Managing Colletotrichum on Fruit Crops: A "Complex" Challenge

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Species from the genus *Colletotrichum* cause destructive diseases on a wide variety of fruit crops. In 2012, the genus was voted one of the top 10 fungal plant pathogens because of its broad host range, devastation of essential crops, and importance as a postharvest pathogen (Dean et al. 2012). Species of *Colletotrichum* are also important because of their use as model organisms (Cannon et al. 2012). In the United States, *Colletotrichum* spp. can cause severe losses on diverse and economically important temperate fruit crops such as apple, peach, grape, blueberry, cranberry, and strawberry (Bernstein et al. 1995; Daykin 1984b; González and Sutton 2004; Howard 1972; Munir et al. 2016). This review will often highlight strawberry anthracnose because it has been extensively studied due to its importance and prevalence. Despite the importance of *Colletotrichum* spp. as pathogens, the vast complexity and diversity of this genus make it difficult to understand and study.

Colletotrichum has been termed "a catalogue of confusion," due to its perpetually changing taxonomy (Bernstein et al. 1995; Hyde et al. 2009b). Obstacles to systematizing the genus are numerous: limited morphological differentiation among species, rare presence of sexual stages, and variation in pathogenicity and cultural morphology (Cannon et al. 2012; Johnson 2018). In addition, hosts and infection courts of different species frequently overlap. Multiple species have been detected infecting the same fruit, and even within the same lesion on apple (Munir et al. 2016). Historically, the two most important *Colletotrichum* species infecting temperate fruit crops were reported as *Colletotrichum acutatum* Simmonds ex Simmonds and *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo (Adaskaveg and Hartin 1997; Bernstein et al. 1995; Cannon et al. 2012; Dean et al. 2012; Freeman et al. 1998). However, the advent of sequencing and molecular identification revealed that these species, along with other species of *Colletotrichum*, are actually "species complexes," composed of numerous diverse and distinct species (Damm et al. 2012; Weir et al. 2012). Some researchers have even suggested classifying *C. siamense*, a species within the *C. gloeosporioides* complex, as a complex within a complex (Sharma et al. 2013; Vieira et al. 2014)! With new advances in sequencing technology, older systematics for naming *Colletotrichum* spp. became obsolete, resulting in decades of publications containing inaccurately identified species and genetic sequences (Hyde et al. 2009b).

Outbreaks caused by these species can be devastating. For example, epidemics of bitter rot of apple, caused by fungi in the *C. acutatum* and *C. gloeosporioides* species complexes, can result in loss of an entire crop during favorable conditions (S. Villani, personal communication). Prior to the use of synthetic fungicides, a bitter rot outbreak in the United States resulted in losses equivalent to approximately $400 million in today's currency based on relative income, and an agricultural bulletin declared, "There is no other disease so enormously destructive to the apple fruit as is the one commonly called bitter rot." (Burrill 1902). More recently, epidemics of strawberry anthracnose have been reported within major strawberry producing states, such as Florida and North Carolina as well as internationally in Israel, Brazil, and Bulgaria (Adaskaveg and Hartin 1997; Delp 1980; Ellis and Omer 2016; Freeman and Katan 1997; Henz 1992; Jelev et al. 2008). Anthracnose outbreaks have also occurred on peach and almond in California (Adaskaveg and Hartin 1997). In tropical and temperate fruit crops, postharvest outbreaks may also result in substantial financial losses to growers (Adaskaveg and Hartin 1997; Arauz 2000; Brown 1975; Prusky 1993; Rosenberger and Cox 2016). These recent outbreaks occurred despite the use of chemical control measures. The limited number of effective fungicides registered for control and the increasing prevalence of fungicide resistance limits chemical management options for diseases caused by *Colletotrichum* spp.

Symptoms and Signs

Anthracnose is the most common name given to diseases caused by *Colletotrichum* spp., though a variety of other names are used as well, including bitter rot on apple and cranberry, and ripe rot on grape and blueberry. The name anthracnose, literally "coal disease," refers to the dark, necrotic lesions typically caused by *Colletotrichum* spp. (Oxford English Dictionary, n.d.). Within these necrotic lesions, acervuli may form under favorable conditions, producing pink to orange mucilaginous masses of conidia (Fig. 1). Some *Colletotrichum* spp., most notably those in the *C. gloeosporioides* species complex (*Glomerella* spp.) may form perithecia on the tissue of temperate fruit hosts.

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Fruit rots are often the most obvious and economically important symptoms of diseases caused by *Colletotrichum* spp. (Fig. 2). However, various species, often in the *C. gloeosporioides* complex, infect leaves, crowns, stems, twigs, and petioles as well (Fig. 3). On apple, 75% of an orchard may be defoliated before harvest due to infections by fungi in the *C. gloeosporioides* complex (González and Sutton 1999; González et al. 2006). All parts of strawberry plants may be infected, including fruit, petioles, crowns, roots, stolons, and leaves (Freeman and Katan 1997; Freeman et al. 2001; Mertely and Legard 2004; Smith 2008; Ureña-Padilla et al. 2002). Highbush blueberry blossoms and fruiting wood may also be severely affected by *C. gloeosporioides* spp., and similar infections of vegetative cranberry tissue such as leaves and stems have also been reported (Doyle et al. 2013; Hartung et al. 1981; Oudemans et al. 1998).

Symptoms do not always appear when plants are colonized by *Colletotrichum* spp. For example, *C. acutatum* has been detected germinating and sporulating on symptomless strawberry and blueberry leaves (Leandro et al. 2001; Yoshida et al. 2007). Quiescent infections on immature fruit are also common (Fig. 3).

Disease Cycle

Nearly every crop worldwide serves as a host for at least one species of *Colletotrichum* (Dean et al. 2012). *Colletotrichum* spp. vary in their infection strategies on different hosts, and species and may preferentially infect different tissues (Fig. 3) (De Silva et al. 2017; González et al. 2006; Ureña-Padilla et al. 2002). However, disease cycles of *Colletotrichum* spp. infecting temperate fruit crops share several overarching similarities.

First, *Colletotrichum* spp. can overwinter in mummified fruit, infected vegetative tissue, or on symptomless plants. For example, in muscadine grape, *C. gloeosporioides* was found overwintering in mummies, pedicels, and fruit spurs. On apple, both sexual and asexual stages of species in the *C. gloeosporioides* complex overwinter in dead wood and perithecia develop on infected fallen leaves. During warm spring rains, ascospores and conidia are released to initiate primary infections on emerging foliage and fruit (Sutton 1983). Though not technically "overwintering," *C. acutatum* may germinate and even sporulate on strawberry leaves, through a process called secondary conidiation, without producing symptoms. In annual production systems, where plants are initially grown in nurseries, this allows it to be unwittingly transferred into the field, providing a continuous source of inoculum at the beginning of the season (Leandro et al. 2001). Weeds have also been shown to harbor *C. acutatum* that can lead to strawberry infections (Freeman et al. 2001) (Fig. 4). However, a multicrop study in Florida indicated that isolates of *C. acutatum* may be host-specific and pose little threat to other crops (MacKenzie et al. 2009b).

Second, the pathogen is typically waterborne, and spreads through rain-splash (Sutton 1983; Yang 1990), and infection is generally favored by warm and moist conditions (Brook 1977; Samuelian et al. 2012; Smith 2008).

Third, quiescent infections of immature fruit, resulting in postharvest disease are common in temperate fruit crops, including peach, grape, strawberry, and apple (Biggs 1995; Daykin 1984b; King et al. 1997; Zaitlin et al. 2000). Quiescent infections on apple may even enlarge during cold storage (Biggs 1995). On grape, *C. gloeosporioides*

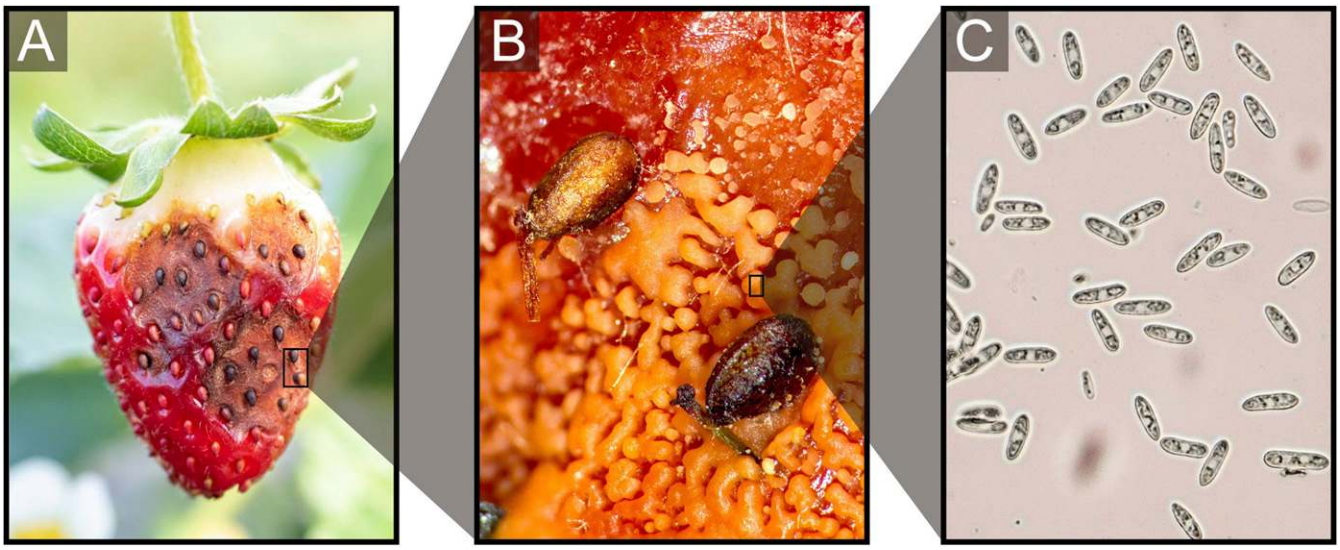

Fig. 1. Symptoms and signs of *Colletotrichum* spp. causing anthracnose fruit rot on strawberry: **A,** ripening strawberry infected by *C. acutatum*; **B,** microscopic image of *C. acutatum* sticky spore masses on anthracnose lesion; **C,** microscopic image of *C. acutatum* spores. Reprinted with permission of Taylor & Francis Ltd.

Fig. 2. Symptoms of diseases caused by *Colletotrichum* spp. on representative temperate fruits: peach, apple, grape, and strawberry.

can penetrate via appressoria, then become dormant, or spores remain dormant on the fruit surface until conditions favor infection (Daykin 1984a).

Lastly, once infection, colonization, and symptom development occur, acervuli or perithecia often appear in lesions, producing inoculum and creating a continuous cycle of disease. Perithecial production has not been detected outside of the laboratory for many fruit-infecting *Colletotrichum* spp., most notably *C. acutatum* (Cannon et al. 2012). However, the sexual stage of *C. gloeosporioides*, formerly called *Glomerella cingulata*, occurs more frequently, and is generally present on plant debris (Peres et al. 2005). Because of the current mycological emphasis on "one fungus, one name," we will refer to all *Colletotrichum* spp. by their asexual names in this review (Miller et al. 2011).

Other reviews have provided excellent summaries of the lifestyles (De Silva et al. 2017; Peres et al. 2005), taxonomy (Damm et al. 2012; Hyde et al. 2009b, a), and biology (Wharton and Diéguez-Uribeondo 2004) of *Colletotrichum* spp. on fruit crops and other crop systems. With the increasing presence of fungicide resistance and the variable response of different species to management methods, this review will emphasize management of diseases caused by *Colletotrichum* spp. on major fruit crops in the United States, focusing on the species involved, disease management strategies, and future outlooks for disease management.

Species Within the *C. acutatum* and *C. gloeosporioides* Complexes Responsible for Disease on Fruit Crops

Four complexes: *C. truncatum*, *C. boninense*, *C. acutatum*, *and C. gloeosporioides* have been reported from temperate fruit crops (Table 1). Of these complexes, *C. acutatum* and *C. gloeosporioides* are by far the most prevalent in literature (Adaskaveg and Hartin 1997; Bernstein et al. 1995; Damm et al. 2012; Dean et al. 2012; Freeman et al. 1998) (Table 1). *C. acutatum* and *C. gloeosporioides* species complexes have been traditionally distinguished by *C. acutatum*'s fusiform conidia with acute ends (Simmonds 1966). Unfortunately, this trait varies widely among *Colletotrichum* species and even among isolates to such a degree that it is unreliable for diagnosis (Damm et al. 2012). However, the ITS region, commonly used for fungal identification, can distinguish the *Colletotrichum* complexes (Cannon et al. 2012; Hyde et al. 2009a) (Fig. 5). Morphological and molecular evidence for subgroups below the complex level has been described in *C. acutatum* for over 20 years (Guerber et al. 2003; Lardner et al. 1999). However, accurate differentiation below the complex level is difficult and currently requires a multigene

