Identifying Species of Pathogens Causing Bitter Rot of Apples in Illinois and Efficacy of Fungicides for Managing the Disease

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Keywords. *Colletotrichum chrysophilum*, *Colletotrichum fioriniae*, *Colletotrichum siamense*, fruit rot, fungal disease, *Malus ×domestica*

Abstract. **Outbreaks of bitter rot disease occurred in Illinois apple (*Malus ×domestica*) orchards during 2010–20. This study was conducted to assess the incidence of bitter rot in commercial apple orchards in Illinois, identify pathogen species that cause bitter rot, and evaluate the efficacy of fungicides for managing the disease. Orchard surveys conducted during 2019–21 showed that fruits with bitter rot were present in most of the orchards in southern and central Illinois, whereas only a few orchards in the northern part of the state had symptomatic fruits. A total of 270 isolates of the pathogens were collected from symptomatic fruits of 14 cultivars, and pathogen species were identified based on the morphological and molecular characteristics of the isolates. GAPDH gene sequence analyses identified species of the pathogens as *Colletotrichum fioriniae*, *C. siamense*, and *C. chrysophilum*. Laboratory and orchards studies were conducted to evaluate the effectiveness of fungicides for managing bitter rot disease. Laboratory studies showed averages of 10.3, 9.6, and 0.24 $mg \cdot L^{-1}$ for the 50% effective concentrations (EC_{50}) of benzovindiflupyr, captan, and fluxapyroxad + pyraclostrobin fungicides, respectively. Orchard experiments involving 'Honeycrisp apples' were conducted in 2019, 2020, and 2021. Benzovindiflupyr, captan, and fluxapyroxad + pyraclostrobin prevented bitter rot development in the treated plots.**

Bitter rot of apples (*Malus ×domestica*) is a disease caused by *Colletotrichum* species including *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* complexes (Braganca et al. 2016; Dowling et al. 2020; Gonzalez and Sutton 2004). It is currently considered the most important apple disease in Illinois because fruit losses of 100% for some apple cultivars have been recorded in several orchards in the state (Babadoost 2022).

Molecular methods have been widely used to accurately identify pathogen species that cause bitter rot of apples (Freeman et al. 1998; Sreenivasaprasad and Talhinhas 2005). Multilocus phylogenetic analyses are reliable for addressing challenges in the identification of *Colletotrichum* species. Investigators first used primers that target the internal transcribed spacer of the rDNA region (Martinez-Culebras et al. 2003; Mills et al. 1992; Sreenivasaprasad et al. 1994). However, it was concluded that the internal transcribed spacer sequences alone could not fully differentiate *Colletotrichum* spp. from each other (Weir et al. 2012). Therefore, sequence analyses of this genetic region and a combination of other genes that code for actin, calmodulin, glyceraldehyde-3-phosphate dehydrogenase, glutamine synthetase, β-tubulin, and histone have been used to accurately identify *Colletotrichum* species (Faedda et al. 2011; Lubbe et al. 2004; Prihastuti et al. 2009; Talhinhas et al. 2002; Wikee et al. 2011).

Although some apple cultivars are less susceptible to bitter rot (i.e., Red Delicious) than others (i.e., Golden Delicious), no highly resistant apple cultivar is commercially available. Thus, chemical management of the disease remains the dominant method for controlling bitter rot of apples. The availability of only a few effective fungicides to control *Colletotrichum* pathogens and the persistent threat of fungicide resistance development in the pathogens make management of bitter rot of apples a difficult task (Dowling et al. 2020). Previous studies have reported variability in fungicide efficacy against bitter rot, which is, to a large extent, related to differences in fungicide sensitivity among *Colletotrichum* species and efficacy among fungicide active ingredients (Chechi et al. 2019; Khodadadi et al. 2020; Martin et al. 2022; Munir et al. 2016). Differences in fungicide sensitivity among species from a single geographical location may be inherent or may have emerged because of fungicide selection pressure influenced by the species-specific inoculum frequency and density and the influx of wild-type phenotypes from nearby hosts (Chen et al. 2016). Excessive use of fungicides poses the threat of fungicide resistance of the pathogens.

Currently, single-site fungicide groups are commonly recommended in the United States for managing *Colletotrichum* spp. (Dowling et al. 2020). These fungicide groups are demethylation inhibitors [DMIs; FRAC 3 (Fungicide Resistance Action Committee)], fluazinam (FRAC 29), methyl benzimidazole carbamates (MBCs; FRAC 1), phenylpyrroles (PPs; FRAC 12), polyoxins (FRAC 19), quinone-outside inhibitors (QoIs; FRAC 11), and succinate dehydrogenase inhibitors (SDHIs; FRAC 7). MBC fungicides affect the fungal cytoskeleton by directly binding to β-tubulin subunits and inhibiting the assembly of microtubules (Angelini et al. 2015). Insensitivity of *C. acutatum* to MBC fungicides has been reported (Chung et al. 2006). The DMI fungicides are registered as either solo or mixture products for various crops. However, their efficacy as solo products against *Colletotrichum* spp. is poor (DeFrancesco et al. 2017). QoI (strobilurin) fungicides target the cytochrome *b* (*cytb*) gene and inhibit fungal mitochondrial respiration (Bartlett et al. 2002; Sauter et al. 1999; Ypema and Gold 1999). Widespread resistance of *C. acutatum* to QoI fungicides in strawberry (*Fragaria ×ananassa*) (Forcelini and Peres 2018) and apple (Chechi et al. 2019; Martin et al. 2022) have been reported. The PP fungicides are useful because there are only a few reported cases of resistance development in fungi to this

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fungicide group (Kilani and Fillinger 2016). Polyoxin has inconsistent and limited efficacy against *Colletotrichum* spp. on fruit crops (McManus and Perry 2017; Pscheidt and Bassinette 2016; Su and Gubler 2009). Fluazinam works by disrupting oxidative phosphorylation, thus inhibiting mitochondrial respiration. This class of fungicide has shown high efficacy against *C. acutatum* and *C. gloeosporioides* species complexes in vitro (Gang et al. 2015; Leroux 1996). Benzovindiflupyr fungicide (of the SDHI group) has been registered for the management of bitter rot of apples in the midwestern states of the United States and has good efficacy against *C. acutatum* (Beckerman et al. 2023; Dowling et al. 2020). The multisite fungicide captan provides satisfactory management of *C. acutatum*; however, it does not have any curative effect (Dowling et al. 2020; Martin et al. 2022; Mertely et al. 2010).

The objectives of this study were to conduct surveys to assess the incidence of bitter rot disease in commercial orchards in Illinois, identify species of pathogens that cause bitter rot disease, and evaluate the efficacy of fungicides for managing bitter rot of apples.

Materials and methods

Incidence of bitter rot disease in commercial orchards

Orchard surveys were conducted in 2019, 2020, and 2021 to assess the incidence of bitter rot of apples in commercial orchards in Illinois. Surveys included 24, 30, and 33 orchards in 2019, 2020, and 2021, respectively (Table 1, Fig. 1). The surveyed orchards were selected based on major apple-producing locations throughout the state. Seventeen cultivars, including Braeburn, Cortland, Empire, Fuji, Gala, Ginger Gold, Golden Delicious, Golden Supreme, Goldrush, Granny Smith, Honeycrisp, Jonagold, Jonathan, McIntosh, Red Delicious, Snow Sweet, and an unknown cultivar, were evaluated to determine the presence of fruits with bitter rot symptoms (Table 2). For each cultivar block at each orchard, five trees were randomly selected and 60 fruits (five fruits in each of the upper, middle, and lower canopies of eastern, northern, western, and southern sides) of each tree were examined to observe symptoms. The size of each cultivar block ranged from 0.25 to more than 2 ha. In each orchard, all existing cultivar blocks were visited. Orchards with no fruit symptomatic of bitter rot were recorded as "orchards without bitter rot"; orchards with at least one cultivar block with bitter rot symptoms were considered "orchards with bitter rot." Five to 10 symptomatic fruits were collected from each cultivar block. The collected fruits were carried in a cooler and kept at 4 °C until associated fungi were isolated within 72 h of collection (Table 3).

Table 1. Commercial apple orchards surveyed to determine the incidence of bitter rot of fruits in northern[i], central[ii], and southern[iii] Illinois.

