowan and Steel's method (3), and oxidase was determined with an oxidase test kit (API, Analytab Products, Inc., Plainview, NY). All diagnostic tests were replicated three times and repeated twice.

Table 1. Relative resistance of 20 tobacco cultivars to soft rot following infiltration of leaf tissue with a water suspension of *E. carotovora* var. *carotovora*

Cultivar	Type	ED_{50}[a] (cells/ml × 10^4)
BU 21	Burley	0.2
Sp. G-28	Flue-cured	0.3
Va. 115	Flue-cured	0.8
NC 2326	Flue-cured	0.9
LAF 53	Flue-cured	1.5
McNair 1040	Flue-cured	1.6
NC 95	Flue-cured	1.6
Coker 347	Flue-cured	2.0
Coker 86	Flue-cured	2.1
Hav. 307	Cigar binder	3.4
NC 12	Flue-cured	3.9
Samsun	Turkish	4.2
L-8	Burley	7.9
Md. 10	Maryland	8.4
BU 49	Burley	11.4
Pennbel	Cigar filler	12.8
Md. 609	Maryland	14.4
Ky. 170	Dark fired	17.0
Fla. 301	Cigar wrapper	19.2
Dixie Shade	Cigar wrapper	24.5
LSD 0.05		13.6

[a] Effective dosage of bacteria in suspension causing 50% of the leaf disk to rot.

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In the study of susceptibility of tobacco cultivars to barn rot, 20 cultivars within eight tobacco types (and including nine flue-cured, three burley, one cigar binder, one Turkish, one dark fired, two Maryland, one cigar filler, and two cigar wrapper) were grown in 15-cm pulp pots in the greenhouse until 30–60 cm tall. A strain of *E. carotovora* var. *carotovora,* Eca 14 isolated from tobacco in North Carolina, was cultured on a NA slant for 24 hr at 27 C, and the bacteria were suspended in sterile distilled water at 10^4, 10^5, 10^6 CFU/ml. Tobacco leaf disks, 7 cm in diameter, were cut from lower leaves of greenhouse-grown plants. Six disks were inoculated with each bacterial suspension by vacuum infiltration (42 mm Hg). The process of pulling and releasing a vacuum was repeated three times to adequately infiltrate the disks.

Infiltrated leaf disks were placed between wet paper towels in plastic refrigerator boxes and incubated 24 hr at 28 C. The leaf disks were observed and numerically rated for soft rot: completely rotted = 1, half rotted = 0.5, not rotted = 0. Combined rating values for the six disks tested at each inoculum concentration resulted in a final rating of 0 to 6. The ED_{50} value (inoculum dose resulting in 50% rot) of the three replicates was calculated for each cultivar by using the regression coefficient obtained by a computer probit analysis (1,13).

Tobacco seeds were evaluated as a source of inoculum. Seeds of flue-cured varieties were obtained from three commercial seed companies and two tobacco breeders. For breeding lines, the flower stalk was covered with a paper bag just before pollination to prevent cross-pollination. These "bagged seeds" were tested to determine whether the protection provided by bagging had altered the bacterial flora associated with the seed. Seeds were obtained and tested primarily from 1977 and 1978 stocks to determine the total number of extractable bacteria and the percentage of seed with pectolytic bacteria. Percent seed germination was computed after counting germinated seeds incubated on moist filter paper in petri dishes.

Total extractable bacteria were determined by adding 1 g of tobacco seed to 10 ml of sterile water in a tube. The mixture was shaken and held for 1 hr. The water was removed by aseptic filtration by using Whatman No. 1 filter paper, and the filtrate was serially diluted and plated on nutrient-dextrose agar. To determine the presence of pectolytic bacteria, the seeds were placed on CVP (100 seeds per plate) and incubated 48 hr at 28 C.

RESULTS

No plant pathogenic fungi were observed in barn rot samples or in cultures from barn rot samples on acidified potato-dextrose agar.

Sixteen strains of pectolytic bacteria were isolated from samples of tobacco with barn rot symptoms. Cells of all strains of pectolytic bacteria reduced nitrates and were gramnegative, single rods, motile by peritrichous flagella, facultative anaerobes, catalase-positive, and oxidase-negative.