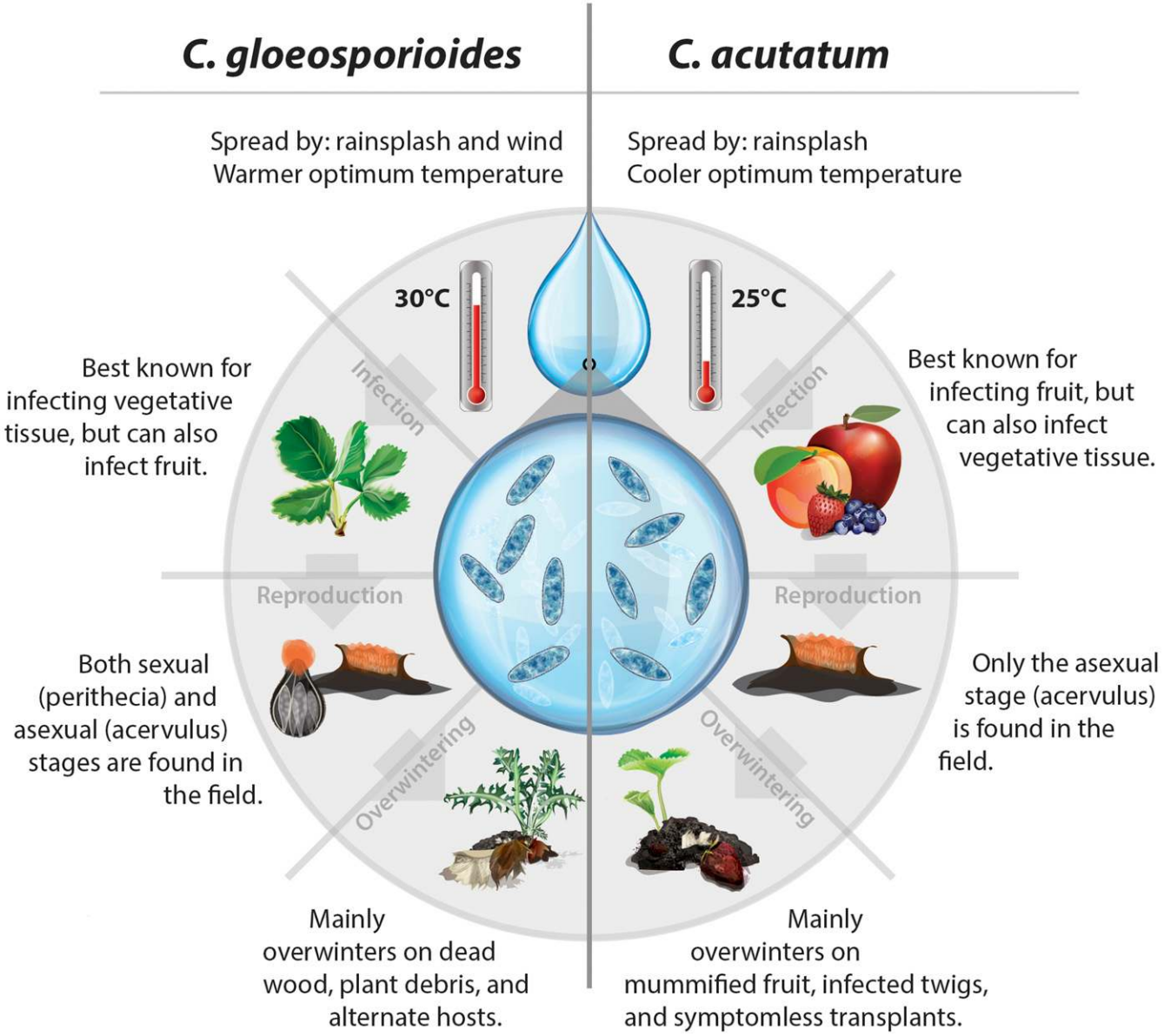

Fig. 3. A comparison of *Colletotrichum acutatum* and *C. gloeosporioides* on temperate fruit crops.

sequencing approach (Damm et al. 2012; Weir et al. 2012) (Table 2; Fig. 5). Fortunately, researchers have made tremendous progress classifying species using multigene sequencing combined with "polyphasic characters," important life traits such as physiology, pathogenicity, and cultural characteristics (Cai et al. 2009). Using these methods, *C. gloeosporioides* and *C. acutatum sensu lato* were separated into 22 and 29 species, respectively, in 2012 (Damm et al. 2012; Weir et al. 2012). While this excellent study has benefited our understanding of *C. acutatum* and *C. gloeosporioides*, there is still more to be done. For example, as one paper states, "there is no consensus as to which loci should be used to infer phylogeny" (Cannon et al. 2012). Some studies use up to nine genes, while others use only two genes (Cannon et al. 2012). This inconsistency and confusion combined with the difficulty and cost of sequencing multiple genes for identification of each isolate makes researchers less apt to distinguish species below the complex level. Vieira et al. (2020) performed an analysis to find a group of markers that would distinguish all species of *Colletotrichum.* Unfortunately, they concluded that such markers do not exist. Markers that were most effective for one complex were least effective for another. However, they propose that for most complexes, three highly variable genes can provide excellent differentiation to the species level (Table 2). To perform initial determination of species complex, Vieira et al. (2020) suggest that a single gene, such as GAPDH or TUB2, be used, and further differentiation be performed using other genes chosen based on complex identity (Fig. 5). However, the large number of species that may infect a single host make experimental design complicated. For example, our literature analysis revealed 21 species of *Colletotrichum* causing bitter rot on apple, 15 causing anthracnose on strawberry, and 12 causing ripe rot on grape worldwide (Table 1).

Though distinguishing species is often challenging, it is necessary due to marked differences in their lifestyle traits (Peres et al. 2005). Here we review the differences between and within the *C. gloeosporioides* and *C. acutatum* species complexes on temperate fruit crops and highlight the importance of accurate species differentiation below the complex level.

Plant organ specificity. *C. gloeosporioides* and *C. acutatum* have long been known to differ in host organ preference on fruit crops. For example, on strawberry, *C. gloeosporioides* is most often associated with crown rot infections, while *C. acutatum* is considered the main fruit rot pathogen, though both species are capable of infecting both crowns and fruit (Maas 1998; Ureña-Padilla et al. 2002). A similar organ preference occurs on apple, where species in the *C. gloeosporioides* complex are most often associated with Glomerella leaf spot symptoms, while species in both complexes are known to cause fruit rot (González et al. 2006; Johnson 2018). Below the complex level, little is known about host organ preferences, though one recent report on apple did not detect organ specificity among species within complexes infecting apple in North Carolina (Hoge 2017).

Pathogen aggressiveness and symptom severity. On a single host organ type, species within each complex often vary in aggressiveness. Within the *C. gloeosporioides* complex, *C. siamense* infections on apple fruit consistently produced deeper and larger lesions than *C. fructicola* or *C. theobromicola* (Munir et al. 2016). Differences among species within the *C. acutatum* complex also occurred on apple, where *C. fioriniae* created more severe lesions than *C. nymphaeae* (Munir et al. 2016). A similar phenomenon was observed on peach fruit within the *C. gloeosporioides* species complex, where *C. fructicola* was more aggressive than *C. siamense* (Hu et al. 2015b). Interestingly, aggressiveness may even vary within a species within a species complex. Two isolates of *C. nymphaeae* on strawberry caused significantly larger lesions on fruit than other *C. nymphaeae* isolates, and these two isolates clustered apart from other isolates of the same species in a

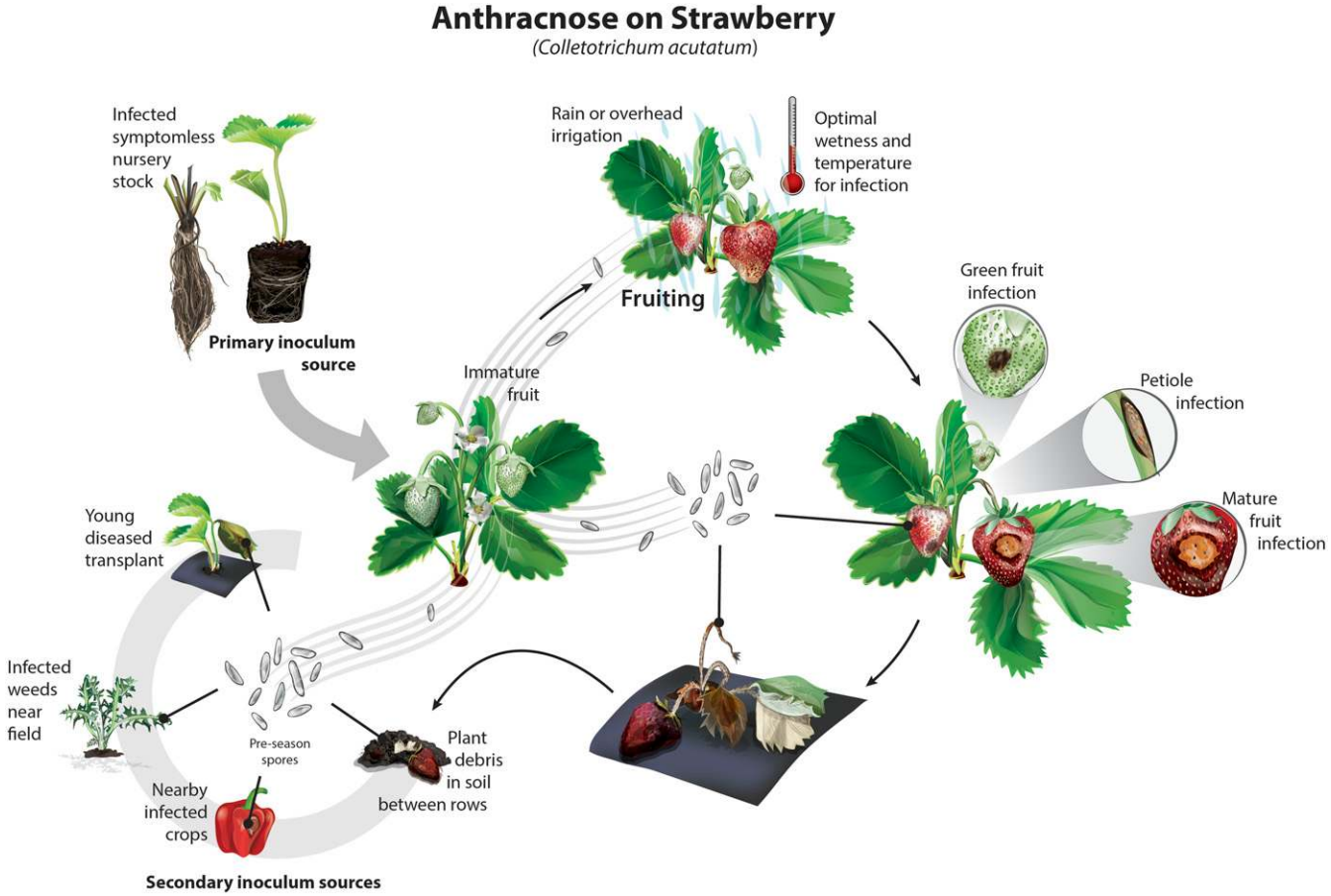

Fig. 4. Representative *Colletotrichum* disease cycle: disease cycle of anthracnose on strawberry caused by *C. acutatum*. Reprinted with permission of Taylor & Francis Ltd.

Table 1. List of *Colletotrichum* spp. on major fruit crops compiled from literature reports. See Supplementary Table S1 for literature sources.

Host	Complex/species	Current name[a]	Location(s)
Apple	*C. acutatum*	*C. fioriniae***[b]	USA*, Belgium, Croatia*, Japan, Korea, France, Slovenia
		*C. godetiae** (syn. *C. clavatum*)	Belgium*, UK, Netherlands, Slovenia, Japan, Croatia, Australia
		*C. nymphaeae**	USA, Belgium, Brazil*, Japan, Korea
		C. cuscutae	Netherlands, New Zealand, USA
		C. salicis	Belgium, Germany, New Zealand
		C. acerbum	New Zealand
		C. acutatum s. str.	Belgium, Australia
		C. melonis	Brazil, Uruguay
		C. rhombiforme	Belgium, China
		C. limetticola	Brazil
		C. paranaense	Brazil
		C. simmondsii	Japan
	C. gloeosporioides	*C. fructicola*****	USA*, Korea, Brazil*, Uruguay*, Japan*, France, China
		*C. siamense**	USA, Korea*, Japan, Pakistan, Argentina
		*C. tropicale**	USA*
		C. alienum	USA, New Zealand
		C. theobromicola (syn. *C. fragariae*)	USA, Uruguay
		C. aenigma	Japan, China
		C. kahawae	USA, Belgium
		C. gloeosporioides s. str.	USA
	C. boninense	*C. karsti*	Brazil
Strawberry	*C. acutatum*	*C. nymphaeae*****	USA*, UK*, Belgium*, Iran*, Italy, France, Colombia, Costa Rica, Switzerland, Australia, Kenya, Portugal, China, Bulgaria, Netherlands, South Africa, Canada, Israel
		C. fioriniae	USA, UK, New Zealand, France, Japan, Belgium
		C. salicis	New Zealand, Belgium
		C. godetiae	UK, Netherlands, Norway, Spain, Belgium, Ireland
		C. cuscutae	New Zealand, UK, USA
		C. acutatum s. str.	Australia
		C. carthami	Japan
		C. simmondsii	Australia, Japan
	C. gloeosporioides	*C. fructicola**	USA, Canada, Japan* (crown), China, Korea
		C. siamense (syn. *C. murrayae**)	USA, Taiwan, Bangladesh, Japan, Brazil, China*
		C. aenigma	UK, Japan, China
		C. alienum	Australia
		C. changpingense	China
		C. gloeosporioides s. str.	China
		C. theobromicola (syn. *C. fragariae*)	USA
		C. viniferum	China
	C. truncatum	*C. truncatum*	China
Peach	*C. acutatum*	*C. fioriniae*	USA, Korea
		C. nymphaeae	USA
		C. simmondsii	Japan
	C. gloeosporioides	*C. siamense**	USA*
		C. fructicola	USA, Korea
	C. truncatum	*C. truncatum*	USA
Grape	*C. acutatum*	*C. fioriniae**	USA*, Japan, Portugal
		C. godetiae	UK, Italy
		C. citri	China
		C. nymphaeae	Brazil
	C. gloeosporioides	*C. viniferum**	Brazil, China*, Korea
		C. gloeosporioides s. str.	USA, Brazil, China
		C. fructicola	Brazil, China
		C. siamense	USA, Brazil
		C. hebeiense	China
		C. aenigma	China
		C. clidemiae	USA
	C. truncatum	*C. truncatum* (syn. *C. capsici*)	Brazil, China
	C. clivae[c]	*C. cliviae*	Brazil
Blueberry	*C. acutatum*	*C. fioriniae*	USA, Japan, Netherlands, New Zealand, Poland
		C. simmondsii	Australia
		C. salicis	Norway
		C. nymphaeae	Japan
	C. gloeosporioides	*C. siamense*	USA
		C. fructicola	USA
	C. boninense	*C. karsti*	Brazil
Cranberry	*C. acutatum*	*C. fioriniae*	USA
	C. gloeosporioides	*C. fructivorum**	USA*, Canada
		C. rhexiae	USA
		C. temperatum	USA
		C. siamense (syn. *C. melanocaulon*)	USA
		C. fructicola	USA

[a] Names based on Damm et al. 2012; Bragança et al. 2016; Cannon et al. 2012; Doyle et al. 2013; Jayawardena 2016; Lei 2016; Weir et al. 2012; Yan et al. 2015.
[b] Number of asterisks indicate the number of studies where this species made up the majority of a collection of isolates.
[c] *C. clivae* is currently considered a species, not a complex or part of a complex.

phylogenetic tree (Wang et al. 2019). Characterizing species and their aggressiveness on specific tissues is important for both laboratory research and field application. In laboratory work, results can be greatly altered depending on the aggressiveness of species used to inoculate plants or detached fruit. In the field, it is critical that management methods target the species causing disease, particularly when fungicide sensitivities and inoculum source may vary among species.

