Occurrence of Bitter Rot on Apple in Michigan

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ABSTRACT

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Although bitter rot is distributed widely in the United States, the disease is virtually unknown in Michigan. Fruit samples of cvs. Empire, McIntosh, Sunrise, Paulared, and Jonagold with bitter rot symptoms were collected from eight apple orchards in 1995 and the *Colletotrichum* spp. determined. The orchards were located in western Michigan, extending from the Indiana border north to Grand Rapids and east to East Lansing. *C. acutatum* and *C. gloeosporioides* were recovered from 81.2 and 18.8% of 165 fruit samples, respectively. All isolates except two of *C. gloeosporioides* grew on potato dextrose agar amended with 5 µg of benomyl per ml. Sequence similarity for expected polymerase chain reaction–amplified internal transcribed spacer region 1 (ITS1) products was high (>95%) among four strains of *C. acutatum* and four strains of *C. gloeosporioides*, but low (62 to 67%) between strains of the two species. All isolates were virulent on apples of cv. Golden Delicious. *Colletotrichum* appears to be endemic in Michigan apple orchards but outbreaks of bitter rot are rare, suggesting that environmental conditions in Michigan are normally unfavorable for disease development.

Bitter rot, a major disease of apple throughout the eastern United States from Pennsylvania west into Indiana and Illinois and south to Alabama and Georgia (7), has not been of concern to the Michigan apple industry. A few infected fruit have been noted on rare occasions in apple orchards near the Indiana-Michigan border, but not elsewhere in Michigan. The *Colletotrichum* spp. causing these rare outbreaks were never identified. In 1995, an unusual outbreak of bitter rot involving 2 to 3% of the fruit occurred in several apple orchards in southwestern Michigan and in central Michigan from East Lansing to Grand Rapids.

Bitter rot is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk) and by *C. acutatum* J. H. Simmonds (1,4,7). In the field, acervuli with orange to salmon conidial masses were often formed in the centers of lesions and in culture most isolates from these fruit produced colonies with scarlet centers when viewed from the underside. Based on these characteristics the causal fungus was tentatively identified as *C. acutatum*. The objective of this study was to establish whether *C. acutatum* was the predominant *Colletotrichum* sp. on apple in Michigan, verify the virulence of Michigan isolates to apple, and determine their susceptibility to benomyl. Knowledge of the identification and distribution of the *Colletotrichum* spp. would be helpful for establishing the value of benomyl for bitter rot control in Michigan since many isolates of *C. gloeosporioides* are sensitive to benomyl while isolates of *C. acutatum* are insensitive to benomyl (1).

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MATERIALS AND METHODS

Apple fruit with suspected bitter rot lesions were collected in late August of 1995 from orchards near Berrien Springs, Eau Claire, Hartford, and Sister Lakes in southwest Michigan; Belding, Conklin, and Lowell in west central Michigan; and East Lansing in central Michigan. A total of 25 to 28 apples with lesions were collected per orchard; fewer apples were collected in two orchards where it was difficult to locate diseased fruit. The fruit were forced open with a large knife to expose decayed tissue extending toward the core. A small amount of decay from the interior of each lesion was placed on potato dextrose agar (PDA) in a petri dish. Isolates previously identified as *C. acutatum* and *C. gloeosporioides* were obtained from E. I. Zehr (Clemson University, Clemson, SC) and from T. B. Sutton (North Carolina State University, Raleigh) for comparison with isolates collected in Michigan (Table 1). An isolate of *C. acutatum* from peach was supplied by K. D. Hickey (Pennsylvania State University Fruit Research Laboratory, Biglerville) and an isolate was obtained from blueberry fruit collected near Grand Junction, MI, by D. C. Ramsdell (Michigan State University, East Lansing). All isolates were transferred to PDA, then the petri dishes were held in darkness at 26°C for 7 days.

The growth of cultures on benomyl-amended (5 µg/ml) and unamended PDA dishes was determined after 7 days at 26°C (1). A 5-mm-diameter mycelial plug from the margin of an actively growing culture on PDA was placed in the center of each dish. Each isolate had three replications. Colony diameter measurements were made after 7 days. The experiment was conducted three times.