Area	Yr	Surveyed orchards, no.	Orchards with bitter rot, no. (%)
Northern	2019	7	0 (0.00)
	2020	9	2 (22.22)
	2021	11	3 (27.27)
	Total	27	5 (18.52)
Central	2019	4	3 (75.00)
	2020	5	3 (60.00)
	2021	6	5 (83.33)
	Total	15	11 (73.33)
Southern	2019	13	11 (84.62)
	2020	16	15 (93.75)
	2021	16	13 (81.25)
	Total	45	39 (86.67)
Total		87	55 (63.22)

[i] Latitudes: 40.90°–42.50°.
[ii] Latitudes: 39.20°–40.90°.
[iii] Latitudes: 37.00°–39.20°.

Isolation and identification of pathogen species

Using sterile blades, small pieces (diameter, 5 mm) of fruit skin and pulp were cut from the outer margins of each lesion of each fruit. The pieces were surface-disinfested by dipping in 95% ethanol for 60 s and then washed three times (1 min each) with sterile distilled water (SDW). Surface-sterilized pieces were dried between sterilized paper tissues and placed on potato dextrose agar (PDA) medium in petri plates (100 mm × 15 mm). The plates were incubated at 24 °C for 5 d in the dark. Pure cultures were obtained by performing single-spore isolation according to the methods reported by Du et al. (2005). Isolated fungal colonies were screened to determine the colony color, conidial shape and size, and growth rate of the colony on PDA. Two single-spore cultures of each isolate were examined after 7 d of growth on PDA. Colony colors were named according to the mycological color chart (Rayner 1970). The size and shape of conidia (fusiform with pointed ends or cylindrical with rounded ends) were examined using a light microscope (Olympus BX41; Olympus Life Science, Tokyo, Japan). The colony growth rate of isolates at 24 °C in the dark was determined by measuring the colony diameter of each isolate daily for 7 d. Isolates were organized into three general groups (morphotypes) based on the colony morphology and color.

All isolates were grown on PDA for 7 d, and genomic DNA was extracted using an E.Z.N.A. MicroElute Genomic DNA kit (Omega Bio Tek, Norcross, GA, USA) according to the manufacturer's protocol. The polymerase chain reaction (PCR) primer pairs GDF1 (5′-GCCGTCAACGACCCCT TCATTGA-3′) and GDR2 (5′-GGGT GGAGTCGTACTTGAGCATGT-3′) for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) amplification (Templeton et al. 1992) was used for PCR amplification. A 25-µL reaction was set up using the GoTaq Green Kit, including 3 µL of genomic DNA, 12.5 µL of GoTaq Green Master Mix, 7.5 µL of DNA-free water, and 1 µL of each of 10 µM GAPDH primers. The PCR amplification was performed using a C1000 Touch thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) with initial denaturation at 95 °C for 2 min, followed by 34 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and final extension for 10 min at 72 °C. From each PCR product, 3 µL was examined in a 1% (weight/volume) agarose gel stained with EtBr in 1× TAE buffer electrophoresed at 96 V

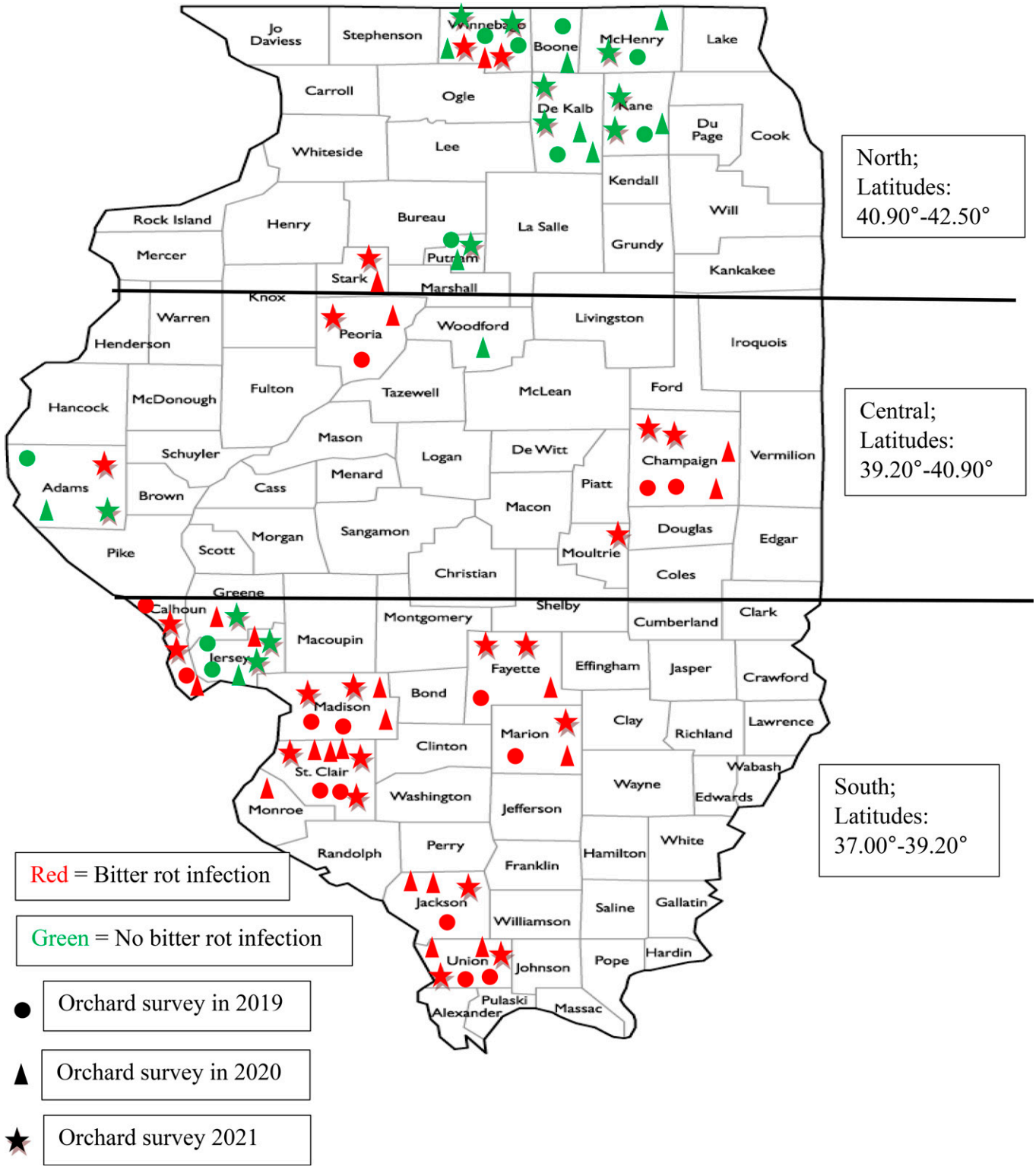

Fig. 1. Locations of surveyed commercial apple orchards in Illinois in 2019, 2020, and 2021.

for 30 min. Then, PCR product purification was performed using a Wizard SV Gel and PCR Clean-Up system (Promega Corporation, Madison, WI, USA), and template DNA was used in 10 µL sequencing reactions with BigDye® Terminator version 3.1 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using the PCR primers. Sequences were generated using an Applied Biosystems 3730XL high-throughput capillary sequencer at the Roy J. Carver Biotechnology Center (University of Illinois, Urbana, IL, USA). Sequences were assembled with Sequencher 5.4 (Gene Codes Corp., Ann Arbor, MI, USA), and their identification was confirmed by comparing type strain sequences using the BLASTn database from the National Center for Biotechnology

Table 2. Apple cultivars in Illinois commercial orchards surveyed to determine the incidence of bitter rot disease during 2019–21.