Species frequency/distribution (United States). To date, studies using multigene methods to differentiate species within the *C. gloeosporioides* and *C. acutatum* complexes indicate that *C. nymphaeae* and *C. fioriniae* are the most commonly reported pathogens infecting many temperate fruit crops in the United States. *C. nymphaeae* is considered the major species within the *C. acutatum* complex infecting strawberry plants in the United States, while *C. fioriniae* has been reported as a major pathogen of grape, apple, and blueberry in the Mid-Atlantic and Southeastern United States, and *C. fructivorum*, within the *C. gloeosporioides* complex, is considered the major pathogen on cranberry (Johnson 2018; Kepner and Swett 2018; Munir et al. 2016; Shi 1996; Waller et al. 2018; Wang et al. 2019). However, other species within both complexes have been implicated causing disease on these crops to a lesser extent, indicating possible host, cultivar, or geographic preference that allows certain species within a complex to dominate over others (Biggs and Miller 2001; Grammen et al. 2019a; López-Moral et al. 2019).

Species frequency/distribution (international). Geographically, there is variation in the species within each complex that is involved in temperate fruit crop diseases. For example, *C. fructicola* is considered the most important species causing apple bitter rot in Uruguay and Brazil, but both *C. fructicola* and *C. fioriniae* are the species most commonly detected in the Mid-Atlantic and Southeastern United States, depending on cultivar, while *C. godetiae* was most commonly found in a study in Belgium (Grammen et al. 2019b; Johnson et al. 2018; Munir et al. 2016; Rockenbach et al. 2016; Shi 1996). Possibly, this variation is due to differences in sampling and identification methods. It may also be due to differences in cultivars surveyed, environmental factors, climate, and management strategies. Because only limited research has yet been performed involving *Colletotrichum* spp. below the complex level, it is difficult to pinpoint the reason for differing reports. Currently, literature provides examples of *Colletotrichum* spp. not reported in the United States that are regularly detected internationally (Table 1). For example, *C. godetiae* is commonly found on apple, strawberry, and grape in Europe, but has never been reported in North or South America (Baroncelli et al. 2014; Grammen et al. 2019a; Munda 2014; Wenneker et al. 2016; Zapparata et al. 2017). Also, *C. viniferum* was the most common species detected in a study in China, and was also detected in Korea and Brazil, but was not detected in a study of *Colletotrichum* spp. causing ripe rot conducted in the Mid-Atlantic United States (Kepner and Swett 2018; Peng et al. 2013). Though these geographical differences in species distribution and frequency may be due to a variety or combination of factors, they are important to consider when performing research using *Colletotrichum* spp. since species-specific management practices in one geographical location may be irrelevant in another. Regional and national surveys on multiple crops are necessary to better understand the geographic distribution of species within each complex as there are significant gaps in our knowledge of species distribution for many crops that are regularly devastated by anthracnose.

Fungicide sensitivity. Understanding fungicide sensitivity differences among species of *Colletotrichum* is critical for developing appropriate management recommendations. Growers have reported variability in fungicide efficacy against bitter rot of apple (Munir et al. 2016) that is likely due to differences in fungicide sensitivity

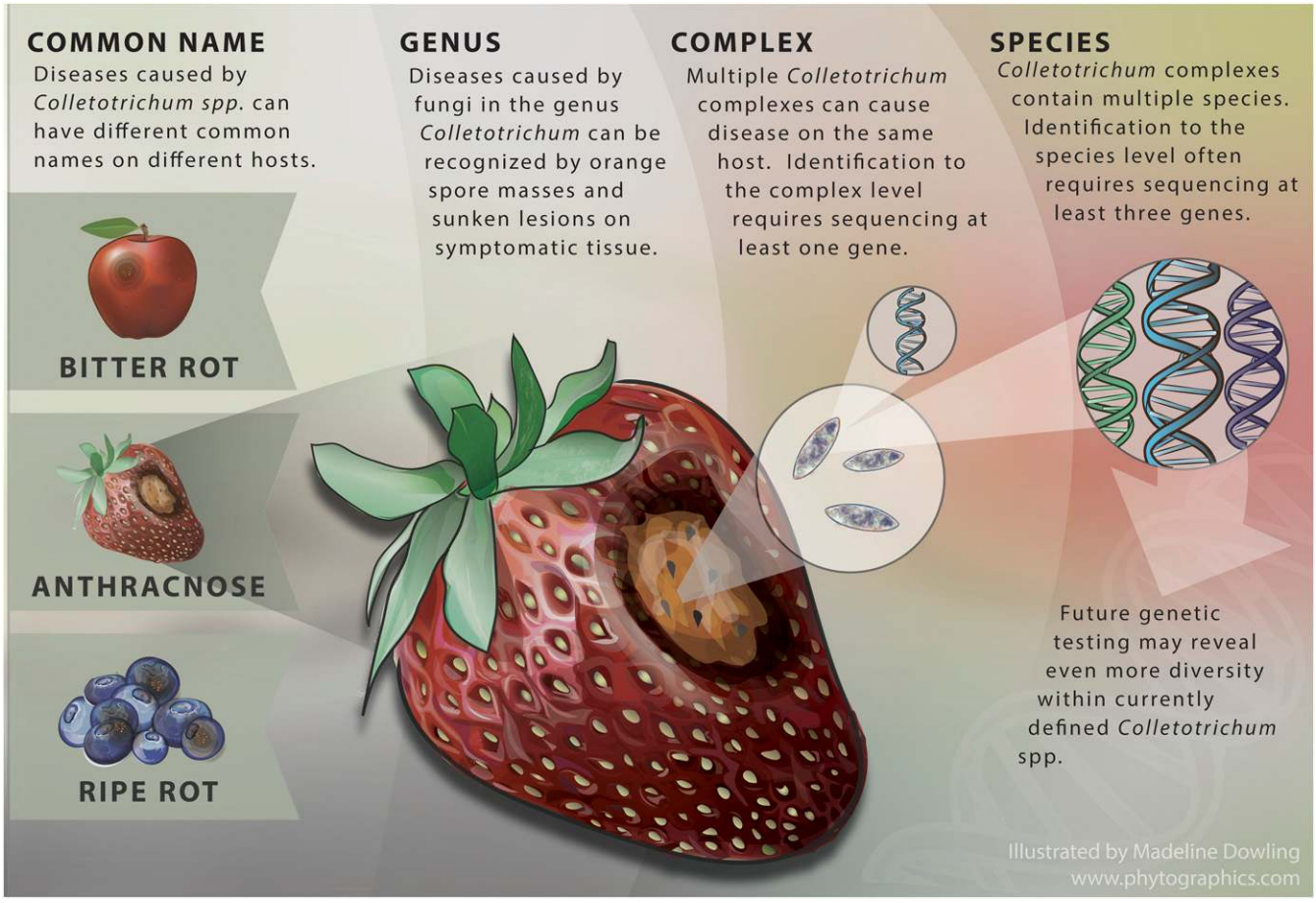

Fig. 5. Levels of complexity in distinguishing *Colletotrichum* spp.

Table 2. Primers to amplify multilocus regions for identification of *Colletotrichum* spp. on fruit crops

Gene	Product name	Complexes distinguished[a]	Primers	Sequence (5′-3′)	PCR conditions[b]	Reference
ACT	Actin	*Cf*[c]	ACT-512F ACT-783R	ATGTGCAAGGCCGGTTTCGC TACGAGTCCTTCTGGCCCAT	94°C (5 min); 40 cycles of 94°C (30 s), 52°C (30 s) and 72°C (30 s); 72°C (7 min)[1]	Carbone and Kohn 1999
APN2	DNA lyase	*Cg*	CgDL_R1 ColDL_F3	GCCCGACGAGCAGAGGACGTAGTC GGGAGAAGCGAACATACCA	95°C (3 min); 35 cycles of 95°C (30 s), 62°C (45 s), and 72°C (60 s); 72°C (10 min)[2]	Rojas et al. 2010
CHS-1	Chitin synthase	None	CHS79-F CHS354R	TGGGGCAAGGATGCTTGGAAGAAG TGGAAGAACCATCTGTGAGAGTTG	94°C (5 min); 40 cycles of 94°C (30 s), 52°C (30 s) and 72°C (30 s); 72°C (7 min)[1]	Carbone and Kohn 1999
CAL	Calmodulin	*Cb*	CL1C CL2C	GAATTCAAGGAGGCCTTCTC CTTCTGCATCATGAGCTGGAC	95°C (4 min); 35 cycles of 95°C (30 s), 59°C (30 s), 72°C (45 s); 72°C (7 min)[3]	Weir et al. 2012
ApMat (APN2/MAT-IGS)	Intergenic spacer between APN2 and MAT1-2-1	*Cg*	AMF (AMF1) AMR (AMR1)	CCAGAAATACACCGAACTTGC TCATTCTACGTATGTGCCCG	94°C (3 min); 30 cycles of 94°C (45 s), 54°C (45 s), and 72°C (60 s); 72°C (7 min)[4]	Silva et al. 2012
			CgDL_F6 CgMAT1_F2	AGTGGAGGTGCGGGACGTT TGATGTATCCCGACTACCG	95°C (3 min); 35 cycles of 95°C (30 s), 62°C (45 s), and 72°C (60 s); 72°C (10 min)[2]	Rojas et al. 2010
GAPDH (G3PDH)	Glyceraldehyde-3-phosphate-dehydrogenase	*Ca*, *Cb*, *Ct*, *Cg*[c]	GDF1 GDR1	GCCGTCAACGACCCCTTCATTGA GGGTGGAGTCGTACTTGAGCATGT	94°C (5 min); 40 cycles of 94°C (30 s), 52°C (30 s) and 72°C (30 s); 72°C (7 min)[1]	Guerber et al. 2003
GAP2-IGS	GAPDH intergenic spacer	*Cg*	GAP-1041 GAP/IGS-204	CTACACCGAGGACGATGTCG TTCTACGGGAAAACCAGGGC	95°C (5 min); 35 cycles of 95°C (30 s), 58°C (30 s), and 72°C (90 s); 72°C (10 min)[2]	Vieira et al. 2017
GS	Glutamine synthase	None	GS64F GS-967R	CCGGAGAATYCTTTWCACGA CTTCAGGTAGACGTCAGAGTTG	95°C (5 min); 35 cycles of 95°C (30 s), 55°C (30 s), and 72°C (90 s); 72°C (10 min)[2]	Vieira et al. 2017
			GSF3 GSR2	GCCGGTGGAGGAACCGTCG GAACCGTCGAAGTTCCAC	95°C (4 min); 35 cycles of 95°C (30 s), 54°C (30 s), 72°C (45 s); 72°C (7 min)[3]	Weir et al. 2012
HIS3	Histone 3	*Ca*, *Cb*	CYLH3F CYLH3R	AGGTCCACTGGTGGCAAG AGCTGGATGTCCTTGGACTG	94°C (5 min); 40 cycles of 94°C (30 s), 52°C (30 s) and 72°C (30 s); 72°C (7 min)[1]	Crous et al. 2004
ITS	Internal transcribed spacers	None	ITS1F	CTTGGTCATTTAGAGGAAGTAA	95°C (4 min); 35 cycles of 95°C (30 s), 52°C (30 s), 72°C (45 s); 72°C (7 min)[3]	Gardes and Bruns 1993
			ITS4	TCCTCCGCTTATTGATATGC		White et al. 1990
TUB2	β-tubulin	*Ca*, *Cb*, *Ct*, *Cg*[c]	T1	AACATGCGTGAGATTGTAAGT	94°C (5 min); 40 cycles of 94°C (30 s), 52°C (30 s) and 72°C (30 s); 72°C (7 min)[1]	O'Donnell and Cigelnik 1997
			Bt-2b	TTCTGGACGTTGCGCATCTG		Glass and Donaldson 1995

[a] Based on Vieira et al. 2020, the complexes listed are best distinguished using the genes specified. *Ca*, *Cb*, *Cg*, and *Ct* refer to *C. acutatum*, *C. boninense*, *C. gloeosporioides*, and *C. truncatum*, respectively.
[b] PCR conditions listed were obtained from [1]Damm et al. (2012), [2]Vieira et al. (2017), [3]Weir et al. (2012), [4]H. Ishii (personal communication).
[c] While not ideal markers, these genes can be used if necessary. GAPDH and TUB2 can be used to distinguish to the complex level for initial screening.

among *Colletotrichum* species and species complexes. Overall, *Colletotrichum* spp. on fruit crops often differ in sensitivity to fungicides such as quinone-outside inhibitors (QoI; FRAC 11), methyl benzimidazole carbamates (MBC; FRAC 1), and demethylation inhibitors (DMI, FRAC 3), though both complexes showed limited sensitivity to the succinate dehydrogenase inhibitor fungicides boscalid, fluxapyroxad, and fluopyram (Chen et al. 2016, 2018; Chung et al. 2006; Ishii et al. 2016; Meazza et al. 2003).