Virulence studies were conducted on apple fruit of cv. Golden Delicious harvested on 29 September 1995. Newly harvested fruit were used in the first experiment. Fruit stored for 3 weeks in a cold room were used to repeat the experiment. Fruit were selected for uniform size and ripeness, then washed and surface sterilized with a 70% ethanol solution. Inoculations were made by cutting into the side of the fruit midway between the blossom and stem ends with a sterile 5-mm-diameter cork bore. Two inoculations were made on opposite sides of each of three apples per isolate. About four isolates were selected at random from each orchard for the virulence studies; additional isolates were then selected for those orchards where some isolates had gray or white rather than scarlet colonies. Mycelial plugs were cut from the margins of actively growing cultures on PDA and were inserted mycelial surface downward into the wounds. Plugs of PDA were inserted into the fruit used as controls. The inoculation sites were covered with a piece of transparent tape, and the fruit were incubated at 28°C in plastic bags. The external diameter of decay lesions was measured after 10 days.

Differences in nucleotide sequence for the internal transcribed spacer region 1 (ITS1) between the 5.8S and 18S ribosomal genes were used to differentiate *C. acutatum* from *C. gloeosporioides*. Mycelial mats taken from cultures grown in minimal medium (dextrose, 10 g; $MgSO_4$, 0.5 g; KNO_3, 3.0 g; KH_2PO_4, 0.5 g; K_2HPO_4, 1.0 g, and distilled water, 1 liter) were dried between paper towels, placed in microcentrifuge tubes, frozen with liquid

nitrogen, and macerated with disposable pellet pestles in microcentrifuge tubes. Total DNA was isolated by a cetyltrimethylammonium bromide (CTAB) miniprep procedure (9). The isolated DNA was then used as template in the PCR. Primers ITS1-F (5'-CTTAGGTCATTTAGAGGAA GTAA) and ITS2 (5'-GCTGCGTTCTTCA TCGATGC) were used to amplify the noncoding region between the 18S and 5.8S rDNA genes (8). The thermal program was as described in Kohn et al. (3). The presence of PCR products in the reaction mixture was confirmed by electrophoresis on 1.5% agarose gels. The PCR reaction product was purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). Automated fluorescent sequencing of the amplified product from PCR was performed with primers ITS1-F and ITS2, the ABI (Applied Biosystems, Inc., Foster City, CA) catalyst for *Taq* cycle sequencing, and the ABI 373A sequencer for analysis of the product at the Michigan State University Department of Energy-Plant Research Laboratory Plant Biochemistry Facility. ITS sequence data

Table 1. Origins of reference strains and 36 out of 165 Michigan apple strains of *Colletotrichum* spp., radial growth on potato dextrose agar (PDA) after 7 days at 26°C and its reduction on PDA amended with benomyl, and lesion development on wound-inoculated fruit of Golden Delicious after 10 days at 28°C