	Orchard block surveyed (no.)									
	2019			2020			2021			Total orchard block
Cultivar	Northern[i]	Central[ii]	Southern[iii]	Northern[i]	Central[ii]	Southern[iii]	Northern[i]	Central[ii]	Southern[iii]	
Braeburn						1 (1)[iv]				1 (1)
Cortland						1 (1)				1 (1)
Empire	5 (0)	1 (1)	3 (3)	7 (0)	3 (1)	2 (2)	5 (1)	2 (1)	3 (3)	31 (12)
Fuji				1 (0)		3 (2)	2 (0)	–	4 (1)	10 (3)
Gala	6 (0)	2 (0)	7 (3)	6 (0)		3 (3)	7 (0)	1 (0)	1 (0)	33 (6)
Ginger Gold							1 (0)			1 (0)
Golden Delicious			3 (2)	2 (0)	2 (0)	8 (6)	9 (0)	3 (0)	11 (3)	38 (11)
Golden Supreme									1 (0)	1 (0)
Goldrush								1 (1)		1 (1)
Granny Smith						1 (1)				1 (1)
Honeycrisp	6 (0)	4 (2)	4 (3)	9 (1)	5 (2)	7 (7)	10 (3)	6 (3)	7 (4)	58 (25)
Jonagold			1 (0)			1 (1)			1 (0)	3 (1)
Jonathan	7 (0)	2 (0)	7 (6)	7 (1)	3 (0)	12 (10)	9 (0)	3 (0)	12 (10)	62 (27)
McIntosh	1 (0)			1 (1)						2 (1)
Red Delicious						1 (0)	2 (0)		7 (2)	10 (2)
Snow Sweet							1 (0)			1 (0)
Unknown									1 (1)	1 (1)
Total orchard block	25 (0)	9 (3)	25 (17)	33 (3)	13 (3)	40 (33)	46 (4)	16 (5)	48 (24)	255 (92)

[i] Latitudes: 40.90°–42.50°.
[ii] Latitudes: 39.20°–40.90°.
[iii] Latitudes: 37.00°–39.20°.
[iv] Numbers outside parentheses indicate the number of orchards surveyed, and numbers inside parentheses indicate the number of surveyed orchards with symptomatic bitter rot fruits.

Information (NCBI, Bethesda, MD, USA).

Pathogenicity of the isolates

Identified *Colletotrichum* isolates from 'Empire', 'Golden Delicious', and 'Honeycrisp' apple fruits were tested to determine their pathogenicity in a laboratory (Table 4). Apple fruits used for this experiment were harvested and stored at 4 °C in the dark for up to 2 weeks before inoculating. Fruits were washed with liquid detergent and tap water to ensure that no fungicide residue remained on the surface. Then, the fruits were surface-disinfested by soaking in 0.5% sodium hypochlorite for 2 min, rinsed in SDW twice (1 min each time), and air-dried on sterile paper towels on a laboratory bench at room temperature (24 °C). Ten fruits of each cultivar were inoculated with each of the identified *Colletotrichum* isolate. Fruits were wounded using syringe needles to produce a puncture depth of 0.5 mm (Velho et al. 2014, 2015). The wounded fruits were inoculated by adding 6 µL of a conidial suspension (1×10^4 mL^{-1} conidia in SDW water, determined using a hemocytometer) to each wounded spot. The conidial suspension was prepared from 7-d-old PDA cultures (Munir et al. 2016). Control fruits were wounded and treated with SDW. The fruits were placed on moist paper towels in translucent plastic boxes and incubated at room temperature (24 °C) with 12 h of light and 12 h of darkness. The diameters of the developed lesions were measured at 3, 6, 9, 12, and 15 d after inoculation.

Table 3. Number of *Colletotrichum* isolates collected from symptomatic apple fruits in commercial orchards in three different areas of Illinois during 2019–21.

			Colletotrichum species identified, no. (%)		
Area	Yr	Isolates collected (no.)	*C. fioriniae*	*C. siamense*	*C. chrysophilum*
Northern[i]	2019	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
	2020	7	5 (71.43%)	2 (28.57%)	0 (0.00%)
	2021	8	6 (75.00%)	2 (25.00%)	0 (0.00%)
	Total	15	11 (73.33%)	4 (26.67%)	0 (0.00%)
Central[ii]	2019	14	6 (42.86%)	8 (57.14%)	0 (0.00%)
	2020	17	7 (41.18%)	10 (58.82%)	0 (0.00%)
	2021	18	2 (11.11%)	11 (61.11%)	5 (27.78%)
	Total	49	15 (30.61%)	29 (59.19%)	5 (10.20%)
Southern[iii]	2019	66	44 (66.67%)	22 (33.33%)	0 (0.00%)
	2020	69	40 (57.97%)	29 (42.03%)	0 (0.00%)
	2021	71	26 (36.62%)	45 (63.38%)	0 (0.00%)
	Total	206	110 (53.40%)	96 (46.60%)	0 (0.00%)
Total		270	136 (50.37%)	129 (47.78%)	5 (1.85%)

[i] Latitudes: 40.90°–42.50°.
[ii] Latitudes: 39.20°–40.90°.
[iii] Latitudes: 37.00°–39.20°.

Fungicide efficacy for managing bitter rot

Effects of fungicides on colony development of *Colletotrichum* isolates in the laboratory. Three isolates of each of *Colletotrichum*

Table 4. Mean lesion diameter of *Colletotrichum* isolates on three apple cultivars 15 d after inoculation in the laboratory.

Species	Mean lesion diam (mm[i]) on apple fruits		
	Empire[ii]	Golden Delicious[ii]	Honeycrisp[ii]
Colletotrichum siamense	55.3 a[iii]	55.4 a	55.0 a
Colletotrichum fioriniae	50.0 a	47.4 a	45.3 a
Colletotrichum chrysophilum	35.1 b	37.2 b	30.8 b

[i] 1 mm = 0.0394 inches.
[ii] Apple cultivars.
[iii] Values within each column followed with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

chrysophilum, *Colletotrichum fioriniae*, and *Colletotrichum siamense* collected from commercial apple orchards in Illinois were included in this study. Isolates of *C. fioriniae* and *C. siamense* were collected from northern (latitudes: 40.90°–42.50°), central (latitudes: 39.20°–40.90°), and southern (latitudes: 37.00°–39.20°) Illinois (one isolate from each area), and all three isolates of *C. chrysophilum* were from central Illinois (the only location where it was collected).

Fungicide sensitivity of the isolates was evaluated against six fungicides registered for use on apple trees. The fungicides were benzovindiflupyr (Aprovia 0.83SC; Syngenta Corp., Greensboro, NC, USA), captan (Captan 80WDG; Loveland Products Inc., Greeley, CO, USA), fluxapyroxad + pyraclostrobin (Merivon 4.18SC; BASF Corp., Research Triangle Park, NC, USA), potassium phosphite (ProPhyt 4.2SC; Luxembourg-Pamol, Inc., Woodbridge, VA, USA), trifloxystrobin (Flint Extra 4.05SC; Bayer CropScience, St. Louis, MO, USA), and zinc dimethyldithiocarbamate (Ziram 76DF; United Phosphorus, Inc., King of Prussia, PA, USA) (Table 5). For the commercial Merivon 4.18SC fungicide, the rates of fluxapyroxad and pyraclostrobin were equal (21.26% each).

Isolates were grown on PDA in petri plates at 24 °C in the dark for 5 d. A mycelial plug (diameter, 3 mm) was cut from the actively growing part of each colony and transferred onto amended PDA in each petri plate. Concentrations of active ingredients in amended PDA were as follows: 8.3, 41.5, 83, and 830 mg·L^{-1} for benzovindiflupyr; 0.8, 8, 80, 400, and 800 mg·L^{-1} for captan; 0.02, 0.11, 2.13, 10.63, 21.26, and 212.6 mg·L^{-1} for fluxapyroxad + pyraclostrobin; 27.25, 54.5, 545, 818.5, and 1090 mg·L^{-1} for potassium phosphite; 4.26, 21.3, 42.6, and 426 mg·L^{-1} for trifloxystrobin; and 7.6, 38, 76, and 380 mg·L^{-1} for zinc dimethyldithiocarbamate. Control PDA plates contained no fungicide. Four replicate plates were included for each fungicide–isolate treatment, and the tests were repeated twice.

The colony diameter of the isolates was measured 5 d after placing the isolate plug onto PDA. The percentage inhibition of each colony was calculated using the following equation:

$$\frac{C-F}{C}*100$$

where C is the colony diameter (in mm) on the control PDA plate and F is the colony diameter (in mm) on the PDA plate amended with fungicide.

After analyzing the data, we used a linear regression model, $\Upsilon_i = \beta_0 + \beta_1 X_i$, to determine the 50% effective concentration (EC_{50}) values of the fungicides. In this model, Υ was the value of the ith dependent variable, β_0 was the intercept parameter, β_1 was the slope parameter, and X_i was value of the ith independent variable. The percent inhibition of the colony diameter was the dependent variable, and the fungicide concentration was the independent variable.