Differences in fungicide sensitivity among species from a single geographical location may be inherent or may have emerged as a result of fungicide selection pressure influenced by species-specific inoculum frequency and density, and influx of wildtype phenotypes from nearby hosts. On peach, multiple isolates of the species *C. nymphaeae* were resistant to the DMI fungicides flutriafol and fenbuconazole while other species tested, including *C. fioriniae*, were sensitive (Chen et al. 2016). A report of *C. fructicola* and *C. siamense* in commercial peach orchards in South Carolina revealed multiple isolates of *C. siamense* were resistant to QoI fungicides and some even dual-resistant to QoI and MBC fungicides, while *C. fructicola* isolates were sensitive (Hu et al. 2015b). A similar study of apple bitter rot in Japan provided opposite results, with *C. fructicola* isolates frequently resistant to QoI fungicides and dual-resistant to MBC and QoI fungicides, while no *C. siamense* isolates in the study were fungicide resistant (Yokosawa et al. 2017). Knowing fungicide resistance profiles of species is important for providing appropriate management recommendations to growers to ensure that only effective fungicides are being applied.

These examples highlight the necessity for differentiating *C. gloeosporioides* and *C. acutatum* spp. further than the complex level. Much work is still necessary to understand the geographic distribution of these species and learn more about their unique and shared traits. Multigene analysis has helped resolve some of the confusion within the *Colletotrichum* genus. However, the high diversity it has revealed within each complex leads to further questions about whether the genes currently used are enough to resolve species with markedly different lifestyles. On strawberry, Wang et al. (2019) discovered *C. nymphaeae* isolates that caused more severe symptoms than other representatives of the *C. nymphaeae* species. These isolates were also genetically different, as evidenced by phylogenetic trees. A similar phenomenon was observed by Chen et al. (2016) on peach, where some "*C. fioriniae*-like" isolates were detected clustering in a separate group from *C. fioriniae* and had different fungicide resistance profiles than typical *C. fioriniae*, but were not differentiated by the molecular markers used. While some light is dawning on the "catalogue of confusion," it is also revealing a wealth of diversity that has not been explored.

Managing *Colletotrichum* spp. on Fruit Crops

Though a variety of integrated management methods have been attempted over the years to combat *Colletotrichum* spp. infecting fruit crops, chemical control remains the major method for managing these diseases. This section will review general cultural and biological control methods, but will focus on chemical control of *Colletotrichum* spp., specifically the chemical classes available for management and current reports of fungicide resistance in *Colletotrichum* spp.

Cultural and biological control. Various cultural practices are routinely employed to manage diseases caused by *Colletotrichum* spp. on temperate fruit crops. These vary with the disease cycle, but in general seek to adjust conditions to decrease inoculum production and spread. For example, in annual strawberry production, management methods involve generating and using clean planting stock, limiting rain-splash and overhead irrigation, adjusting soil nutrients, managing weeds, and using cultivars with limited susceptibility to the disease (Coelho et al. 2008; Freeman 2008; Maas 1998; Walter et al. 2008). Production of clean planting stock is paramount, but also difficult since symptomless infections are frequent and difficult to detect (Debode et al. 2015; Leandro et al. 2001, 2003). Methods such as LAMP and quantitative PCR assays are being refined that allow detection of symptomless infections (Rahman et al. 2019; Zhang et al. 2016). However, application at the large scale of strawberry plant production is challenging. Symptomless infections by members of the *C. acutatum* species complex may also occur on apple leaves, providing an additional source of inoculum (Børve and Stensvand 2017). For most fruit crops, removing diseased and mummified fruit and symptomatic vegetative tissue is generally recommended (Feil et al. 2003; Stensvand et al. 2017; Sutton et al. 2014; Wilcox et al. 2015). Removing whole sections of strawberry fields affected by anthracnose crown rot is recommended to decrease the spread of disease in nurseries or commercial production fields (Rahman et al. 2015). However, in regions with warm temperatures such as Florida, sanitation of strawberry fields may not be helpful since berries on the orchard floor are not considered a major source of inoculum (Ureña-Padilla et al. 2001). Instead, alternate hosts are the major source of inoculum for *C. gloeosporioides* strawberry crown infections in Florida, and high temperatures prevent the inoculum from over-summering (Ureña-Padilla et al. 2001). Breeding for host-resistance is an environmentally friendly and cost-effective method for managing disease if the resistant cultivars have other desired traits. However, while several cultivars of apple, strawberry, and blueberry have moderate resistance to *C. acutatum* or *C. gloeosporioides*, none of the commercially marketable cultivars provide complete protection against the disease. A case study discussing host resistance breeding against diseases caused by *Colletotrichum* spp. is provided later in this review.

Many biological controls and biofungicides have been tested against *Colletotrichum* spp. on fruit crops, but none have reliably provided field efficacy. Numerous organisms have been tested including fungi such as *Trichoderma* spp., avirulent strains of *Colletotrichum*, and yeasts, as well as bacteria such as *Bacillus subtilus* and *Streptomyces lydicus* (Chalfoun et al. 2011; Conway et al. 2004; Freeman et al. 2004; Kim and Hwang 2007; Salazar et al. 2007, 2013). The most promising organisms, biofungicides, and systemic acquired resistance inducers have been formulated for testing in field trials, but they have provided inconsistent control even when combined with traditional chemical products (Cosseboom et al. 2018; Mertely and Peres 2013; Mertely et al. 2011, 2015, 2017; Shuman and Bratsch 2004; Yoder et al. 2017). Therefore, though most commercial growers use applicable cultural controls, they mainly rely on chemical control to manage *Colletotrichum* spp.

Chemical control. Multisite protectant fungicides, including mancozeb, thiram, ziram, captan, and chlorothalonil, have historically provided moderate to good efficacy against plant diseases, including diseases caused by *Colletotrichum* spp. However, increasingly stringent export regulations for these fungicides and restrictions on applications despite wetter and longer growing seasons has necessitated the incorporation of modern locally systemic fungicides into *Colletotrichum* management programs.

There are currently seven single single-site chemistries recommended in United States' spray guides for *Colletotrichum* control (Table 3): methyl benzimidazole carbamates (MBC; FRAC 1), demethylation inhibitors (DMI; FRAC 3), succinate dehydrogenase inhibitors (SDHI; FRAC 7), quinone-outside inhibitors (QoI; FRAC 11), phenylpyrroles (PP, FRAC 12), polyoxins (FRAC 19), and fluazinam (FRAC 29). Of this list, MBCs, DMIs, SDHIs, QoIs, polyoxins, and fluazinam are available as solo products.

QoI (FRAC 11) fungicides. While there are many single-site options registered for controlling *Colletotrichum* spp. on fruit crops, QoI fungicides are arguably the most consistently effective mode of action registered against *Colletotrichum* spp. Ten of the 22 commonly used single-site fungicides and mixtures registered for managing *Colletotrichum* spp. on fruit crops are QoIs or QoI mixtures (Table 2), and there is at least one QoI or QoI mixture registered for every major fruit crop.

Unfortunately, this high efficacy has produced a dependence on QoI fungicides that has led to selection for fungicide resistance. *Colletotrichum* spp. resistant to FRAC 11 (QoI) fungicides have already been

reported on multiple commercial temperate fruit crops in the United States including apple, strawberry, peach, and blueberry (Chechi et al. 2019; Forcelini et al. 2016; Hu et al. 2015b; Johnson 2018). Widespread field resistance resulting in lack of control and disease outbreaks has occurred on strawberry, apple, and peach crops (Chechi et al. 2019; Forcelini et al. 2016; Hu et al. 2015a).

QoIs act by binding to the cytochrome bc_1 complex and blocking electron transfer, thus inhibiting mitochondrial respiration (Bartlett et al. 2002). Practical resistance to QoI fungicides is typically caused by mutations in the cytochrome b gene (*CYTB*) which prevent fungicide binding (Ma and Michailides 2005). The most commonly reported mutation, G143A, results in qualitative resistance to QoI fungicides, whereas other mutations, such as F129L, typically result in moderate or partial resistance (Fernandez-Ortuno et al. 2008; Fisher et al. 2004). The G143A mutation has been reported to confer high resistance to isolates of *C. acutatum* and *C. gloeosporioides* collected from apple, strawberry, blueberry, and peach in the United States (Chechi et al. 2019; Forcelini et al. 2016; Hu et al. 2015b; Johnson 2018), whereas the F129L mutation was reported in isolates of *C. acutatum* infecting strawberry (Forcelini et al. 2016). Interestingly, *C. siamense* (*C. gloeosporioides* complex) from apple fruit from Illinois lacked any mutation in the *CYTB* gene but was resistant to the QoI fungicide azoxystrobin with an EC_{50} value greater than 100 µg/ml (Chechi et al. 2019). Reduced sensitivity to QoI fungicides and resistance in the absence of *CYTB* mutations has also been reported in other pathogens, indicating that other resistance mechanisms may be involved (Gisi et al. 2000; Olaya et al. 1998).

MBC (FRAC 1) fungicides. In the past, reports stated that MBC fungicides provided good efficacy against *C. gloeosporioides* spp. (MacKenzie et al. 2009a). As mentioned previously, fungi in the *C. acutatum* complex have inherent resistance or insensitivity to MBC fungicides (Chung et al. 2006). However, resistance to MBCs has also developed in *C. gloeosporioides*, threatening to make this chemical class ineffective as a chemical control option (Table 3).

MBC fungicides attack the fungal cytoskeleton by directly binding to β-tubulin subunits and inhibiting the assembly of microtubules (De Miccolis Angelini et al. 2015). Mutations in the β-tubulin gene can decrease the ability of the fungicide to bind, resulting in fungicide resistance (Davidse 1986; De Miccolis Angelini et al. 2015). Resistance to this fungicide class is widespread in many plant pathogens, and though many resistance mutations have been reported in various fungi, the most frequently reported mutations that confer resistance are E198A, E198G, E198K, and F200Y (De Miccolis Angelini et al. 2015). Low, intermediate, and high MBC fungicide resistance levels have been reported for *C. gloeosporioides* on pear in Japan (E198A, F200Y); peach (E198A), blueberry (E198A), apple (E198A), and strawberry (E198A) in the United States; and grape in Korea and China (F200Y) (Chechi et al. 2019; Chen et al. 2013; Chung et al. 2010; Hu et al. 2015a; Hwang et al. 2010).

DMI (FRAC 3) fungicides. A variety of DMI fungicides are registered as solo and mixture products for different fruit crops. Solo products show poor to good efficacy against *Colletotrichum* spp., though they do not typically perform as well as QoI fungicides (Defrancesco et al. 2018). However, on blueberry in the southeastern United States, metconazole is reported as having excellent efficacy (Burrack et al. 2019). Most of the mixtures available are combinations with QoI fungicides and perform well against diseases on registered crops. A recent in vitro study on *C. nymphaeae* and *C.*

Table 3. Currently registered chemicals for management of diseases caused by *Colletotrichum* spp. on fruit crops

Fungicide type	Fungicide category	FRAC code	Chemical class[a]	Resistance risk[b]	Active ingredient(s)	Registration
Solo	Single-site	1	MBC	H	Thiophanate-methyl	Strawberry (CR[c]), apple
	Single-site	3	DMI	M	Fenbuconazole	Cranberry, blueberry
	Single-site	3	DMI	M	Metconazole	Blueberry
	Single-site	3	DMI	M	Propiconazole	Strawberry (CR)
	Single-site	7	SDHI	M-H	Benzovindiflupyr	Apple
	Single-site	7	SDHI	M-H	Penthiopyrad	Apple (state restricted)
	Single-site	11	QoI	H	Azoxystrobin	Strawberry, blueberry, cranberry, peach
	Single-site	11	QoI	H	Fluoxastrobin	Strawberry, cranberry
	Single-site	11	QoI	H	Pyraclostrobin	Strawberry
	Single-site	11	QoI	H	Trifloxystrobin	Apple, strawberry
	Single-site	19	Polyoxin	M	Polyoxin D zinc salt	Apple (state restricted), strawberry, grape, cranberry, blueberry
	Single-site	29	N/A	L	Fluazinam	Apple, blueberry
	Multisite	M3	Dithiocarbamate	L	Mancozeb	Cranberry, apple
	Multisite	M3	Dithiocarbamate	L	Thiram	Strawberry (state restricted)
	Multisite	M3	Dithiocarbamate	L	Ziram	Apple, grape, blueberry
	Multisite	M4	Phthalimide	L	Captan	Apple (state restricted), strawberry
	Multisite	M5	Chloronitrile	L	Chlorothalonil	Cranberry
Mixture	Single-site	1/3	MBC/DMI	M	Thiophanate-methyl/ propiconazole	Strawberry (FR)
	Single-site	3/9	DMI/AP	M	Difenoconazole/cyprodinil	Peach, blueberry, strawberry
	Single-site	3/11	DMI/QoI	M	Difenoconazole/azoxystrobin	Strawberry, peach, blueberry
	Single-site	3/11	DMI/QoI	M	Propiconazole/azoxystrobin	Strawberry, peach
	Single-site	7/11	SDHI/QoI	M-H	Boscalid/pyraclostrobin	Apple, strawberry, grape, blueberry, peach
	Single-site	7/11	SDHI/QoI	M-H	Fluopyram/trifloxystrobin	Apple, strawberry
	Single-site	7/11	SDHI/QoI	M-H	Fluxapyroxad/pyraclostrobin	Apple, Strawberry, peach
	Single-site	7/12	SDHI/PP	M-H	Pydiflumetofen/fludioxonil	Grape, blueberry, strawberry
	Single-site	9/12	AP/PP	L-M	Cyprodinil/fludioxonil	Strawberry, blueberry
	Single/multisite	17/M4	KRI/ phthalimide	L-M	Fenhexamid/captan	Strawberry, blueberry

[a] Chemical classes MBCs: methyl benzimidazole carbamates, DMIs: demethylation inhibitors, SDHIs: succinate dehydrogenase inhibitors, QoIs: quinone outside inhibitors, APs: anilinopyrimidines, PPs: phenylpyrroles, KRIs: ketoreductase inhibitors.
[b] Designations refer to high (H), medium (M), and low (L) resistance risk.
[c] For strawberry, registrations are separated by crown rot (CR) or fruit rot (FR) unless the chemical is registered for both diseases.

fioriniae indicates that mixtures of two DMI fungicides can have a synergistic effect against *Colletotrichum* spp., presumably due to differential binding to paralogous CYP51 proteins (Chen et al. 2020). An in vitro study of *C. truncatum* from peach revealed that this species had resistance or reduced sensitivity to most DMI fungicides registered for anthracnose management (Chen et al. 2018). Fortunately, *C. truncatum* is currently not commonly found on temperate fruit crops.