		Colonies on PDA[a]		Reduction in growth (%) on PDA + 5 µg of benomyl per ml[b]	Lesions on fruit[c]	
Reference isolates	Origin and source	Color	Diameter (cm)		Diameter (mm)	Acervuli (mm) in lesions
C. acutatum						
SC4	E. Zehr, Sandy Springs, SC, apple	Crimson	6.0	60.7	25.8	Yes
SC5	B. Smith, strawberry	Crimson	6.3	69.1	24.3	Yes
NC1837	T. Sutton, NC, apple	Crimson	6.4	65.1	24.1	Yes
PA1	K. Hickey, PA, peach	Crimson	6.3	63.8	29.7	Yes
BB1	Grand Junction, MI, blueberry	Crimson	4.7	64.0	19.3	Yes
C. gloeosporioides						
SC1	E. Zehr, Pendleton, SC, apple	White/orange	6.5	85.3	16.6	No
SC2	L. Pusey, Byron, GA, apple	White/orange	6.5	58.9	29.7	Yes
SC3	L. Pusey, Byron, GA, peach	White/orange	8.6	91.0	30.9	Yes
NC1894	T. Sutton, NC, apple	White/orange	8.2	90.0	32.4	Yes
NC1869	T. Sutton, NC, apple	White	8.4	NG[d]	23.5	No
C. acutatum[e]						
S5	Eau Claire, MI, Empire	Crimson	5.4	66.9	23.9	Yes
S15	Eau Claire, MI, Empire	Crimson	5.5	62.5	25.4	Yes
S18	Eau Claire, MI, Empire	Crimson	5.4	63.1	22.2	Yes
S25	Eau Claire, MI, Empire	Crimson	5.2	63.7	24.1	Yes
H7	Hartford, MI, McIntosh	Crimson	5.4	63.0	25.6	Yes
H17	Hartford, MI, McIntosh	Crimson	5.8	66.5	24.9	Yes
H20	Hartford, MI, McIntosh	Crimson	5.9	65.5	25.1	Yes
B7	Berrien Springs, MI, Sunrise	Crimson	6.3	63.7	28.0	Yes
B12	Berrien Springs, Mi, Sunrise	Crimson	5.4	65.0	23.5	Yes
B20	Berrien Springs, MI, Sunrise	Crimson	5.5	61.1	23.0	Yes
I6	Sister Lakes, MI, Jonagold	Crimson	6.0	62.9	19.9	Yes
I7	Sister Lakes, MI, Jonagold	Crimson	6.0	66.0	24.7	Yes
I20	Sister Lakes, MI, Jonagold	Crimson	6.0	71.8	28.7	Yes
G2	Belding, MI, Empire	Crimson	6.4	72.4	23.6	Yes
G11	Belding, MI, Empire	Crimson	6.5	62.5	28.4	Yes
T7	Lowell, MI, Empire	Crimson	5.6	64.4	13.0	Yes
T18	Lowell, MI, Empire	Crimson	5.8	60.9	27.4	Yes
T21	Lowell, MI, Empire	Crimson	6.7	71.1	26.7	Yes
Bg1	Belding, MI, Paula Red	Crimson	5.8	64.5	25.4	Yes
Bg2	Belding, MI, Paula Red	Crimson	4.8	60.4	24.2	Yes
Bt2	East Lansing, MI, Empire	Crimson	5.7	65.8	25.5	Yes
Bt3	East Lansing, MI, Empire	Crimson	5.8	66.0	25.5	Yes
Bt5	East Lansing, MI, Empire	Crimson	5.9	65.4	23.9	Yes
C. gloeosporioides[e]						
B1	Berrien Springs, MI, Sunrise	Gray/orange	5.6	54.1	25.8	Yes
B3	Berrien Springs, MI, Sunrise	Gray/orange	4.7	63.6	25.2	Yes
B10	Berrien Springs, MI, Sunrise	Gray/orange	6.2	65.8	30.1	Yes
I26	Sister Lakes, MI, Jonagold	Gray	8.6	87.3	35.9	No
G10	Belding, MI, Empire	Gray	8.1	84.8	32.1	Yes
G14	Belding, MI, Empire	Gray	6.3	85.2	19.6	No
G15	Belding, MI, Empire	Gray	5.7	80.8	22.8	No
T5	Lowell, MI, Empire	Gray	6.9	77.7	34.2	No
T6	Lowell, MI, Empire	Gray	4.4	57.0	12.4	No
T9	Lowell, MI, Empire	White/orange	5.5	50.9	27.1	Yes
T15	Lowell, MI, Empire	Gray	8.2	NG	33.3	Yes
U2	Conklin, MI, McIntosh	Gray	5.1	71.9	22.4	No
U3	Conklin, MI, McIntosh	Gray	5.5	71.5	19.8	No

[a] Color and radial growth measurements are for three colonies from one experiment. Results were similar in two additional experiments.
[b] Average of three colonies.
[c] Average of lesion diameter of six inoculation sites on a total of three fruit.
[d] No growth.
[e] From Michigan apple orchards.

were aligned and compared with the MEGALIGN module of the Lasergen Sequence Analysis System (DNASTAR Inc., Madison, WI).