Efficacy of fungicides on managing bitter rot in the orchard. Orchard experiments were conducted in 2019, 2020, and 2021, to evaluate the effectiveness of the selected fungicides for managing bitter rot and other summer diseases of 'Honeycrisp' apples. The experiments were conducted at the University of Illinois Fruit Research Farm in Urbana, IL, USA (lat. 40°4.91′ N, long. 88°12.96 W, elevation 222 m). The trees were 10 years old in 2019; they were planted in 24 rows, with each row comprising 10 trees. Bitter rot had

Table 5. Concentration of selected fungicides for the EC_{50}[i] of colony development of *Colletotrichum* spp. from apple orchards in Illinois on PDA[ii] medium amended with the fungicides.

Colletotrichum species (area)	Fungicide EC_{50} (mg·L^{-1})[iii]					
	Benzovindiflupyr	Captan	Fluxapyroxad + pyraclostrobin	Potassium phosphite	Trifloxystrobin	Zinc dimethyldithio carbamate
C. chrysophilum (central)	6.4	6.2	0.07	625.9	57.8	31.5
C. chrysophilum (central)	6.4	6.2	0.07	650.0	57.8	30.0
C. chrysophilum (central)	6.1	6.2	0.08	625.9	55.4	30.0
C. fioriniae (northern)	9.7	9.2	0.13	938.9	87.8	41.5
C. fioriniae (central)	9.9	9.0	0.12	862.9	87.8	43.1
C. fioriniae (southern)	9.9	9.5	0.13	938.9	90.1	43.1
C. siamense (northern)	8.9	8.5	0.12	890.7	83.2	36.5
C. siamense (central)	8.7	8.5	0.12	866.6	78.5	41.5
C. siamense (southern)	8.7	8.8	0.13	866.6	85.1	41.5
Mean	8.3	8.0	0.11	818.5	76.1	37.6

[i] Effective fungicide concentration in reducing colony diameter of the fungal isolates by 50%.
[ii] Potato dextrose agar.
[iii] 1 mg·L^{-1} = 1 ppm.

Table 6. Fungicides tested in 2019 for the management of bitter rot caused by *Colletotrichum* spp. in the Honeycrisp apple orchard in Illinois.

Fungicide (active ingredient)	FRAC[i] code	Spray interval (days)	Fungicide rate, active ingredient ($kg \cdot ha^{-1}$)[ii]	Incidence of fruit with bitter rot (%)
Benzovindiflupyr[iii]	7	7	0.05	0.00
Captan[iv]	M4	7	4.48	0.00
Fluxapyroxad + pyraclostrobin[v]	7 + 11	7	0.20	0.00
(Fluxapyroxad + pyraclostrobin) + captan	7 + 11, M4	7	0.20 + 4.48	0.00
(Fluxapyroxad + pyraclostrobin) + captan	7 + 11, M4	10	0.20 + 4.48	0.00
(Fluxapyroxad + pyraclostrobin) + captan	7 + 11, M4	14	0.20 + 4.48	0.00
Monopotassium and dipotassium phosphite[vi]	P07	7	2.89	22.20
Potassium phosphite[vii]	P07	10	3.73	24.87
Potassium phosphite + captan	P07, M4	10	3.73 + 4.48	0.00
Thiophanate-methyl[viii] + captan	1, M4	10	0.78 + 4.48	0.00
Trifloxystrobin[ix] + captan	11, M4	10	0.10 + 4.48	0.00
Zinc dimethyldithiocarbamate[x] + captan	M3, M4	10	5.11 + 4.48	0.00

[i] Fungicide Resistance Action Committee.
[ii] 1 $kg \cdot ha^{-1}$ = 0.8922 lb/acre.
[iii] Commercial product: Aprovia 0.83SC (Syngenta Crop., Greensboro, NC, USA).
[iv] Commercial product: Captan 80WDG (Loveland Products Inc., Greeley, CO, USA).
[v] Commercial product: Merivon 4.18SC (BASF Corp., Research Triangle Park, NC, USA).
[vi] Commercial product: Reliant 5.17SC (Quest Products, LLC, Greeley, CO, USA).
[vii] Commercial product: ProPhyt 4.2SC (Luxembourg-Pamol, Inc., Woodbridge, VA, USA).
[viii] Commercial product: Topsin-M 70WSB (United Phosphorus, Inc., King of Prussia, PA, USA).
[ix] Commercial product: Flint Extra 4.05SC (Bayer CropScience, St. Louis, MO, USA).
[x] Commercial product: Ziram 76DF (United Phosphorus, Inc., King of Prussia, PA, USA).

occurred in orchards during the previous 5 years.

In 2019, eight fungicides (Table 6) in 12 treatments were evaluated. For each treatment, two rows with up to 20 trees were included. To control spring diseases (powdery mildew, rusts, scab), the trees were sprayed with mancozeb (Manzate PRO Stick 0.75WP; United Phosphorus, Inc.) plus difenoconazole + cyprodinil (Inspire Super 2.82SC; Syngenta Corp.) and alternated with mancozeb plus penthiopyrad (Fontelis 1.67SC; Corteva Agriscience LLC, Indianapolis, IN, USA) at weekly intervals from the green-tip growth stage to 1-week before petal fall. Applications of the fungicides (Table 6) for managing summer diseases began at petal fall on 20 May and ended on 19 Aug (21 d before harvesting fruits). Fungicides were spray-applied at 7-, 10-, and 14-d intervals with an SR 450 motorized backpack sprayer (STIHL Corp., Virginia Beach, VA, USA) using 2 L of spray suspension per tree.

Four trees in each treatment were randomly selected to evaluate the incidence of summer diseases. The incidence of diseases was determined on 28 Jul and 9 Sep. The percentage of symptomatic fruits in each tree was determined by examining 60 fruits, including five fruits from each of the upper, middle, and lower canopies on each of the northern, eastern, southern, and western sides. A total of 240 fruits in each treatment were examined for disease symptoms.

In 2020 and 2021, experiments were conducted at the same orchard block of 2019. Management of spring diseases was as described for the 2019 experiment. Selected fungicides for evaluations and spray intervals were considered after analyzing the results of the experiment in 2019.

Tested fungicides were benzovindiflupyr, captan, fluxapyroxad + pyraclostrobin, potassium phosphite, thiophanate-methyl (Topsin-M 70WSB; United Phosphorus, Inc.), trifloxystrobin, and zinc dimethyldithiocarbamate (Tables 7 and 8). The experiment was performed using a randomized complete block design with four replications. Each plot had two trees. Fungicides were spray-applied at 10- and 14-d intervals. Applications of the fungicides for managing summer diseases in 2020 began at petal fall on 18 May and ended on 26 Aug (17 d before harvesting fruits). Applications of the fungicides in 2021 began at petal fall on 14 May and

Table 7. Fungicides tested in the orchard in 2020 and 2021 to determine their effectiveness for the management of *Colletotrichum* spp. and incitants of bitter rot disease of apples in Illinois.

Fungicide		
Common name (active ingredient)	Commercial name (manufacturer)	FRAC[i] code
Benzovindiflupyr	Aprovia 0.83SC (Syngenta Corp., Greensboro, NC, USA)	7
Captan	Captan 80WDG (Loveland Products Inc., Greeley, CO, USA)	M4
Fluxapyroxad + pyraclostrobin	Merivon 4.18SC (BASF Corp., Research Triangle Park, NC, USA)	7 + 11
Potassium phosphite	ProPhyt 4.2SC (Luxembourg-Pamol, Inc., Woodbridge, VA, USA)	P07
Thiophanate-methyl	Topsin-M 70WSB (United Phosphorus, Inc., King of Prussia, PA, USA)	1
Trifloxystrobin	Flint Extra 4.05SC (Bayer CropScience, St. Louis, MO, USA)	11
Zinc dimethyldithiocarbamate	Ziram 76DF (United Phosphorus, Inc., King of Prussia, PA, USA)	M3

[i] Fungicide Resistance Action Committee.

Table 8. Efficacy of selected fungicides for the management of bitter rot of 'Honeycrisp' apples in Illinois.