SDHI (FRAC 7) fungicides. Several SDHI fungicides are registered for use on fruit crops either as solo products or in mixtures. Benzovindiflupyr is registered for the suppression of apple bitter rot in the Midwestern U.S. and reported as having excellent efficacy against the disease caused by members of the *C. acutatum* species complex (Beckerman et al. 2020). However, the same product showed only moderate efficacy against bitter rot and Glomerella leaf spot on apple caused by *C. fructicola* in the southeast (Johnson et al. 2018; Ritchie et al. 2016). In 2020, benzovindiflupyr is not yet registered for anthracnose fruit rot of strawberry, but has showed good efficacy against the disease in preliminary field trials (Mertely et al. 2018, 2019). In laboratory studies, *C. gloeosporioides s.l.* and *C. acutatum s.l.* isolates were naturally resistant to boscalid, fluxapyroxad, and fluopyram (Ishii et al. 2016) (Table 3). However, in field trials, a mixture of pyraclostrobin (a QoI fungicide) and fluxapyroxad was consistently one of the most effective compounds against bitter rot and Glomerella leaf spot on apple, performing better than the products containing just pyraclostrobin or fluxapyroxad. That suggests that fluxapyroxad may have at least some activity against *Colletotrichum* spp. in the field, or that the two fungicides may function synergistically (Brannen et al. 2018; Villani et al. 2018). An older SDHI fungicide, penthiopyrad, demonstrated moderate to good efficacy in vitro and in field trials for management of Glomerella leaf spot and bitter rot caused by *C. fructicola*, and was granted a special registration for apple in certain states (Villani et al. 2019). Incomplete cross-resistance among some SDHI fungicides has been reported in several fungal species (Ishii et al. 2011). However, the fungicide resistance action committee does not recommend rotating multiple chemicals within this class since selection for mutations conferring resistance to multiple SDHIs (such as P225F in the *SDHB* gene) has occurred in other crops (Hermann and Stenzel 2019). Development of newer SDHIs such as benzovindiflupyr provides opportunities for greater intrinsic efficacy against pathogens.

PP (FRAC 12). The combination of the FRAC 12 fungicide, fludioxonil, and the FRAC 9 fungicide, cyprodinil, is registered for controlling *Colletotrichum* spp. causing strawberry crown rot and fruit rot as well as blueberry anthracnose. This mixture has shown similar field efficacy to the QoI fungicide pyraclostrobin for anthracnose management on strawberry (Wedge et al. 2007). Preplant strawberry dip treatments using this chemical were also effective (Haack et al. 2018; Mertely et al. 2010). The combination of pydiflumetofen and fludioxonil is registered for grape ripe rot. In 2019, a special registration of this combination was enacted for blueberry and strawberry, and it showed consistently high efficacy against *C. acutatum* crown rot on strawberry in dip treatments, the same or better than that of azoxystrobin against sensitive isolates in preplant dip tests (Haack et al. 2018). However, this activity is likely due to fludioxonil, since solo pydiflumetofen did not typically provide significant control of the disease compared with the untreated control (Haack et al. 2018; Mertely et al. 2010). Phenylpyrrole fungicides are particularly useful since very few instances of resistance development have occurred in this class of fungicides (Kilani and Fillinger 2016).

Polyoxin (FRAC 19) fungicides. Though it is registered for use against anthracnose diseases on most fruit crops and showed promising results during in vitro tests (Hao et al. 2017), field tests of polyoxin d zinc salt indicate that it has inconsistent and often limited efficacy against *Colletotrichum* spp. on these crops (McManus and Perry 2017; Pscheidt and Bassinette 2016; Su and Gubler 2009).

Fluazinam (FRAC 29). Fluazinam is registered for use on apple, blueberry, and highbush cranberry. In addition, extension specialists have requested a special registration of this product for strawberry nurseries (Forcelini and Peres 2018). Fluazinam acts by uncoupling oxidative phosphorylation, thereby inhibiting mitochondrial respiration, and has shown efficacy against *C. gloeosporioides* and *C. acutatum* species complexes in vitro (Gang et al. 2015; Leroux 1996). It also has particularly low risk of resistance development.

On apple, it performed well in multiple field trials, providing similar levels of control to the commercial mixture of pyraclostrobin and fluxapyroxad (Brannen et al. 2018; Ritchie et al. 2016). On blueberry and strawberry, its efficacy was moderate, comparable to captan (Beckerman et al. 2020; Mertely et al. 2018).

Outlook for Management of *Colletotrichum* spp. on Fruit Crops

While seven single-site chemistries are available for control of *Colletotrichum* spp. on fruit crops as a whole, the loss of QoI and MBC fungicides to resistance would leave few effective single-site chemistries available for resistance management on several important crops (Table 3). For example, on apple, only SDHI fungicides and fluazinam would be available, while blueberry anthracnose management would be limited to DMIs and an SDHI/PP mixture. The limited number of effective fungicide chemical classes, the threat of fungicide resistance, the limitations of sanitation practices, and lack of reliability of biological control agents justify the development of more tools for an integrated disease management approach. These efforts include host resistance breeding, registration of new chemistries, and resistance monitoring. This section will focus on the current status and future directions of fungicide resistance management in *Colletotrichum* spp.

Resistance breeding (strawberry as a case study). One of the most promising, environmentally friendly, and potentially cost-effective means to reduce infection pressure and manage fungicide resistant *Colletotrichum* isolates is to develop resistant cultivars. While some cultivars of blueberry, apple, and strawberry are reported to be "less susceptible" or "resistant" to *Colletotrichum* spp., chemical control is still a necessity. In addition, cultivars "resistant" to anthracnose are often susceptible to other diseases or have other characteristics that limit their marketability. Ideally, breeding programs can overcome some of these obstacles to create fruit with resistance to disease without a wide variety of negative traits attached. Here we will review the history of breeding efforts for strawberry anthracnose at the University of Florida (UF).

For more than 70 years, the UF strawberry breeding program has used recurrent phenotypic selection to develop strawberry cultivars with high yields (Whitaker et al. 2011), desirable fruit quality traits, and disease resistance (Whitaker et al. 2012b) including resistance to *C. acutatum* and *C. gloeosporioides*. Broad variability for resistance to anthracnose fruit rot (AFR) exists in the UF breeding germplasm, with most cultivars being moderately to highly resistant (Chandler et al. 2006; Seijo et al. 2008; Whitaker et al. 2012a, b, 2015, 2018). A single locus, *FaRCa1*, appears to account for the variability in resistance to *C. acutatum* in the UF breeding program (Salinas et al. 2019). This locus explained at least 50% of phenotypic variance for AFR incidence in two distinct QTL discovery populations and was validated in advanced selections and cultivars (Salinas et al. 2019). For *C. gloeosporioides*, a quantitative trait locus that accounts for most of the genetic variation for resistance in the discovery sets was found and named *FaRCg1* (Anciro et al. 2018). For both diseases, high-throughput marker assays have been developed for the most significant SNPs that correlated with the mode of the QTL region. The discovery and characterization of these loci has been used to develop molecular tools that are used by the breeding program to achieve increased genetic gains for resistance. The cultivars developed by the UF breeding program with moderate to high levels of resistance to anthracnose fruit and crown rots have been successfully adopted by the Florida strawberry growers, in combination with cultural and chemical control methods, to manage the diseases caused by *Colletotrichum* spp. However, these cultivars might not always perform well in other climates.

Fungicide resistance management strategies. One means to avoid selection of resistant isolates is to reduce the number of fungicide applications, especially single-site fungicide applications.

MacKenzie and Peres (2012) developed a threshold for timing fungicide applications based on leaf wetness duration and the temperature during that period from Wilson et al. (1990). The web-based Strawberry Advisory System (StAS) was launched in 2009 and provides growers with SMS and/or emails advising when to apply fungicides as well as recommended products (Pavan et al. 2011). Following the recommendations by StAS has reduced the number of fungicide applications for control of anthracnose as well as gray mold on strawberry in Florida by about half (Cordova et al. 2017, 2018b; Zhang et al. 2019). Adoption in some other states has been challenging, though. The model determines past infection risk and depends on growers to apply fungicides as soon as possible after infection has occurred. While on Florida's fast-draining, sandy soils this may be feasible, getting a tractor back into fields with soil of higher clay proportion may be a challenge or even impossible. Nevertheless, where applicable, the system should reduce selection for resistance to fungicides prone to that problem and could potentially be applied in other crops.

Registration of unique chemistries. Natamycin (FRAC 48), an active ingredient historically used for food preservation, has shown

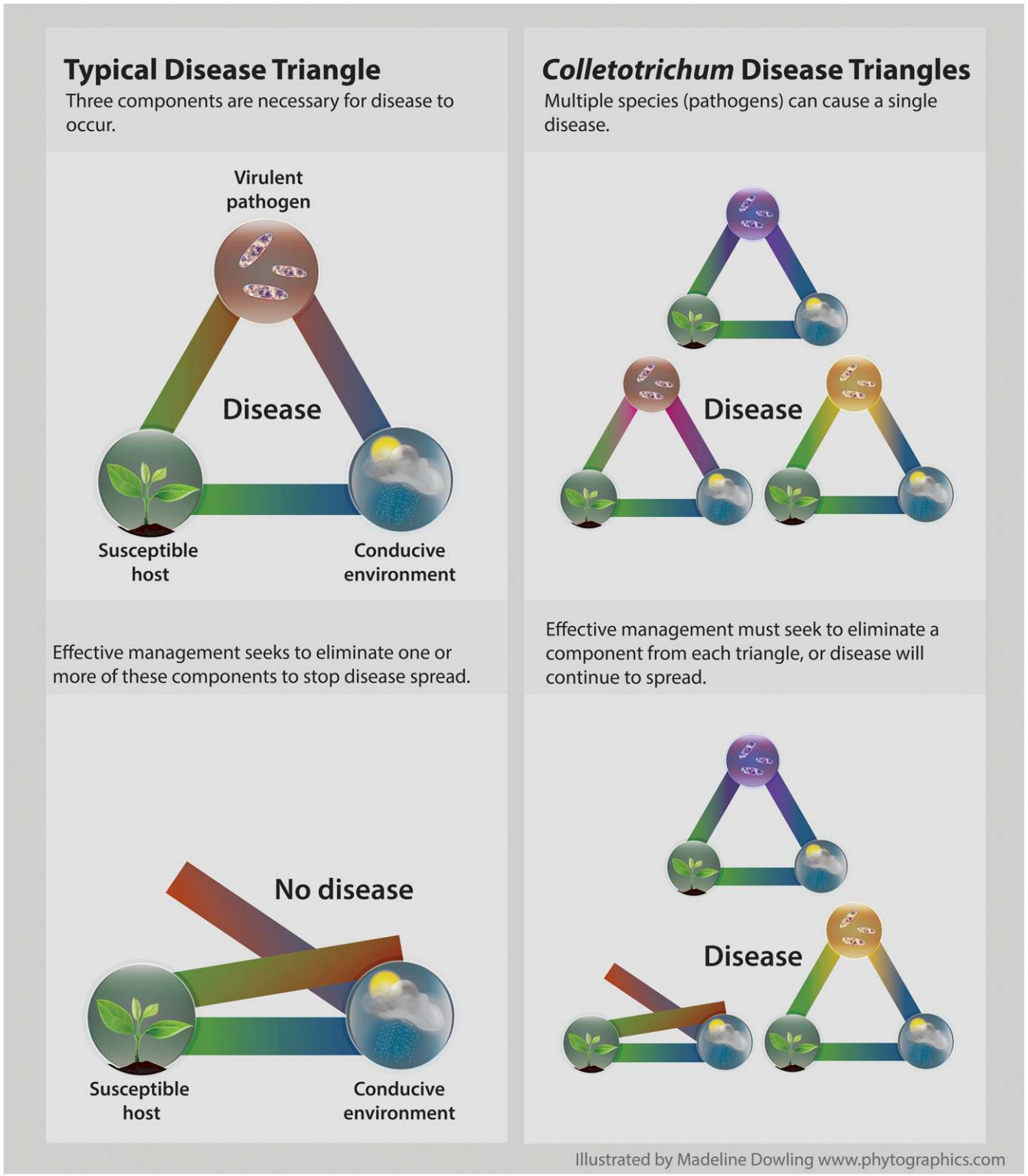

Fig. 6. Complexity of managing *Colletotrichum* spp. explained using disease cycles.

excellent potential against *Colletotrichum* spp. on fruit crops. This chemistry is particularly interesting due to its seemingly limited risk of resistance development. Natamycin acts by binding to ergosterol and disrupting the fungal membrane (te Welscher et al. 2008, 2010). It has such low toxicity in mammals that it has a long history of use as a food and beverage preservative and is used as an antifungal medication for human ophthalmic mycoses (Aparicio et al. 2016). Since its introduction as a preservative in 1967, no resistance has been reported in agricultural crops (Aparicio et al. 2016). It is currently registered as a postharvest treatment for citrus, pome, and stone fruits. It has also shown good efficacy as a preplant dip treatment against strawberry anthracnose crown rot caused by *C. acutatum* in field trials in both California and Florida, has been registered as a dip treatment in these states, and registration will likely be expanded soon (Cordova et al. 2018a; Cosseboom et al. 2018). When used in dip applications, its efficacy was excellent on both QoI resistant and sensitive isolates of *C. acutatum*, comparable to industry standard QoI fungicides on sensitive *C. acutatum* isolates (Haack et al. 2018). Limited information is currently available about the efficacy of natamycin against *C. gloeosporioides* on field applications since the currently available formulation is UV sensitive. However, in three separate human fungal eye infection cases, *C. gloeosporioides* isolates showed intermediate resistance to natamycin, indicating that this species may have inherent or acquired resistance (Shiraishi et al. 2011). Interestingly, each of these three mycoses resulted from injury in an agricultural setting, leading clinical researchers to believe that the fungi originated from infected plants, since *C. gloeosporioides* is not a common human pathogen (Shiraishi et al. 2011).