Fourteen strains produced cream-colored colonies on NA and on YDC and produced acid from lactose but not from maltose or α-methylglucoside. None utilized sodium malonate or produced indole, and all were phosphatase-negative. Strains in this group were considered to be *E. carotovora* (Jones) Bergey et al var. *carotovora.*

Two strains produced colonies 4–6 mm in diameter with a fine granular, white to light tan color when grown for 24–48 hr on YDC. Neither produced acid from lactose or maltose, and both produced indole, were phosphatase-positive, and utilized sodium malonate. The strains in this group were considered to be *E. chrysanthemi* Burkholder, McFadden, and Dimock. Koch's postulates were satisfied with all 16 strains from tobacco.

Wounded leaf disks sprayed or vacuum-infiltrated with various water suspensions of *E. carotovora* var. *carotovora* or *E. chrysanthemi* soft rotted within 24 hr of incubation. Although there were significant differences among some of the cultivars, all were considered susceptible to these bacterial pathogens (Table 1).

Most commercial tobacco seed and tobacco seed produced by noncommercial plant breeders was infested with large numbers of extractable bacteria (Tables 2 and 3). These bacteria survived on the seed during storage. The number of CFU per seed sampled varied from 2 to 3,358 in 1977 and 1978 seeds. Colony types were recorded for bacteria extracted from four 1978 cultivars (Table 4). In addition to pectolytic bacteria, as determined on CVP medium, three colony types were readily and consistently discernible on nutrient-dextrose agar: large cream, transparent, and small white. These types were considerably more numerous than the pectolytic bacteria.

Pectolytic bacteria were detected on up

Table 2. Total bacteria (colony forming units) extracted with water from tobacco seed samples (1977 test)

Cultivar[y]	Accession	Year produced	Bacteria per seed[z]
OP 4264	Ox(GRG)	1976	3358 a
OP 5215	Ox(GRG)	1976	1973 b
C 254	76-32	1976	1489 bc
C 258	76-33	1976	1489 bc
NC 2326	76-21	1976	1108 bcd
C 347	76-30	1976	1039 bcd
C 319	76-34	1976	866 bcd
C 347 B	Ox(GRG)	1976	716 cd
Sp. G-28	6	1976	672 cd
C 86	76-38	1976	523 cd
NC 13	76-43	1976	523 cd
C 411	76-31	1976	454 cd
Sp. G-23	5	1975	343 d
Sp. G-140	3	1973	284 d
C 319 B	Ox(GRG)	1976	284 d
Sp. G-28	(GRG)	1976	197 d
Sp. G-33	1	1971	113 d
Sp. G-28	5	1975	101 d
McN 944	873061	1976	88 d
Sp. G-7 B	Ox(GRG)	1976	59 d
McN 1040	87418	1976	58 d
McN 20	873871	1976	37 d
C 411 B	Ox(GRG)	1976	25 d
McN 944	(2 of 2)	1976	23 d
McN 944	(1 of 2)	1976	21 d
McN 30	871332	1976	20 d
4031 B	Kin(GRG)	1976	5 d
4264B	Kin(GRG)	1976	2 d

[y] Italic type and letter B indicate that the flower was covered with a bag immediately before pollination.

[z] Average of two replicates; numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Percentages of germination and of seed with pectolytic bacteria in tobacco seed produced in 1977 (1978 test)

Cultivar[x]	Accession	Percent seed	
		Germination[y]	Pectolytic bacteria[z]
C 48		93	15 a
C 86		87	13 ab
C 319		91	10 bc
McN 944	88106	92	8 cd
Sp. G-28		95	6 d
Sp. G-140		97	5 de
C 347		91	5 de
McN 944	88107	88	5 de
McN 944	88105	93	5 de
McN 944	88104	90	3 ef
NC 13		93	3 ef
C-114-4 B	JFC	90	1 f
315 B	JFC	85	0 f
C-42-1 B	JFC	78	0 f
C-78-5 B	JFC	90	0 f

[x] Italic type and letter B indicate that the flower was covered with a bag immediately before pollination.

[y] Average, two replications.

[z] Average of five replicates; numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Total bacteria extracted with water from 1977 tobacco seed and number exhibiting pectolytic activity or forming other major colony types (1978 test)

Cultivar	Colony type (CFU/seed)[z]				
	Pecto-lytic	Large cream	Trans-parent	Small white	Total
C 48	48 a	240 ab	260 a	561 a	1109 a
McN 944	3 b	409 a	172 ab	459 a	1043 a
Sp. G-28	3 b	174 b	47 ab	149 b	373 b
C 78-5	0 b	88 b	7 b	0 b	95 b

[z] Average of four replicates; numbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

to 21% of the seed in a sample tested in 1977 (Table 5) and 15% in 1978 (Table 3). In 1978 tests, bagged seed had 1% or less seed with pectolytic bacteria. Germination was high. Strongly pectolytic bacteria such as *E. carotovora* var. *carotovora* and *E. chrysanthemi* produced distinct pits, whereas weakly pectolytic bacteria produced shallow concavities on CVP as shown in Table 5.