Fungicide, active ingredient rate (kg·ha^{-1})[ii]	Spray interval (d)	Application dates		Incidence of fruit with bitter rot (%)[i]							
				1 Aug		22 Aug		5 Sep		12 Sep	
		2020[iii]	2021[iv]	2020	2021	2020	2021	2020	2021	2020	2021
Untreated check				8.1 a[v]	7.4 a	79.4 a	98.2 a	100 a	100 a	100 a	100 a
Benzovindiflupyr (0.05) + captan (4.48) *alt* trifloxystrobin (0.10) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.8 bc	0.0 b	2.5 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48)	10	(1,2,4,6,7,9,11, 12,13,15,17)	(1,2,4,6,7,9,10, 12,13,14,16)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	1.4 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48)	14	(1,3,5,8,10,12, 14,16)	(1,3,5,8,9,11, 13,15,17)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	0.5 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48) *alt* trifloxystrobin (0.10) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.6 bc	0.0 b	1.7 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48) *alt* potassium phosphite (3.73) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	1.8 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48) *alt* thiophanate-methyl (0.78) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.9 bc	0.0 b	2.4 b
Fluxapyroxad + pyraclostrobin (0.20) + captan (4.48) *alt* zinc dimethyldithiocarbamate (5.11) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b	2.3 b
Thiophanate-methyl (0.78) + captan (4.48) *alt* trifloxystrobin (0.10) + captan (4.48)	14	(1,5,10,14) *alt* (3,8,12,16)	(1,5,9,13,17) *alt* (3,8,11,15)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	1.9 b	0.0 b	2.6 b

[i] Percent of fruits with bitter rot was determined by examining fruits of four trees of each treatment. In each tree, 60 fruits were examined, including five fruits from each of the upper, middle, and lower canopies on each of the northern, eastern, southern, and western sides.

[ii] 1 kg·ha^{-1} = 0.8922 lb/acre.

[iii] Application dates in 2020: 1 = 18 May; 2 = 28 May; 3 = 1 Jun; 4 = 7 Jun; 5 = 15 Jun; 6 = 17 Jun; 7 = 27 Jun; 8 = 29 Jun; 9 = 7 Jul; 10 = 13 Jul; 11 = 17 Jul; 12 = 27 Jul; 13 = 6 Aug; 14 = 10 Aug; 15 = 16 Aug; 16 = 24 Aug; and 17 = 26 Aug.

[iv] Application dates in 2021: 1 = 14 May; 2 = 24 May; 3 = 28 May; 4 = 3 Jun; 5 = 11 Jun; 6 = 13 Jun; 7 = 23 Jun; 8 = 25 Jun; 9 = 3 Jul; 10 = 13 Jul; 11 = 17 Jul; 12 = 23 Jul; 13 = 26 Jul; 14 = 5 Aug; 15 = 10 Aug; 16 = 15 Aug; and 17 = 20 Aug.

[v] Values within each column followed with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

ended on 20 Aug (17 d before harvest). Spray applications of fungicides were as described for the 2019 experiment. One tree in each plot with a good fruit load was selected to evaluate disease symptoms. In 2020, the incidence of diseases was assessed on 25 Jul; 1, 8, 15, 22, and 29 Aug; and 5 and 12 Sep. In 2021, the incidence of diseases was assessed on 11, 18, and 25 Jul; 1, 8, 15, 22, and 29 Aug; and 5 and 12 Sep. The percentage of symptomatic fruits in each tree was determined by examining 60 fruits per tree, as previously described.

Results

Incidence of bitter rot disease in commercial orchards

Bitter rot incidence was recorded in 14 of 24 orchards visited in 2019, 20 of 30 orchards visited in 2020, and 21 of 33 orchards visited in 2021 (Fig. 1). During the three years of surveys, fruits with bitter rot were observed in 55 of 87 (63.22%) of the commercial orchards (Table 1). Overall, 18.52%, 73.33%, and 86.67% of visited apple orchards in northern, central, and southern Illinois, respectively, had fruits with bitter rot symptoms (Table 1). The incidence of bitter rot in the orchards ranged from 0% to 63% in 2019, and from 0% to 100% in both 2020 and 2021. The highest percents of fruits (100%) with bitter rot symptoms were observed for 'Honeycrisp' in central Illinois and 'Empire' in southern Illinois in 2019, for 'Golden Delicious' and 'Empire' in southern Illinois, and for 'Honeycrisp' and 'Empire' in central Illinois in 2020 and 2021. Overall, the incidence of fruits with bitter rot symptoms of Empire, Golden Delicious, Honeycrisp, and Jonathan apples was higher than that of other apple cultivars (Table 2).

Identification of pathogen species

The GAPDH sequences of 270 bp confirmed that all isolates tested were *Colletotrichum* species. Based on the colony morphology, 270 isolates were divided into morphotype groups 1, 2, and 3. Morphotype group 1, which comprised 136 isolates (Table 3), produced a salmon to pink colony on PDA after 7 d that was visible on the top and bottom of the colony (Fig. 2A and B). Conidia produced by isolates in this group were fusiform with acute ends. Based on these morphological

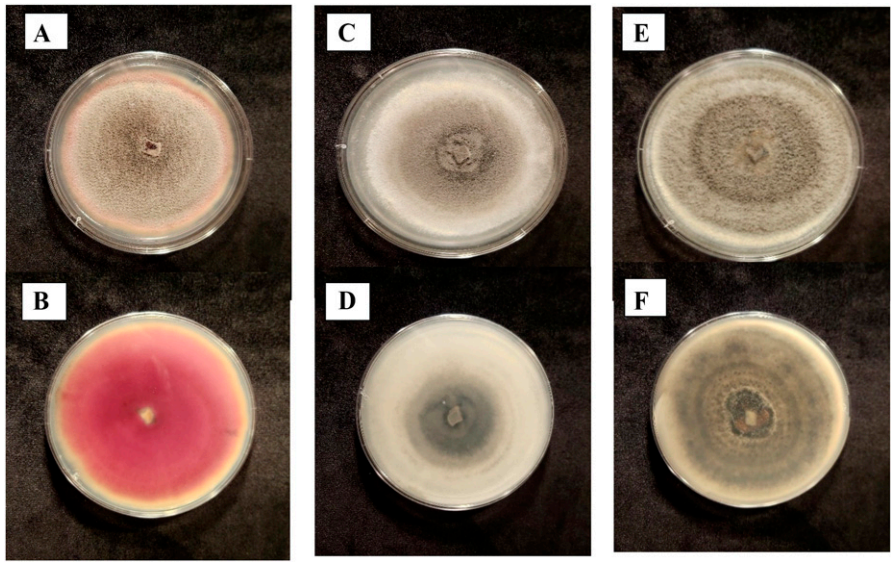

Fig. 2. Colonies of *Colletotrichum* species identified in fruits with bitter rot symptoms in commercial apple orchards in Illinois during 2019–21. Cultures on potato dextrose agar in petri plates were 15 d old. (A, C, and E) The top of the culture plates of *Colletotrichum fioriniae*, *C. siamense*, and *C. chrysophilum*, respectively. (B, D, and F) The bottom of the culture plates of *Colletotrichum fioriniae*, *C. siamense*, and *C. chrysophilum*, respectively.

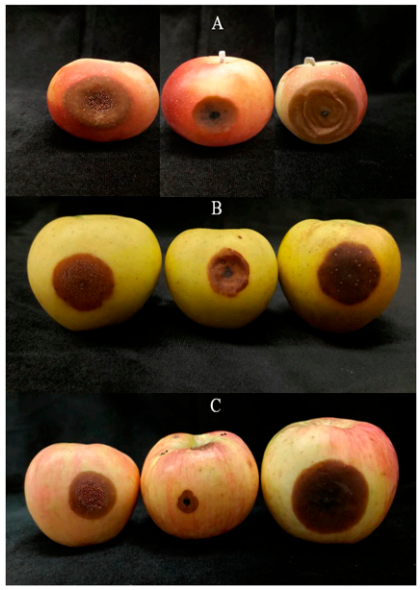

Fig. 3. Symptoms of bitter rot disease on inoculated apple fruits of 'Empire' (A), 'Golden Delicious' (B), and 'Honeycrisp' (C) in the laboratory. *Colletotrichum fioriniae* (left), *C. chrysophilum* (center), and *C. siamense* (right). Acervuli were produced by *C. fioriniae* on 'Empire', 'Golden Delicious', and 'Honeycrisp' apples.

characteristics, isolates in this group corresponded to *C. fioriniae* in the *C. acutatum* complex. Morphotype group 2, which comprised 129 isolates (Table 3), produced a white to light gray colony with light grey centers on PDA (Fig. 2C and D). Conidia were cylindrical with rounded ends. Colonies of isolates in morphotype group 2 grew faster than colonies of isolates in morphotype group 1. These characteristics of morphotype group 2 were consistent with descriptions of *C. siamense* in the *C. gloeosporioides* complex. Morphotype group 3, which comprised five isolates (Table 3), produced a dark gray colony on PDA (Fig. 2E and F). These isolates produced cylindrical conidia with rounded ends. Colonies of this group grew faster than isolates in morphotype groups 1 and 2. Colony characteristics of this group were consistent with descriptions of *C. chrysophilum*, which is also in the *C. gloeosporioides* complex. *C. fioriniae* produced salmon to pink coloration on PDA, whereas neither of the other two species produced any pigment.