Fungicide resistance monitoring. Location-specific resistance monitoring allows for rapid assessment of resistance risk. Monitoring can aid in the design of resistance management programs, prolonging the functional lifetime of chemicals, and preventing the use of fungicides rendered ineffective by resistance. Timely monitoring requires a rapid testing method for determining resistance, an effective sampling method for collecting isolates, and a reliable communication channel for providing resistance management advice to producers. Pilot monitoring programs using bioassays have been extremely useful for other diseases such as gray mold on strawberry (*Botrytis cinerea*) and grapevine powdery (*Erysiphe necator*) and downy (*Plasmopara viticola*) mildews (Corio-Costet 2015; Schnabel et al. 2015). A similar program has been developed for QoI resistance monitoring of *C. acutatum* on strawberry in the United States. This program has already provided many important insights about the distribution of resistance within the United States, the frequency of resistance, and the importance of nurseries to resistance development (Forcelini and Peres 2018).

Rapid and accurate disease diagnosis and detection of fungicide-resistant isolates are important for effective monitoring. To inform strawberry growers of disease diagnostic results in time for effective deployment of chemical control practices, a multiplex high-resolution melting (HRM) assay was developed to detect strawberry crown rot pathogens and differentiate *Colletotrichum* from other pathogens (Forcelini et al. 2018). The HRM assay showed excellent specificity in the presence of DNA from individual isolates, with each primer pair generating the specific melting peak corresponding to the target pathogen and without any primer interactions. An end point assay was further developed to differentiate *C. acutatum* isolates resistant and sensitive to QoI fungicides (Forcelini et al. 2018). In the future, it may be possible to evaluate isolates in a specific planting and advise growers of the presence of resistance early in the season.

Though much of the current focus of monitoring involves QoI fungicide resistance, it is equally important to monitor resistance and sensitivity to other fungicides with efficacy against *Colletotrichum* species, such as fluazinam, fludioxonil/cyprodinil mixture, and natamycin.

Conclusions

Effective disease management requires an understanding of the host, pathogen, and environment. Unfortunately, for *Colletotrichum* spp., each of these aspects is complex and multidimensional (Fig. 6). While it is difficult to prescribe a way to examine all three aspects, we propose focusing on three critical "need areas" that can improve our understanding of the *Colletotrichum* pathogen and ultimately lead to improved management: 1) pathogen identification, 2) larger-scale field sampling, and 3) fungicide sensitivity testing. Pathogen identification is the first and most critical step to designing appropriate disease management strategies. Studying complexes without differentiating individual species will result in skewed data and inaccurate comparisons due to species-specific sensitivity profiles. Simple but reliable techniques must be developed and employed for accurate species differentiation. Secondly, while many studies have researched traits of small groups of select *Colletotrichum* isolates, relatively few have investigated larger collections of field isolates. Studies using isolates that more closely represent field populations provide more accurate information for the development of science-based management strategies. Lastly, *Colletotrichum* spp. sensitivity testing has historically focused on QoI and MBC fungicides. An expansion of fungicide sensitivity information to include other promising single-site fungicides would advance the design of better management strategies. It would also expose potential threats of reduced sensitivity or resistance to other fungicides and inherent resistance in individual species.

Ultimately, no single method can fully control anthracnose. However, a better understanding of *Colletotrichum* species involved and the efficacy of different management tools will enable growers to make more successful integrated management decisions. Improving existing methods for species identification, applying resistance management practices, registering new modes of action, and breeding tolerant cultivars will further increase the available options for integrated management.

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Literature Cited

Adaskaveg, J. E., and Hartin, R. J. 1997. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology 87:979-987.

Anciro, A., Mangandi, J., Verma, S., Peres, N., Whitaker, V. M., and Lee, S. 2018. FaRCg1: a quantitative trait locus conferring resistance to *Colletotrichum* crown rot caused by *Colletotrichum gloeosporioides* in octoploid strawberry. Theor. Appl. Genet. 131:2167-2177.

Aparicio, J. F., Barreales, E. G., Payero, T. D., Vicente, C. M., de Pedro, A., and Santos-Aberturas, J. 2016. Biotechnological production and application of the antibiotic pimaricin: biosynthesis and its regulation. Appl. Microbiol. Biotechnol. 100:61-78.

Arauz, L. F. 2000. Mango anthracnose: economic impact and current options for integrated management. Plant Dis. 84:600-611.

Baroncelli, R., Sreenivasaprasad, S., Thon, M. R., and Sukno, S. A. 2014. First report of apple bitter rot caused by *Colletotrichum godetiae* in the United Kingdom. Plant Dis. 98:1000.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Manag. Sci. 58:649-662.

Beckerman, J., Bessin, R., Strang, J., Gauthier, N., Lewis-Ivey, M., and Tucker, T. 2020. 2019-2020 Midwest Fruit Pest Management Guide. Purdue University, West Lafayette, IN.

Bernstein, B., Zehr, E., Dean, R. A., and Shabi, E. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan, and other hosts. Plant Dis. 79:478.

Biggs, A. R. 1995. Detection of latent infections in apple fruit with paraquat. Plant Dis. 79:1062.

Biggs, A. R., and Miller, S. S. 2001. Relative susceptibility of selected apple cultivars to *Colletotrichum acutatum*. Plant Dis. 85:657-660.

Børve, J., and Stensvand, A. 2017. *Colletotrichum acutatum* occurs asymptomatically on apple leaves. Eur. J. Plant Pathol. 147:943-948.

Bragança, C. A. D., Damm, U., Baroncelli, R., Massola Júnior, N. S., and Crous, P. W. 2016. Species of the *Colletotrichum acutatum* complex associated with anthracnose diseases of fruit in Brazil. Fungal Biol. 120:547-561.

Brannen, P., Glenn, T., Covington, R., and Rogers, D. 2018. Evaluation of fungicide programs for control of bitter rot of apples in Georgia, 2017. University of Georgia, Athens, GA.
Brook, P. J. 1977. *Glomerella cingulata* and bitter rot of apple. N. Z. J. Agric. Res. 20:547-555.
Brown, G. E. 1975. Factors affecting postharvest development of *Colletotrichum gloeosporioides* in citrus fruits. Phytopathology 65:404-409.
Burrack, H., Brannen, P., and Cline, B. 2019. 2019 Southeast Regional Blueberry Integrated Management Guide. North Carolina State University, Raleigh, NC.

Madeline Dowling

Madeline Dowling is a postdoctoral scientist in the Department of Plant and Environmental Sciences at Clemson University. Her research involves fungicide resistance of fruit pathogens, particularly *B. cinerea*, *Colletotrichum* spp., and *M. fructicola*. For her research work, she was named Clemson's graduate student researcher of the year in 2017 and one of the "Schroth Faces of the Future" by the American Phytopathological Society in 2019. She is also combining extension with her previous experience in graphic design and photography to create scientific illustrations, photographs, and animations explaining complex scientific concepts to a general audience. She has received grants, fellowships, and awards for this work, including a grant from the Southern IPM Center and the R.J. Tarleton Fellowship from the American Phytopathological Society. She aspires to be like one of her favorite plant pathologists, George Washington Carver, with whom she shares faith in God, love for art, and passion for extension. Like Carver, she hopes to encourage growers to further utilize scientific concepts in the field, resulting in application of more effective management practices in the future.

Natalia Peres

Dr. Natalia Peres is a professor at the University of Florida Gulf Coast Research and Education Center (GCREC). She has an active applied research and extension program focused primarily on the management of fungal diseases of strawberry. She is also responsible for the Diagnostic Clinic at GCREC serving the strawberry, ornamental, and vegetable industries in the area. Her research and extension programs focus on the development of integrated management approaches to reduce losses to growers in Florida, but recommendations extend across regions affecting many growers, including strawberry nurseries. She has developed the widely adopted web-based Strawberry Advisory System, which provides recommendations for strawberry growers on the need for fungicide applications based on the weather conditions. Her program is internationally known and has attracted students and visiting scientists from many countries. She has authored or co-authored over 100 peer-reviewed articles in scientific journals as well as technical extension bulletins and published multiple book chapters. In addition to numerous awards from the University of Florida, she received the APS William Boright Hewitt and Maybelle Ellen Ball Hewitt Award (2007), the APS Lee Hutchins Award for Excellence in Fruit Research (2014), and was recently recognized as an APS Fellow (2020).

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Dr. Sara Villani is an assistant professor and extension specialist in the Department of Entomology and Plant Pathology at North Carolina State University. She received a B.S. degree in chemistry from SUNY Geneseo in 2005 and a Ph.D. in plant pathology from Cornell University in 2016. Dr. Villani's current research interests include understanding mechanisms driving practical fungicide and antibiotic resistance, understanding the effect of abiotic stressors on disease development, and the development of chemical, biological, and cultural strategies for the management of economically important diseases on fruit and woody ornamentals in the southeastern United States. Dr. Villani authored or co-authored over 20 peer-reviewed articles and book chapters. Her recent extension activities include co-developing apple disease content for the MyIPM smartphone app, developing the NC Appalachian Apples Extension Portal, and developing an apple bitter rot and Glomerella leaf spot management program for NC apple growers. For these efforts, Sara was the recipient the NC Cooperative Extension 2019 Dr. Joseph and Mrs. Lisé Zublena Program Impact Award.

Guido Schnabel

Dr. Guido Schnabel is a professor and extension plant pathologist at Clemson University with over 20 years of experience working with pathogens of fruit crops. He has an active research and extension program focused on management of small fruit and stone fruit diseases. His basic research program investigates the occurrence and molecular mechanisms of fungicide resistance in fruit crop pathogens, and his applied research program includes the improvement of fruit disease management, fungicide resistance management, cultural disease management, and identification and management of abiotic disorders such as peach skin bronzing. He is the creator of the MyIPM smartphone app that, with the help of many of his colleagues, provides practical pest and disease diagnostics and management information for fruit growers. His involvement in USAID-funded outreach in Indonesia, the Philippines, and Cambodia helped improve the lives of dozens of farm families. He has authored or co-authored over 100 peer-reviewed articles in premier scientific journals and published multiple book chapters. He received the APS Lee Hutchins Award for Excellence in Tree Fruit Research (2011), the APS Excellence in Extension Award, the Clemson University Godley-Snell Award for Excellence in Agricultural Research (2015), and the Clemson University Centennial Professorship Award (2017).