DISCUSSION

The recent epidemic of barn rot in North Carolina coincides with an increase in mechanized harvesting and curing. Mechanical leaf harvesters injure and wound tobacco leaves, and conveyors probably spread the pathogens. Newly developed bulk-curing barns handled by inexperienced operators may provide the humidity and temperature conditions that favor soft rot. Tobacco barn rot was observed in every North Carolina county that produces flue-cured tobacco, and in some instances losses to individual growers amounted to thousands of dollars.

The pectolytic bacteria isolated from tobacco samples with characteristic symptoms were *E. carotovora* var. *carotovora* and the related species *E. chrysanthemi*. Efforts to isolate the pathogen were hindered until it was realized that by fully curing tobacco, thermal death of the bacterial pathogens resulted. The final stages of flue-curing use a temperature near 72 C for 5 hr, which kills *Erwinia* soft-rot bacteria. *Erwinia* spp. were readily isolated from samples of rotting leaf tissue obtained from curing barns before temperature elevation.

In North Carolina, *E. carotovora* var. *carotovora* was more prevalent than *E. chrysanthemi*. Barn rot (soft rot) symptoms produced by *E. carotovora* var. *carotovora* and *E. chrysanthemi* under laboratory conditions were similar to field symptoms. The relationship or relative importance of these two bacterial species to each other and to the distribution or severity of barn rot is unknown.

Table 5. Percent of seed in sample infested with bacteria and percent with pectolytic-positive bacteria (1977 test)

Cultivar[a]	Percent seed[b]		
	Bacteria	Pectolytic bacteria	
		Pits	Concavities
Sp. G-28 (6)	100	10	14
C 319	100	21	25
C 86	100	15	20
NC 2326	100	13	29
OP 4264 (GRG)	100	7	9
OP 5215 (GRG)	100	9	9
C 254	99	12	19
C 347 (GRG) B	98	1	2
C 319 (GRG) B	98	3	22
McN 1040	97	16	25
C 258	96	13	20
McN 30	94	8	20
C 347	91	4	15
C 411 B	91	11	28
Sp. G-33 (1)	90	15	26
Sp. G-28 (5)	89	11	16
Sp. G-140	89	15	21
NC 13	89	4	15
Sp. G-23	88	5	15
C 411 (GRG)	87	3	5
Sp. G-7 (GRG) B	87	2	7
Sp. G-28 (GRG)	83	0	7
McN 944 (2)	82	9	14
McN 944 (1)	80	11	11
McN 944 #873061	72	7	20
McN 20	69	7	13
4264 (GRG) B	14	1	1
4301 (GRG) B	13	1	2
LSD 0.05	15	6	10
0.01	21	8	14

[a] Italic type and letter B indicate that the flower was covered with a bag immediately before pollination.
[b] Average of two replicates.

The resistance of the 20 tobacco cultivars tested was considered too low for field control. Other sources of germ plasm must be tested to find effective resistance to this disease. Because significant differences in susceptibility were detectable, we consider the technique suitable for evaluating resistance or other disease characteristics.

Commercial tobacco seed was infested with bacteria, and some had high populations. Also, a significant portion of the seed (3–15%) had pectolytic-positive bacteria that were pathogenic *E. carotovora* var. *carotovora* and *E. chrysanthemi*. Our results (*unpublished*) and those of others showed that soft-rot bacteria do not survive well in soil but do survive in association with plant roots and, as with the hollow-stalk *E. carotovora* var. *carotovora*, survive on seed (6,10). Thus, we conclude that, for barn rot of flue-cured tobacco, seed may be an important source of inoculum or possibly the primary source. If seedborne bacteria prove to be a source of primary inoculum, they can be eliminated by microwave radiation (6), hot water therapy (10), or chemical treatment (Echandi and Spurr, *unpublished*).

Careful management of tobacco during harvesting and curing can decrease injury to leaves and control the spread of the bacterial barn rot pathogens, thus decreasing disease. Good practices to follow include avoiding harvesting and handling of wet tobacco, injury to tobacco, and overpacking of tobacco, especially lower stalk leaves, and raising the curing temperature before removing free moisture from the leaves.

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