One hundred representative isolates from the three morphotype groups were selected for the molecular study. These represented the different geographical locations of the orchards and variations in apple cultivars. Sequence similarity searches of only type strains using BLASTn indicated that all isolates in morphotype group 1 matched with *C. fioriniae*, that those in morphotype group 2 matched *C. siamense*, and that those in morphotype group 3 matched *C. chrysophilum*. The ranges of sequence variations within each species compared with the type sequence were 0% to 2.1% for *C. fioriniae* (JQ948622), 0% to 2.8% for *C. siamense* (JX009924), and 0% to 0.9% for *C. chrysophilum* (KX094183). Five unique genotype sequences were deposited in NCBI for *C. fioriniae* (OR140159–OR140163), four were deposited for *C. siamense* (OR137130–OR137133), and two were deposited for *C. chrysophilum* (OR142761–OR142762).

Pathogenicity of the isolates

All 270 isolates of *Colletotrichum* species were pathogenic and produced typical bitter rot symptoms on inoculated apple fruits of 'Empire', 'Golden Delicious', and 'Honeycrisp'. The symptoms exhibited noticeably sunken lesions with orange or dark acervuli from infections by *C. fioriniae* (Fig. 3). In the cross-section, necrotic V-shaped symptomatic tissues were observed beneath the surface lesions. We conducted the mean separation procedure using Fischer's least significant difference to compare diameters of lesions developed following inoculation of apple fruits. There was a significant ($P = 0.015$) difference between the mean lesion diameters of *C. siamense* and *C. chrysophilum*, a significant ($P = 0.05$) difference between those of *C. fioriniae* and *C. chrysophilum*, and no significant ($P > 0.05$) difference between those of *C. siamense* and *C. fioriniae* (Table 4). *C. chrysophilum* produced deeper lesions on 'Empire' and 'Golden Delicious' fruits than the other two pathogens. Only isolates of *C. fioriniae* produced acervuli on the lesions (Fig. 3). Pathogens were re-isolated from the lesions and re-identified as *Colletotrichum* spp. using the aforementioned methods. Fruits treated with SDW did not develop any symptoms.

Fungicide efficacy

Laboratory fungicide evaluations. The mean EC_{50} values of benzovindiflupyr, captan, fluxapyroxad + pyraclostrobin, potassium phosphite, trifloxystrobin, and zinc dimethyldithiocarbamate were 8.3, 8.0, 0.11, 818.5, 76.1, and 37.6 mg·L^{-1}, respectively, for all nine isolates tested (Table 5).

Orchard evaluations. During the orchard experiments, bitter rot was the only disease that developed in the plots. In 2019, the disease was first

observed on 21 Jul, and its incidence increased as the season progressed. Benzovindiflupyr, captan, and fluxapyroxad + pyraclostrobin were the most effective fungicides for preventing the development of bitter rot on fruits. Benzovindiflupyr and captan were effective at all three spray intervals (7, 10, and 14 d). Benzovindiflupyr was effective at the 7-d interval (only sprayed interval) for protecting against disease development. Potassium phosphite and monopotassium and dipotassium phosphite (Reliant; Quest Products, LLC, Greeley, CO, USA) were the least effective fungicides for preventing disease development. The incidence of bitter rot of fruits was significantly ($P = 0.01$) higher in untreated plots than in the treated plots. Detailed results of the experiment in 2019 are not presented because some fungicides did not effectively protect fruits against bitter rot disease (Table 6), and there were no significant differences in the applications of benzovindiflupyr, captan, and fluxapyroxad + pyraclostrobin at 7-, 10-, and 14-d intervals.

In 2020, bitter rot was observed only in untreated plots. The disease was first observed on 25 Jul in two untreated plots, and its incidence in untreated plots increased as the season progressed. The disease incidence in untreated plots was 100% on 12 Sep. Bitter rot and other diseases of apples were not observed in the treated plots. All fungicide combinations, applied either at 10-d or 14-d intervals, were effective for preventing disease development (Table 8).

In 2021, fruits with bitter rot were first observed on 11 Jul in two untreated plots, and its incidence in untreated plots increased toward the end of the season. Bitter rot and other diseases of apple were not observed in treated plots until 5 Sep (16 d after the last fungicide application). Symptomatic fruits were observed in some treated plots on 5 Sep (Table 8). On 12 Sep (21 d after the last fungicide applications), bitter rot was observed in most of the plots. The lowest incidence of symptomatic fruits (0.5%) was observed in the plots sprayed with fluxapyroxad + pyraclostrobin plus captan (Table 8). More rainfall than usual occurred from 20 Aug to 15 Sep in 2021.

Discussion

This was the first field survey to assess the incidence of bitter rot disease in commercial apple orchards in Illinois. The results of our studies showed that some apple cultivars (Empire, Golden Delicious, Honeycrisp, and Jonathan) in Illinois are more susceptible than other cultivars. Studies in other apple-growing areas have also shown that 'Honeycrisp and 'Golden Delicious' are more susceptible to bitter rot pathogens (Biggs and Miller 2001). Additionally, the disease was more prevalent in southern Illinois (with warmer and more humid conditions) than in northern Illinois (with cooler and less humid conditions), consistent with the results of Martin et al. (2021), who also reported that warmer temperatures and moist conditions are more conducive for bitter rot development.

In previous studies, combinations of morphology and molecular methods have been used to achieve accurate identification of *Colletotrichum* species (Gonzalez and Sutton 2004; Mills et al. 1992). The results of our study confirmed that using both colony morphology and molecular methods are necessary for the accurate identification of *Colletotrichum* species.

Our research identified *Colletotrichum* species that cause bitter rot of apples in Illinois orchards belong to the following three species: *C. chrysophilum* (1.85% of isolates), *C. fioriniae* (50.37% of isolates), and *C. siamense* (47.78% of isolates). These species belong to the *C. acutatum* and *C. gloeosporioides* species complexes and have been caused bitter rot of apples in other apple-growing areas in the world (Dowling et al. 2020; González et al. 2006; Martin and Peter 2021; Munir et al. 2016; Park et al. 2018; Peres et al. 2007). In 2021, *C. chrysophilum* was identified as a causal agent of bitter rot in only one orchard in central Illinois. This finding represents the first report of *C. chrysophilum* as a cause of bitter rot of apples in the Midwestern United States and the third report of *C. chrysophilum* as a pathogen of apples in the United States. *C. chrysophilum* was first reported as an incitant of bitter rot of apples in New York in 2020 (Khodadadi et al. 2020). *C. fioriniae* was the dominant species in the northern area, whereas *C. siamense* was more prevalent in the central and southern parts of the state. Both species were found within the same orchard and on the same fruit in several locations across the state. Overall, *C. fioriniae* was the most prevalent species identified. This is consistent with the findings of previous studies in Kentucky (Munir et al. 2016) and New York (Khodadadi et al. 2020), which reported *C. fioriniae* as the dominant species in both states.

All three identified *Colletotrichum* species caused bitter rot in inoculated apple fruits in the laboratory. On average, *C. siamense* produced larger lesions than *C. fioriniae* and *C. chrysophilum* on all three apple cultivars tested. This result is similar to that of previous studies in Kentucky (Munir et al. 2016) and South Korea (Oo et al. 2018). *C. fioriniae* isolates were the only ones to consistently exude red pigment on PDA, thus serving as a reliable morphological feature used for *C. fioriniae* identification.