Burrill, T. 1902. Bitter Rot of Apples. University of Illinois, Urbana-Champaign, IL.
Cai, L., Hyde, K. D., Taylor, P., Weir, B. S., Waller, J. M., Abang, M. M., et al. 2009. A polyphasic approach for studying *Colletotrichum*. Fungal Divers. 39: 183-204.
Cannon, P. F., Damm, U., Johnston, P. R., and Weir, B. S. 2012. *Colletotrichum* – current status and future directions. Stud. Mycol. 73:181-213.
Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553-556.
Chalfoun, N. R., Castagnaro, A. P., and Díaz Ricci, J. C. 2011. Induced resistance activated by a culture filtrate derived from an avirulent pathogen as a mechanism of biological control of anthracnose in strawberry. Biol. Control 58:319-329.
Chandler, C. K., Mertely, J. C., and Peres, N. 2006. Resistance of selected strawberry cultivars to anthracnose fruit rot and *Botrytis* fruit rot. Acta Hortic. 708:123-126.
Chechi, A., Stahlecker, J., Dowling, M. E., and Schnabel, G. 2019. Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples. Pestic. Biochem. Physiol. 158:18-24.
Chen, D., Shi, H. J., Wu, H. M., Xu, Z. H., and Zhang, C. Q. 2013. Resistance of *Colletotrichum gloeosporioides* causing grape ripe rot to thiophanate-methyl and tebuconazole in Zhejiang. J. Fruit Sci. 30:665-668.
Chen, S., Hu, M., Schnabel, G., Yang, D., Yan, X., and Yuan, H. 2020. Paralogous CYP51 genes of *Colletotrichum* spp. mediate differential sensitivity to sterol demethylation inhibitors. Phytopathology 110:615-625.
Chen, S., Wang, Y., Schnabel, G., Peng, C. A., Lagishetty, S., Smith, K., et al. 2018. Inherent resistance to 14α-demethylation inhibitor fungicides in *Colletotrichum truncatum* is likely linked to CYP51A and/or CYP51B gene variants. Phytopathology 108:1263-1275.
Chen, S. N., Luo, C. X., Hu, M. J., and Schnabel, G. 2016. Sensitivity of *Colletotrichum* species, including *C. fioriniae* and *C. nymphaeae*, from peach to demethylation inhibitor fungicides. Plant Dis. 100:2434-2441.
Chung, W.-H., Chung, W.-C., Peng, M.-T., Yang, H.-R., and Huang, J.-W. 2010. Specific detection of benzimidazole resistance in *Colletotrichum gloeosporioides* from fruit crops by PCR-RFLP. N. Biotechnol. 27:17-24.
Chung, W.-H., Ishii, H., Nishimura, K., Fukaya, M., Yano, K., and Kajitani, Y. 2006. Fungicide sensitivity and phylogenetic relationship of anthracnose fungi isolated from various fruit crops in Japan. Plant Dis. 90:506-512.
Coelho, M. V. S., Palma, F. R., and Café-Filho, A. C. 2008. Management of strawberry anthracnose by choice of irrigation system, mulching material, and host resistance. Int. J. Pest Manage. 54:347-354.
Conway, W. S., Leverentz, B., Janisiewicz, W. J., Blodgett, A. B., Saftner, R. A., and Camp, M. J. 2004. Integrating heat treatment, biocontrol, and sodium bicarbonate to reduce postharvest decay of apple caused by *Colletotrichum acutatum* and *Penicillium expansum*. Postharvest Biol. Technol. 34:11-20.
Cordova, L., Mertely, J. C., and Peres, N. A. 2018a. Evaluation of dip treatments for control of *Colletotrichum acutatum* on strawberry transplants, 2017-18. Plant Dis. Manage. Rep. 12:PF073.
Cordova, L. G., Ellis, M. A., Wilson, L. L., Madden, L. V., and Peres, N. A. 2018b. Evaluation of the Florida Strawberry Advisory System for control of *Botrytis* and anthracnose fruit rots in Ohio. Plant Health Prog. 19:182-187.
Cordova, L. G., Madden, L. V., Amiri, A., Schnabel, G., and Peres, N. A. 2017. Meta-analysis of a web-based disease forecast system for control of anthracnose and *Botrytis* fruit rots of strawberry in southeastern United States. Plant Dis. 101:1910-1917.
Corio-Costet, M.-F. 2015. Monitoring resistance in obligate pathogens by bioassays relating to field use: grapevine powdery and downy mildews. Pages 251-279 in: Fungicide resistance in plant pathogens. H. Ishii and D. Hollomon, eds. Springer, Tokyo.
Cosseboom, S. D., Ivors, K., Schnabel, G., and Holmes, G. 2018. First report of *Botrytis mali* causing gray mold on strawberry in California. Plant Dis. 102:679.
Crous, P. W., Groenewald, J. Z., Risède, J. M., Simoneau, P., and Hywel-Jones, N. L. 2004. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Stud. Mycol. 50:415-430.
Damm, U., Cannon, P. F., Woudenberg, J. H. C., and Crous, P. W. 2012. The *Colletotrichum acutatum* species complex. Stud. Mycol. 73:37-113.
Davidse, L. C. 1986. Benzimidazole fungicides: mechanism of action and biological impact. Annu. Rev. Phytopathol. 24:43-65.
Daykin, M. E. 1984a. Histopathology of ripe rot caused by *Colletotrichum gloeosporioides* on muscadine grape. Phytopathology 74:1339.
Daykin, M. E. 1984b. Ripe rot of muscadine grape caused by *Colletotrichum gloeosporioides* and its control. Phytopathology 74:710.
De Miccolis Angelini, R. M., Pollastro, S., and Faretra, F. 2015. Genetics of Fungicide Resistance. Pages 13-34 in: Fungicide Resistance in Plant Pathogens. H. Ishii and D. W. Hollomon, eds. Springer, Tokyo, Japan.
De Silva, D. D., Crous, P. W., Ades, P. K., Hyde, K. D., and Taylor, P. W. J. 2017. Lifestyles of *Colletotrichum* species and implications for plant biosecurity. Fungal Biol. Rev. 31:155-168.
Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414-430.
Debode, J., Van Hemelrijck, W., Xu, X.-M., Maes, M., Creemers, P., and Heungens, K. 2015. Latent entry and spread of *Colletotrichum acutatum* (species complex) in strawberry fields. Plant Pathol. 64:385-395.
Defrancesco, J., Pscheidt, J., and Yang, W. 2018. 2018 Blueberry Pest Management Guide for the Willamette Valley. Oregon State University Extension Service, Corvallis, OR.
Delp, B. R. 1980. Control of strawberry anthracnose with captafol. Plant Dis. 64:1013.
Doyle, V. P., Oudemans, P. V., Rehner, S. A., and Litt, A. 2013. Habitat and host indicate lineage identity in *Colletotrichum gloeosporioides s.l.* from wild and agricultural landscapes in North America. PLoS One 8:e62394.
Ellis, M., and Omer, E. 2016. Anthracnose of Strawberry Factsheet. Ohio State University, Columbus, OH.
Feil, W. S., Butler, E. E., Duniway, J. M., and Gubler, W. D. 2003. The effects of moisture and temperature on the survival of *Colletotrichum acutatum* on strawberry residue in soil. Can. J. Plant Pathol. 25:362-370.
Fernandez-Ortuno, D., Tores, J. A., de Vicente, A., and Perez-Garcia, A. 2008. Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. Int. Microbiol. Off. J. Span. Soc. Microbiol. 11:1-9.
Fisher, N., Brown, A. C., Sexton, G., Cook, A., Windass, J., and Meunier, B. 2004. Modeling the Qo site of crop pathogens in *Saccharomyces cerevisiae* cytochrome b. Eur. J. Biochem. 271:2264-2271.
Forcelini, B. B., Lee, S., Oliveira, M. S., and Peres, N. A. 2018. Development of high-throughput SNP genotyping assays for rapid detection of strawberry *Colletotrichum* species and the G143A mutation. Phytopathology 108: 1501-1508.
Forcelini, B. B., and Peres, N. A. 2018. Widespread resistance to QoI fungicides of *Colletotrichum acutatum* from strawberry nurseries and production fields. Plant Health Prog. 19:338-341.
Forcelini, B. B., Seijo, T. E., Amiri, A., and Peres, N. A. 2016. Resistance in strawberry isolates of *Colletotrichum acutatum* from Florida to quinone-outside inhibitor fungicides. Plant Dis. 100:2050-2056.
Freeman, S. 2008. Management, survival strategies, and host range of *Colletotrichum acutatum* on strawberry. HortScience 43:66-68.
Freeman, S., Horowitz, S., and Sharon, A. 2001. Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. Phytopathology 91:986-992.
Freeman, S., and Katan, T. 1997. Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. Phytopathology 87:516-521.
Freeman, S., Katan, T., and Shabi, E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Dis. 82:596-605.
Freeman, S., Minz, D., Kolesnik, I., Barbul, O., Zveibil, A., Maymon, M., et al. 2004. *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. Eur. J. Plant Pathol. 110:361-370.
Gang, G.-H., Cho, H. J., Kim, H. S., Kwack, Y.-B., and Kwak, Y.-S. 2015. Analysis of fungicide sensitivity and genetic diversity among *Colletotrichum* species in sweet persimmon. Plant Pathol. J. 31:115-122.
Gardes, M., and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113-118.
Gisi, U., Chin, K. M., Knapova, G., Färber, R. K., Mohr, U., Parisi, S., et al. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Prot. 19:863-872.
Glass, N. L., and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61:1323-1330.
González, E., and Sutton, T. B. 1999. First report of Glomerella leaf spot (*Glomerella cingulata*) of apple in the United States. Plant Dis. 83:1074.
González, E., and Sutton, T. B. 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. 88:1335-1340.
González, E., Sutton, T. B., and Correll, J. C. 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 96:982-992.
Grammen, A., Van Campenhout, J., Geeraerd, A., and Keulemans, W. 2019a. Susceptibility of apple fruits (*Malus* × *domestica* Borkh.) to the postharvest pathogen *Colletotrichum fioriniae*: cultivar differences and correlation with fruit ripening characteristics. Eur. J. Plant Pathol. 155:801-816.
Grammen, A., Wenneker, M., Van Campenhout, J., Pham, K. T. K., Van Hemelrijck, W., Bylemans, D., et al. 2019b. Identification and pathogenicity assessment of *Colletotrichum* isolates causing bitter rot of apple fruit in Belgium. Eur. J. Plant Pathol. 153:47-63.
Guerber, J. C., Liu, B., Correll, J. C., and Johnston, P. R. 2003. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95:872-895.
Haack, S. E., Ivors, K. L., Holmes, G. J., Förster, H., and Adaskaveg, J. E. 2018. Natamycin, a new biofungicide for managing crown rot of strawberry caused by QoI-resistant *Colletotrichum acutatum*. Plant Dis. 102:1687-1695.
Hao, X. J., Hu, M. J., Chen, S. N., and Schnabel, G. 2017. Challenges in assessing efficacy of polyoxin-d zinc salt against *Colletotrichum* species. J. Plant Pathol. 99:513-516.
Hartung, J. S., Burton, C. L., and Ramsdell, D. C. 1981. Epidemiological studies of blueberry anthracnose disease caused by *Colletotrichum gloeosporioides*. Phytopathology 71:449-453.