RESULTS AND DISCUSSION

Colletotrichum was isolated from all collected fruit: 25, 25, 27, and 26 apples collected from four orchards in southwest Michigan; 28, 25, and 3 apples collected from three orchards in west central Michigan; and six apples collected in central Michigan. The isolates exhibited considerable variation in colony color and morphology after 7 days in darkness at 26°C (Table 1). Some colonies were gray, others white with aerial mycelium and orange masses of conidia near the center of the colony. The majority of the colonies were scarlet, particularly when viewed from the underside, with scarlet-gray compact aerial mycelium. The gray and white colonies with aerial mycelium corresponded to the gray types of *C. gloeosporioides* and the scarlet to gray colonies with compact mycelium to the pink types of *C. acutatum* described by Bernstein et al. (1). Among the isolates from southwest Michigan, 100 were tentatively identified as *C. acutatum* and three as *C. gloeosporioides;* among those from west central Michigan, 28 were *C. acutatum* and 28 were *C. gloeosporioides;* and all six isolates from central Michigan were *C. acutatum*. The predominance of *C. acutatum* in our survey suggests that this species was more important than *C. gloeosporioides* in the outbreak of bitter rot in Michigan. Although inoculum may have come from nearby plantings of small fruit crops, little is known about the distribution of different *Colletotrichum* spp. on these crops in Michigan. Previously, *C. gloeosporioides* was reported, based on the identification of a single isolate, as a common pathogen of blueberries in Michigan (2); the Michigan blueberry isolate (BB1) examined in this study was *C. acutatum* (Table 1).

The mean colony diameter for the isolates of *C. gloeosporioides* from Michigan was 6.2 cm versus 5.8 cm for the 22 isolates of *C. acutatum* (Table 1). Isolates of *C. gloeosporioides* are often sensitive to benomyl (1), but due to an overlap in response it was not possible to distinguish isolates of *C. gloeosporioides* from *C. acutatum* collected in Michigan based on their reaction to benomyl. Colony diameters for isolates of *C. gloeosporioides* and *C. acutatum* on benomyl-amended media were reduced by about 70 and 65%, respectively, compared with those for isolates on nonamended PDA. Benzimidazole fungicides are relatively ineffective for bitter rot control (7). Our sensitivity tests indicate that benomyl would not control the majority of isolates of *Colletotrichum* found in Michigan apple orchards.

In the virulence study, all isolates produced symptoms typical of bitter rot while no symptoms developed on noninoculated wounded fruit. Decayed areas ranged from 16.6 to 35.9 mm after 10 days and there was no apparent difference in lesion diameter among isolates of the two species. Lesions on all fruit inoculated with isolates of *C. acutatum* contained acervuli with orange masses of conidia. Several isolates of *C. gloeosporioides* did not sporulate and may have been perithecial strains; otherwise the symptoms produced by isolates of *C. acutatum* and *C. gloeosporioides* were identical. Although bitter rot was not observed on Golden Delicious in Michigan in 1995, all Michigan isolates were highly virulent on Golden Delicious; bitter rot is a common summer rot disease of Golden Delicious in the southern United States (1, 4,7).

The ITS1 was 171 and 180 bp for *C. gloeosporioides* and *C. acutatum*, respec-

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Colletotrichum acutatum
  <--18S          10          20          30          40          50          60
473      CTGAGTTACC GCTCTATAAC CCTTTGTGAA CGTACCTA** ACCGTTGCTT CGGCGGGCAG
SC4      A......... .A........ .......... ........** .......G.. ..........
S18      .......... .......... .......... ........** .......... ..........
T9       .......... .......... .......... .A......** .......... ..........
B7       .......... AA.......T .......... ........** .......G.. ..........
C. gloeosporioides
G201     .......TA. .......... .......... .A......TA ..T....... ...T...T..
I26      .......TA. .......... .......... .A......TA ..T....... ...C...T..
G10      .......TA. .......... .......... .A......TA ..T....... ...C...T..
T5       .......TA. .......... .......... .A......TA ..T....... ...T...T..
NC1869   .......TA. ......C..A ....A..... .A......TA ..T....... ...T...T..