Effective management of bitter rot requires timely cultural practices and applications of effective fungicides. Because species of bitter rot pathogens may vary in their sensitivity to fungicides (Chechi et al. 2019; Dowling et al. 2020; Gonzalez and Sutton 2004; Khodadadi et al. 2020; Martin et al. 2022; Wang et al. 2015), we evaluated the effectiveness of the potential fungicides in the laboratory against isolates of three identified species of *Colletotrichum* that had been identified in Illinois. Both laboratory and field studies showed that benzovindiflupyr, captan, and pyraclostrobin + fluxapyroxad were highly effective for managing *Colletotrichum* species that cause bitter rot disease of apples. Similar results have been reported by other studies of the management of bitter rot of apples (Chechi et al. 2019; Chen et al. 2016; Khodadadi et al. 2020; Martin et al. 2022; Munir et al. 2016) and *Colletotrichum* spp. on different crops (Adaskaveg and Hartin 1997; Bernstein et al. 1995; Freeman et al. 1998; Peres et al. 2007; Valero et al. 2010; Velho et al. 2014, 2015).

Results from our orchard trials revealed that benzovindiflupyr, captan, and fluxapyroxad + pyraclostrobin applied at either 10- or 14-d intervals were highly effective for managing bitter rot disease on 'Honeycrisp' apple. We did not have access to other apple cultivars susceptible to bitter rot for additional orchard experiments. Investigating the effectiveness of these fungicides for managing bitter rot and other summer diseases of apples such as 'Empire', 'Golden Delicious', and 'Jonathan' would provide more information regarding the chemical management of

bitter rot disease of apples. However, commercial apple growers in Illinois who used the findings of our studies during 2020–2023 regarding various apple cultivars expressed their high satisfaction with the results.

Preharvest intervals of benzovindiflupyr (FRAC 7), captan (FRAC M4), and fluxapyroxad + pyraclostrobin (FRAC 7 + 11) are 30, 0, and 0 d, respectively. Thus, we suggest using benzovindiflupyr during the early summer season. Mixed sprays of captan with trifloxystrobin (FRAC 11), a phosphonate compound (i.e., potassium phosphite or monopotassium and dipotassium phosphite; FRAC P07), thiophanate-methyl (FRAC 1), or zinc dimethyldithiocarbamate (FRAC M) were effective for protecting apple fruits against bitter rot and other summer diseases. Spray applications of any of these mixed compounds in alternation with fluxapyroxad + pyraclostrobin plus captan are effective. For some of the treatments used for our orchard experiments, the numbers of applications and/or maximum rates of fungicides per season were more than those recommended by the labels. These procedures were performed for research purposes to determine the effectiveness of the fungicides. The label recommendations regarding the numbers of applications and total amount per season for each fungicide must be followed. During the past 3 years, we have been monitoring applications of the recommended fungicides according to the label directions in commercial apple orchards in Illinois. The recommended treatments in commercial orchards have been highly effective for managing bitter rot and other diseases of apples in Illinois.

Conclusions and recommendations

The results of our study and those of studies by other investigators have shown that bitter rot disease caused by *Colletotrichum* spp. is one of the most serious diseases of apples in areas with moist conditions. To develop effective management of bitter rot in each apple-growing area, the following actions are necessary: determine the susceptibility of the cultivars; identify the species of the causal agent(s); test the potential fungicides to determine their efficacy; and, after considering preharvest intervals specified on the fungicide labels, spray trees with effective fungicides at 14-d intervals until a maximum of 2 weeks before fruit harvest. All apple growers in Illinois who conducted timely cultural practices (removed mummified fruit, dead wood, and fire-blighted twigs before bloom) and applied the recommended fungicides in a timely manner reduced the incidence of bitter rot in their orchards from up to 100% fruit losses to 0% or negligible losses in most of the orchards with a history of bitter rot.

References cited

Adaskaveg JE, Hartin RJ. 1997. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology. 87(9): 979–987. https://doi.org/10.1094/PHYTO.1997.87.9.979.

Angelini RM, Pollastro S, Faretra F. 2015. Genetics of fungicide resistance, p 13-34. In: Ishii H, Holloman DW (eds). Fungicide resistance in plant pathogens. Springer, Tokyo, Japan.

Babadoost M. 2022. Bitter rot of apple. Report on Plant Disease, No. 805. Dept Crop Sci., Univ. Illinois, Urbana, IL, USA. http://extension.cropsciences.illinois.edu/fruitveg/pdfs/805-Apple%20Bitter%20Rot-2022.pdf.

Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B. 2002. The strobilurin fungicides. Pest Manag Sci. 58(7):649–662. https://doi.org/10.1002/ps.520.

Beckerman J, Bessin R, Strang J. 2023. Midwest fruit pest management guide. Purdue Univ Ext ID-465. West Lafayette, IN, USA. https://view.officeapps.live.com/op/view.aspx.

Bernstein B, Zehr EI, Dean RA, Shabi E. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan, and other hosts. Plant Dis. 79(5):478–482. https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1995Articles/PlantDisease79n05_478.PDF.

Biggs AR, Miller SS. 2001. Relative susceptibility of selected apple cultivars to *Colletotrichum acutatum*. Plant Dis. 85(6):657–660. https://doi.org/10.1094/PDIS.2001.85.6.657.

Braganca CAD, Damn U, Baroncelli R, Massola NS Jr, Crous PW. 2016. Species of the *Colletotrichum acutatum* complex associated with anthracnose diseases of fruit in Brazil. Fungal Biol. 120(4):547–561. https://doi.org/10.1016/j.funbio.2016.01.011.

Chechi A, Stahlecker J, Dowling ME, Schnabel G. 2019. Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples. Pestic Biochem Physiol. 158:18–24. https://doi.org/10.1016/j.pestbp.2019.04.002.

Chen SN, Luo CX, Hu MJ, Schnabel G. 2016. Sensitivity of *Colletotrichum* species, including *C. fioriniae* and *C. nymphaeae*, from peach to demethylation inhibitor fungicides. Plant Dis. 100(12):2434–2441. https://doi.org/10.1094/PDIS-04-16-0574-RE.

Chung WH, Ishii H, Nishimura K, Fukaya M, Yano K, Kajitani Y. 2006. Fungicide sensitivity and phylogenetic relationship of anthracnose fungi isolated from various fruit crops in Japan. Plant Dis. 90(4): 506–512. https://doi.org/10.1094/PD-90-0506.

DeFrancesco JT, Pscheidt JW, Yang W. 2017. Blueberry: 2017 pest management guide for the Willamette Valley. Oregon State Univ Ext Serv. Corvallis, OR, USA. https://ir.library.oregonstate.edu/concern/eesc_publications/d791sn15g.

Dowling M, Peres N, Villani S, Schnabel G. 2020. Managing *Colletotrichum* on fruit crops: A "complex" challenge. Plant Dis. 104(9):2301–2316. https://doi.org/10.1094/PDIS-11-19-2378-FE.

Du M, Schardl CL, Nuckles EM, Vaillancourt LJ. 2005. Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexes. Mycologia. 97(3):641–658. https://doi.org/10.1080/15572536.2006.11832795.

Faedda R, Agosteo GE, Schena L, Mosca S, Frisullo S, Magnano G, Cacciola SO. 2011. *Colletotrichum clavatum* sp. nov. identified as the causal agent of olive anthracnose in Italy. Phytopathol Mediterr. 50(2):283–302. https://doi.org/10.14601/PHYTOPATHOL_MEDITERR-9547.

Forcelini BB, Peres NA. 2018. Widespread resistance to QoI fungicides of *Colletotrichum acutatum* from strawberry nurseries and production fields. Plant Health Prog. 19(4):338–341. https://doi.org/10.1094/PHP-08-18-0050-RS.

Freeman S, Katan T, Shabi E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruit. Plant Dis. 82(6):596–605. https://doi.org/10.1094/PDIS.1998.82.6.596.

Gang GH, Cho HJ, Kim HS, Kwack YB, Kwak YS. 2015. Analysis of fungicide sensitivity and genetic diversity among *Colletotrichum* species in sweet persimmon. Plant Pathol J. 31(2):115–122. https://doi.org/10.5423/PPJ.OA.03.2015.0033.

González E, Sutton T. 2004. Population diversity within isolates of *Colletotrichum* spp. causing *Glomerella* leaf spot and

bitter rot of apples in three orchards in North Carolina. Plant Dis. 88(12):1335–1340. https://doi.org/10.1094/PDIS.2004.88.12.1335.