C. acutatum     70          80          90          100         110         120
473      GGGAAGCCTC TCGCGGGCCT CCCCTCCCGG CGCCGGCCCC CACCACGGGG ACGGGGCGCC
SC4      .......... .......... .......... ......A... .......... ..........
S18      .......... .......... .......... ......A... .......... ..........
T9       .......... .......... .......... ......A... .....G.... ..........
B7       .......... .......... .......... T.....A... .......... ..........
C. gloeosporioides
G201     ..****T... *....A**** *......... .CT.**..G. .T..G*..C. GGTC......
I26      ..****T... *....A**** *......... .CT.**..G. .T..G*..C. GGTC......
G10      ..****T... *....A**** *......... .CT.**..G. .T..G*..C. GGTC..A...
T5       ..****T... *....A**** *......... .CT.**..G. .T..G*..C. GGTC......
NC1869   ..****T... *....A**** *......... GCT.**..G. .C..G*..C. GGTC..A...

C. acutatum    130         140         150         160         170         180     5S-->
473      CGCCGGAGGA *AACCAAACT CTATTTACAC GACGTCTCTT CTGAGTGGCA CAAGCAAATA ATTA
SC4      .......... *......... .......... .......... .......... .......... ....
S18      .......... *......... .......... .......... .......... .......... ....
T9       .......... *......... .......... .......... .......... .......... ....
B7       .......... *......... ......TT.. .......... .......... .......... ....
C. gloeosporioides
G201     .......... T......... ..G...TA.. .....T.... ........T. .......... ..C.
I26      .......... T......... ..G...TA.. .....T.... ........T. .......... ..C.
G10      .......... T......... ..G...TA.. .....T.... ........T. .......... ..CT
T5       .......... T......... ..G...TA..  ....T.... ........T. .......... ..C.
NC1869   .......... T......... ..G...TA.. .....T.... ....A...T. .......... ..C.
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Fig. 1. Comparison of the nucleotide sequences of the internal transcribed spacer region 1 (ITS1) for isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from apples collected in Michigan, with published sequence data for *C. acutatum* isolate 473 (6) and *C. gloeosporioides* isolate G201 (5). In the aligned sequences a dot = a match, an upper-case letter = a mismatch with sequence for *C. acutatum* isolate 473, and an asterisk = a gap.

tively, and the sequence data for each of the eight isolates were in agreement with sequence data from previous studies (Fig. 1). The similarity of the ITS1 sequence data for *C. acutatum* isolate 473 (6) and isolates SC4, S18, T9, and B7 and for *C. gloeosporioides* isolate G201 (5) and isolates I26, G10, T5, and NC1869 was high (>95%), while the similarity between isolates of the two species was low (62 to 67%). The separation of ITS1 sequences for isolates collected in Michigan into two previously identified and distinct groups confirms the initial identification of these isolates based on colony color and morphology.

Bitter rot is particularly important in the southeastern United States (7), where temperatures during the growing season are typically higher than in Michigan. The outbreak of bitter rot in several Michigan orchards was associated with the unusually high temperatures in Michigan during the summer of 1995. Temperatures in southwestern Michigan in 1995 exceeded the 30-year average by 1.8, 1.5, and 3.4°C in June, July, and August, respectively, at a National Weather Bureau observation station maintained near Eau Claire, MI. Also, fire blight was present in many of the affected orchards and fire blight–infected twigs colonized by *Colletotrichum* may have served as an inoculum source (7). Reduced usage of the ethylenebisdithiocarbamate fungicides since 1989, particularly after the petal fall stage of bud development, probably contributed to the current outbreak of bitter rot. Except for being associated with certain cultivars, fruit infected with bitter rot were uniformly distributed in affected orchards. This suggests that the pathogen is endemic in many orchards but symptoms are rare due to unfavorable weather in most years.

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