González E, Sutton TB, Correll JC. 2006. Clarification of the etiology of *Glomerella* leaf spot and bitter rot of apple caused by *Colletotrichum* spp. based on morphology and genetic, molecular, and pathogenicity tests. Phytopathology. 96(9):982–992. https://doi.org/10.1094/PHYTO-96-0982.

Khodadadi F, González JB, Martin PL, Giroux E, Bilodeau GJ, Peter KA, Doyle VP, Aćimović SG. 2020. Identification and characterization of *Colletotrichum* species causing apple bitter rot in New York and description of *C. noveboracense* sp. nov. Sci Rep. 10:1–19. https://doi.org/10.1038/s41598-020-66761-9.

Kilani J, Fillinger S. 2016. Phenylpyrroles: 30 years, two molecules and (nearly) no resistance. Front Microbiol. 7:1–10. https://doi.org/10.3389/fmicb.2016.02014.

Leroux P. 1996. Recent developments in the mode of action of fungicides. Pestic Sci. 47(2):191–197. https://doi.org/10.1002/(SICI)1096-9063(199606)47:2<191::AID-PS415>3.0.CO;2-I.

Lubbe CM, Denman S, Cannon PF, Groenewald JZ, Lamprecht SC, Crous PW. 2004. Characterization of *Colletotrichum* species associated with diseases of Proteaceae. Mycologia. 96(6):1268–1279. https://doi.org/10.1080/15572536.2005.11832877.

Martin PL, Peter KA. 2021. Quantification of *Colletotrichum fioriniae* in orchards and deciduous forests indicates it is primarily a leaf endophyte. Phytopathology. 111(2):333–344. https://doi.org/10.1094/PHYTO-05-20-0157-R.

Martin PL, Krawczyk T, Khodadadi F, Aćimović SG, Peter KA. 2021. Bitter rot of apple in the Mid-Atlantic United States: Causal species and evaluation of the impact of regional weather patterns and cultivar susceptibility. Phytopathology. 111(6):966–981. https://doi.org/10.1094/PHYTO-09-20-0432-R.

Martin PL, Krawczyk T, Pierce K, Thomas C, Khodadadi F, Aćimović SG, Peter KA. 2022. Fungicide sensitivity of *Colletotrichum* species causing bitter rot of apple in the Mid-Atlantic United States. Plant Dis. 106(2):549–563. https://doi.org/10.1094/PDIS-06-21-1142-RE.

Martinez-Culebras PV, Querol A, Suarez-Fernandez MB, Garcia-Lopez MD, Barrio E. 2003. Phylogenetic relationships among *Colletotrichum* pathogens of strawberry and design of PCR primers for their identification. J Phytopathol. 151(3):135–143. https://doi.org/10.1046/j.1439-0434.2003.00694.x.

McManus P, Perry RS. 2017. Evaluation of fungicides for control of cranberry fruit rot in Wisconsin, 2016. Plant Dis Manag Rep. 11:SMF012. https://doi.org/10.1094/PDMR11.

Mertely JC, Seijo TE, Peres NA. 2010. Effect of pre-and post-plant fungicide and fertilizer treatments on infection by *Colletotrichum acutatum*, plant survival, and yield of annual strawberry in Florida. Plant Health Prog. 11(1):1–7. https://doi.org/10.1094/PHP-2010-0125-01-RS.

Mills PR, Sreenivasaprasad S, Brown AE. 1992. Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. FEMS Microbiol Lett. 77(1-3):137–143. https://doi.org/10.1016/0378-1097(92)90145-e.

Munir M, Amsden B, Dixon E, Vaillancourt L, Gauthier NAW. 2016. Characterization of *Colletotrichum* species causing bitter rot of apples in Kentucky orchards. Plant Dis. 100(11):2194–2203. https://doi.org/10.1094/PDIS-10-15-1144-RE.

Oo MM, Yoon HY, Jang HA, Oh SK. 2018. Identification and characterization of *Colletotrichum* species associated with bitter rot disease of apple in South Korea. Plant Pathol J. 34(6):480–489. https://doi.org/10.5423/PPJ.FT.10.2018.0201.

Park MS, Kim BR, Park IH, Hahm SS. 2018. First report of two *Colletotrichum* species associated with bitter rot on apple fruit in Korea – *C. fructicola* and *C. siamense*. Mycobiol. 46(2):154–158. https://doi.org/10.1080/12298093.2018.1478220.

Peres NAR, Souza NL, Peever TL, Timmer LW. 2007. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. Plant Dis. 88(2):125–130. https://doi.org/10.1094/PDIS.2004.88.2.125.

Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD. 2009. Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Divers. 39:89–109. https://www.researchgate.net/publication/228478718.

Pscheidt JW, Bassinette JP. 2016. Fungicide management of blueberry rots, 2015. Plant Dis Manag Rep. 10:SMF027. https://doi.org/10.1094/PDMR10.

Rayner RW. 1970. A Mycological colour chart. British Mycol. Soc. Kew, Surrey, UK.

Sauter H, Steglich W, Anke T. 1999. Strobilurins: Evolution of a new class of active substances. Angew Chem Int Ed. 38(10):1328–1349. https://doi.org/10.1002/(SICI)1521-3773(19990517)38:10<1328::AID-ANIE1328>3.0.CO;2-1.

Sreenivasaprasad S, Mills PR, Brown AE. 1994. Nucleotide sequence of the rDNA spacer 1 enables identification of isolates of *Colletotrichum* as *C. acutatum*. Mycol Res. 98(2):186–188. https://doi.org/10.1016/S0953-7562(09)80184-6.

Sreenivasaprasad S, Talhinhas P. 2005. Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. Mol Plant Pathol. 6(4):361–378. https://doi.org/10.1111/j.1364-3703.2005.00291.x.

Su H, Gubler WD. 2009. Fungicide control of Botrytis and anthracnose fruit rot on strawberry in California, 2008 – trial I. Plant Dis Manag Rep. 3:SMF022. https://doi.org/10.1094/PDMR03.

Talhinhas P, Sreenivasaprasad S, Neves-Martins J, Oliveira H. 2002. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. Phytopathology. 92(9):986–996. https://doi.org/10.1094/PHYTO.2002.92.9.986.

Templeton M, Rikkerink E, Solon S, Crowhurst R. 1992. Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase-encoding gene and cDNA from the plant pathogenic fungus *Glomerella cingulata*. Gene. 122(1):225–230. https://doi.org/10.1016/0378-1119(92)90055-T.

Valero M, García-Martínez S, Giner MJ, Alonso A, Ruiz JJ. 2010. Benomyl sensitivity assays and species-specific PCR reactions highlight association of two *Colletotrichum gloeosporioides* types and *C. acutatum* with rumple disease on Primofiori lemons. Eur J Plant Pathol. 127(3):399–405. https://doi.org/10.1007/s10658-010-9606-0.

Velho AC, Stadnik MJ, Casanova L, Mondino P, Alaniz S. 2014. First report of *Colletotrichum nymphaeae* causing apple bitter rot in southern Brazil. Plant Dis. 98(4):567. https://doi.org/10.1094/PDIS-06-13-0671-PDN.

Velho AC, Alaniz S, Casanova L, Mondino P, Stadnik ML. 2015. New insight into the characterization of *Colletotrichum* species associated with apple diseases in southern Brazil and Uruguay. Fungal Biol. 119(4):229–244. https://doi.org/10.1016/j.funbio.2014.12.009.

Wang B, Li BH, Dong XL, Wang CX, Zhang ZF. 2015. Effects of temperature, wetness duration, and moisture on the conidial germination, infection, and disease incubation period of *Glomerella cingulata*. Plant Dis. 99(2):249–256. https://doi.org/10.1094/PDIS-04-14-0361-RE.

Weir BS, Johnston PR, Damm U. 2012. The *Colletotrichum gloeosporioides* species

complex. Stud Mycol. 73(1):115–180. https://doi.org/10.3114/sim0011.

Wikee S, Cai L, Pairin N, McKenzie EH, Su YY, Chukeatirote E, Thi HN, Bahkali AH, Moslem MA, Abdelsalam K, Hyde KD. 2011. *Colletotrichum* species from Jasmine (*Jasminum sambac*). Fungal Divers. 46(1):171–182. https://doi.org/10.1007/s13225-010-0049-x.

Ypema HL, Gold RE. 1999. Kresoxim-methyl: Modification of a naturally occurring compound to produce a new fungicide. Plant Dis. 83(1):4–19. https://doi.org/10.1094/PDIS.1999.83.1.4.