Henz, G. P. 1992. Outbreak of strawberry anthracnose caused by *Colletotrichum acutatum* in central Brazil. Plant Dis. 76:212A.
Hermann, D., and Stenzel, K. 2019. FRAC mode-of-action classification and resistance risk of fungicides. Pages 589-608 in: Modern Crop Protection Compounds. P. Jeschke, M. Witschel, W. Krämer, and U. Schirmer, eds. Wiley-VCH Verlag GmbH & Co., Weinheim, Germany.
Hoge, B. L. 2017. Characterization of the genetic diversity and developmental biology of the *Colletotrichum gloeosporioides* species complex in NC apple orchards. M.S. thesis, North Carolina State University, Raleigh, NC.
Howard, C. M. 1972. A strawberry fruit rot caused by *Colletotrichum fragariae*. Phytopathology 62:600.
Hu, M.-J., Grabke, A., Dowling, M. E., Holstein, H. J., and Schnabel, G. 2015a. Resistance in *Colletotrichum siamense* from peach and blueberry to thiophanate-methyl and azoxystrobin. Plant Dis. 99:806-814.
Hu, M.-J., Grabke, A., and Schnabel, G. 2015b. Investigation of the *Colletotrichum gloeosporioides* species complex causing peach anthracnose in South Carolina. Plant Dis. 99:797-805.
Hwang, S.-Y., Kim, H.-R., Kim, J.-H., Park, J.-H., Lee, S.-B., Cheong, S.-R., et al. 2010. Sensitivity of *Colletotrichum* spp. isolated from grapes in Korea to carbendazim and the mixture of carbendazim plus diethofencarb. Plant Pathol. J. 26:49-56.
Hyde, K. D., Cai, L., Cannon, P. F., Crouch, J. A., Crous, P. W., Damm, U., et al. 2009a. *Colletotrichum*—names in current use. Fungal Divers. 39:147-183.
Hyde, K. D., Cai, L., McKenzie, E. H. C., Yang, Y. L., Zhang, J. Z., and Prihastuti, H. 2009b. *Colletotrichum*: a catalogue of confusion. Fungal Divers. 39:1-17.
Ishii, H., Miyamoto, T., Ushio, S., and Kakishima, M. 2011. Lack of cross-resistance to a novel succinate dehydrogenase inhibitor, fluopyram, in highly boscalid-resistant isolates of *Corynespora cassiicola* and *Podosphaera xanthii*. Pest Manag. Sci. 67:474-482.
Ishii, H., Zhen, F., Hu, M., Li, X., and Schnabel, G. 2016. Efficacy of SDHI fungicides, including benzovindiflupyr, against *Colletotrichum* species. Pest Manag. Sci. 72:1844-1853.
Jayawardena, R. 2016. An account of *Colletotrichum* species associated with strawberry anthracnose in China based on morphology and molecular data. Mycosphere 7:1147-1163.
Jelev, Z. J., Bobev, S. G., Minz, D., Maymon, M., and Freeman, S. 2008. Characterization of *Colletotrichum* species causing strawberry anthracnose in Bulgaria. J. Phytopathol. 156:668-677.
Johnson, K. A. 2018. Characterization and fungicide efficacy of North Carolina *Colletotrichum* populations causing Glomerella leaf spot and fruit rot on apple. M. S. thesis, North Carolina State University, Raleigh, NC.
Johnson, K. A., Kreis, R., Justus, C., and Villani, S. 2018. Fungicide efficacy and molecular characterization of North Carolina *Colletotrichum* populations causing Glomerella leaf spot and fruit rot on apple. Phytopathology 108:S1.298.
Kepner, C., and Swett, C. L. 2018. Previously unrecognized diversity within fungal fruit rot pathosystems on *Vitis vinifera* and hybrid white wine grapes in Mid-Atlantic vineyards. Australas. Plant Pathol. 47:181-188.
Kilani, J., and Fillinger, S. 2016. Phenylpyrroles: 30 years, two molecules and (nearly) no resistance. Front. Microbiol. 7:2014.
Kim, B. S., and Hwang, B. K. 2007. Microbial fungicides in the control of plant diseases. J. Phytopathol. 155:641-653.
King, W. T., Madden, L. V., Ellis, M. A., and Wilson, L. L. 1997. Effects of temperature on sporulation and latent period of *Colletotrichum* spp. infecting strawberry fruit. Plant Dis. 81:77-84.
Lardner, R., Johnston, P. R., Plummer, K. M., and Pearson, M. N. 1999. Morphological and molecular analysis of *Colletotrichum acutatum* sensu lato. Mycol. Res. 103:275-285.
Leandro, L. F. S., Gleason, M. L., Nutter, F. W., Wegulo, S. N., and Dixon, P. M. 2001. Germination and sporulation of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 91:659-664.
Leandro, L. F. S., Gleason, M. L., Nutter, F. W., Wegulo, S. N., and Dixon, P. M. 2003. Influence of temperature and wetness duration on conidia and appressoria of *Colletotrichum acutatum* on symptomless strawberry leaves. Phytopathology 93:513-520.
Lei, Y. 2016. Identification and characterization of *Colletotrichum* species causing grape ripe rot in southern China. Mycosphere 7:1177-1191.
Leroux, P. 1996. Recent developments in the mode of action of fungicides. Pestic. Sci. 47:191-197.
López-Moral, A., Agustí-Brisach, C., Lovera, M., Luque, F., Roca-Castillo, L. F., Arquero, O., et al. 2019. Effects of cultivar susceptibility, fruit maturity, leaf age, fungal isolate, and temperature on infection of almond by *Colletotrichum* spp. Plant Dis. 103:2425-2432.
Ma, Z., and Michailides, T. J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24:853-863.
Maas, J. L. 1998. Compendium of Strawberry Diseases, 2nd Ed. American Phytopathological Society, St. Paul, MN.
MacKenzie, S. J., Mertely, J. C., and Peres, N. A. 2009a. Curative and protectant activity of fungicides for control of crown rot of strawberry caused by *Colletotrichum gloeosporioides*. Plant Dis. 93:815-820.
MacKenzie, S. J., and Peres, N. A. 2012. Use of leaf wetness and temperature to time fungicide applications to control anthracnose fruit rot of strawberry in Florida. Plant Dis. 96:522-528.
MacKenzie, S. J., Peres, N. A., Barquero, M. P., Arauz, L. F., and Timmer, L. W. 2009b. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. Phytopathology 99:620-631.
McManus, P., and Perry, R. S. 2017. Evaluation of fungicides for control of cranberry fruit rot in in Wisconsin, 2016. Plant Dis. Manag. Rep. 11:SMF012. doi:10.1094/PDMR11
Meazza, G., Dayan, F. E., and Wedge, D. E. 2003. Activity of quinones on *Colletotrichum* species. J. Agric. Food Chem. 51:3824-3828.
Mertely, J., and Peres, N. A. 2013. Evaluation of products for anthracnose and Botrytis fruit rot control in annual strawberry, 2012-13. Plant Dis. Manag. Rep. 8:SMF013. doi:10.1094/PDMR08
Mertely, J., Seijo, T., Martin, R., and Peres, N. A. 2011. Evaluation of products for the control of anthracnose fruit rot in annual strawberry, 2010-2011. Plant Dis. Manag. Rep. 6:SMF014. doi:10.1094/PDMR06
Mertely, J., Seijo, T., and Peres, N. A. 2015. Evaluation of products for anthracnose and Botrytis fruit rot control in annual strawberry, 2014-2015. Plant Dis. Manag. Rep. 9:SMF019. doi:10.1094/PDMR09
Mertely, J., Seijo, T., and Peres, N. A. 2018. Evaluation of products for anthracnose fruit rot and Gnomonia leaf blotch control in strawberry, 2016-2017. Plant Dis. Manag. Rep. 12:PF046. doi:10.1094/PDMR12
Mertely, J. C., Forcelini, B. B., and Peres, N. A. 2017. Root necrosis of strawberry caused by *Colletotrichum acutatum*. Publ No PP-211 Univ. IFAS EDIS, Gainesville, FL.
Mertely, J. C., and Legard, D. E. 2004. Detection, isolation, and pathogenicity of *Colletotrichum* spp. from strawberry petioles. Plant Dis. 88:407-412.
Mertely, J. C., Seijo, T., and Peres, N. 2019. Evaluation of products and programs for anthracnose fruit rot in annual strawberry, 2018-2019. Plant Dis. Manag. Rep. 8:SMF013. doi:10.1094/PDMR08
Mertely, J. C., Seijo, T. E., and Peres, N. A. 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. 11:30.
Miller, J. S., Funk, V. A., Wagner, W. L., Barrie, F., Hoch, P. C., and Herendeen, P. 2011. Outcomes of the 2011 botanical nomenclature section at the XVIII International Botanical Congress. PhytoKeys 5:1-3.
Munda, A. 2014. First report of *Colletotrichum fioriniae* and *C. godetiae* causing apple bitter rot in Slovenia. Plant Dis. 98:1282.
Munir, M., Amsden, B., Dixon, E., Vaillancourt, L., and Gauthier, N. A. W. 2016. Characterization of *Colletotrichum* species causing bitter rot of apple in Kentucky orchards. Plant Dis. 100:2194-2203.
O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogenet. Evol. 7:103-116.
Olaya, G., Zheng, D., and Köller, W. 1998. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. Pestic. Sci. 54:230-236.
Oudemans, P. V., Caruso, F. L., and Stretch, A. W. 1998. Cranberry fruit rot in the northeast: a complex disease. Plant Dis. 82:1176-1184.
Oxford English Dictionary. Anthracnose n.8401. Oxford English Dictionary, Oxford University Press.
Pavan, W., Fraisse, C. W., and Peres, N. A. 2011. Development of a web-based disease forecasting system for strawberries. Comput. Electron. Agric. 75:169-175.
Peng, L.-J., Sun, T., Yang, Y.-L., Cai, L., Hyde, K. D., Bahkali, A. H., et al. 2013. *Colletotrichum* species on grape in Guizhou and Yunnan provinces, China. Mycoscience 54:29-41.
Peres, N. A., Timmer, L. W., Adaskaveg, J. E., and Correll, J. C. 2005. Lifestyles of *Colletotrichum acutatum*. Plant Dis. 89:784-796.
Prusky, D. 1993. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. Plant Dis. 77:114.
Pscheidt, J. W., and Bassinette, J. P. 2016. Fungicide management of blueberry rots, 2015. Plant Dis. Manag. Rep. 10:SMF027. doi:10.1094/PDMR10
Rahman, M., Islam, T., Schwegel, R., and Louws, F. J. 2019. Simultaneous detection of *Colletotrichum acutatum* and *C. gloeosporioides* from quiescently infected strawberry foliage by real-time PCR based on high resolution melt curve analysis. Am. J. Plant Sci. 10:382-401.
Rahman, M., Ojiambo, P., and Louws, F. 2015. Initial inoculum and spatial dispersal of *Colletotrichum gloeosporioides*, the causal agent of strawberry anthracnose crown rot. Plant Dis. 99:80-86.
Ritchie, D. F., Parker, M. L., Hoge, B., Obermiller, J. D., and Holmberg, C. 2016. Evaluation of selected fungicides and their time of application for management of Glomerella leaf spot on Gala apples, 2015. Plant Dis. Manag. Rep. 10:PF017. doi:10.1094/PDMR10
Rockenbach, M. F., Velho, A. C., Gonçalves, A. E., Mondino, P. E., Alaniz, S. M., and Stadnik, M. J. 2016. Genetic structure of *Colletotrichum fructicola* associated to apple bitter rot and Glomerella leaf spot in southern Brazil and Uruguay. Phytopathology 106:774-781.
Rojas, E. I., Rehner, S. A., Samuels, G. J., Bael, S. A. V., Herre, E. A., Cannon, P., et al. 2010. *Colletotrichum gloeosporioides s.l.* associated with *Theobroma cacao* and other plants in Panamá: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102:1318-1338.
Rosenberger, D., and Cox, K. 2016. Preventing bitter rot in apples: bitter with the sweet. Scaffolds Fruit J. 25:1-4.
Salazar, S. M., Castagnaro, A. P., Arias, M. E., Chalfoun, N., Tonello, U., and Díaz Ricci, J. C. 2007. Induction of a defense response in strawberry mediated by an avirulent strain of *Colletotrichum*. Eur. J. Plant Pathol. 117:109-122.

Salazar, S. M., Grellet, C. F., Chalfoun, N. R., Castagnaro, A. P., and Díaz Ricci, J. C. 2013. Avirulent strain of *Colletotrichum* induces a systemic resistance in strawberry. Eur. J. Plant Pathol. Dordr. 135:877-888.

Salinas, N., Verma, S., Peres, N., and Whitaker, V. M. 2019. FaRCa1: a major subgenome-specific locus conferring resistance to *Colletotrichum acutatum* in strawberry. Theor. Appl. Genet. 132:1109-1120.

Samuelian, S. K., Greer, L. A., Savocchia, S., and Steel, C. C. 2012. Overwintering and presence of *Colletotrichum acutatum* (ripe rot) on mummified bunches, dormant wood, developing tissues and mature berries of *Vitis vinifera*. Vitis 51:33-37.

Schnabel, G., Hu, M., and Fernández-Ortuño, D. 2015. Monitoring resistance by bioassay: relating results to field use using culturing methods. Pages 281-293 in: Fungicide Resistance in Plant Pathogens. H. Ishii and D. W. Hollomon, eds. Springer, Tokyo, Japan.

Seijo, T. E., Chandler, C. K., Mertely, J. C., Moyer, C., and Peres, N. A. 2008. Resistance of strawberry cultivars and advanced selections to anthracnose and *Botrytis* fruit rots. Pages 246-248 in: Proceedings of the Florida State Horticultural Society 121.

Sharma, G., Kumar, N., Weir, B. S., Hyde, K. D., and Shenoy, B. D. 2013. The ApMat marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. Fungal Divers. 61:117-138.

Shi, Y. 1996. Frequency of *Colletotrichum* species causing bitter rot of apple in the southeastern United States. Plant Dis. 80:692.

Shiraishi, A., Araki-Sasaki, K., Mitani, A., Miyamoto, H., Sunada, A., Ueda, A., et al. 2011. Clinical characteristics of keratitis due to *Colletotrichum gloeosporioides*. J. Ocul. Pharmacol. Ther. 27:487-491.

Shuman, J. L., and Bratsch, A. D. 2004. Actigard reduces anthracnose on field-grown strawberry. HortScience 39:824.

Silva, D. N., Talhinhas, P., Várzea, V., Cai, L., Paulo, O. S., and Batista, D. 2012. Application of the *Apn2/MAT* locus to improve the systematics of the *Colletotrichum gloeosporioides* complex: an example from coffee (*Coffea* spp.) hosts. Mycologia 104:396-409.

Simmonds, J. H. 1966. A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. Queensl. J. Agric. Anim. Sci 22:437-459.

Smith, B. J. 2008. Epidemiology and pathology of strawberry anthracnose: a North American perspective. HortScience 43:69-73.

Stensvand, A., Børve, J., and Talgø, V. 2017. Overwintering diseased plant parts and newly infected flowers and fruit as sources of inoculum for *Colletotrichum acutatum* in sour cherry. Plant Dis. 101:1207-1213.

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

Sutton, T. B. 1983. Epidemiology of the perfect stage of *Glomerella cingulata* on apples. Phytopathology 73:1179.

Sutton, T. B., Aldwinckle, H. S., Agnello, A. M., and Walgenbach, J. F. 2014. Compendium of apple and pear diseases and pests. American Phytopathological Society, St. Paul, MN.

te Welscher, Y. M., Jones, L., van Leeuwen, M. R., Dijksterhuis, J., de Kruijff, B., Eitzen, G., et al. 2010. Natamycin inhibits vacuole fusion at the priming phase via a specific interaction with ergosterol. Antimicrob. Agents Chemother. 54: 2618-2625.

te Welscher, Y. M., Napel, H. H., Balagué, M. M., Souza, C. M., Riezman, H., Kruijff, B., et al. 2008. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane. J. Biol. Chem. 283:6393-6401.

Ureña-Padilla, A. R., MacKenzie, S. J., Bowen, B. W., and Legard, D. E. 2002. Etiology and population genetics of *Colletotrichum* spp. causing crown and fruit rot of strawberry. Phytopathology 92:1245-1252.

Ureña-Padilla, A. R., Mitchell, D. J., and Legard, D. E. 2001. Oversummer survival of inoculum for *Colletotrichum* crown rot in buried strawberry crown tissue. Plant Dis. 85:750-754.

Vieira, W. A. S., Bezerra, P. A., da Silva, A. C., Silva Veloso, J., Paz Saraiva Câmara, M., and Patrick Doyle, V. 2020. Optimal markers for the identification of *Colletotrichum* species. Evol. Biol. 143:106694.

Vieira, W. A. S., Lima, W. G., Nascimento, E. S., Michereff, S. J., Câmara, M. P. S., and Doyle, V. P. 2017. The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with *Musa*. Mycologia 109:912-934.

Vieira, W. A. S., Michereff, S. J., de Morais, M. A., Hyde, K. D., and Câmara, M. P. S. 2014. Endophytic species of *Colletotrichum* associated with mango in northeastern Brazil. Fungal Divers. 67:181-202.

Villani, S. M., Johnson, K. A., Kreis, R. A., and Justus, C. 2019. Evaluation of fungicides for the management of Glomerella leaf spot and bitter rot on 'Gala' apple in NC, 2018. Plant Dis. Manag. Rep. 13:PF062. doi:10.1094/PDMR13

Villani, S. M., Johnson, K. A., Kreis, R. A., and Justus, J. D. 2018. Evaluation of fungicides for the management of Glomerella leaf spot and bitter rot on "Gala" apple in NC, 2017. Plant Dis. Manag. Rep. 12:PF025. doi:10.1094/PDMR12

Waller, T. J., Vaiciunas, J., Constantelos, C., and Oudemans, P. V. 2018. Evidence that blueberry floral extracts influence secondary conidiation and appressorial formation of *Colletotrichum fioriniae*. Phytopathology 108:561-567.

Walter, M., Braithwaite, B., Smith, B. J., and Langford, G. I. 2008. Nutrient nitrogen management for disease control in strawberry. N. Z. Plant Prot. 61:70-79.

Wang, N.-Y., Forcelini, B. B., and Peres, N. 2019. Anthracnose fruit and root necrosis of strawberry are caused by a dominant species within the *Colletotrichum acutatum* species complex in the United States. Phytopathology 109:1293-1301.

Wedge, D. E., Smith, B. J., Quebedeaux, J. P., and Constantin, R. J. 2007. Fungicide management strategies for control of strawberry fruit rot diseases in Louisiana and Mississippi. Crop Prot. 26:1449-1458.

Weir, B. S., Johnston, P. R., and Damm, U. 2012. The *Colletotrichum gloeosporioides* species complex. Stud. Mycol. 73:115-180.

Wenneker, M., Pham, K. T. K., Lemmers, M. E. C., de Boer, F. A., van der Lans, A. M., van Leeuwen, P. J., et al. 2016. First report of *Colletotrichum godetiae* causing bitter rot on 'Golden Delicious' apples in the Netherlands. Plant Dis. 100:218.

Wharton, P. S., and Diéguez-Uribeondo, J. 2004. The biology of *Colletotrichum acutatum*. An. Jardín Botánico Madr.:20.

Whitaker, V. M., Chandler, C. K., Peres, N., do Nascimento Nunes, M. C., Plotto, A., and Sims, C. A. 2015. Sensation™ 'Florida127' Strawberry. HortScience 50:1088-1091.

Whitaker, V. M., Chandler, C. K., Santos, B. M., Peres, N., do Nascimento Nunes, M. C., Plotto, A., and Sims, C. A. 2012a. Winterstar™ ('FL 05-107') Strawberry. HortScience 47:296-298.

Whitaker, V. M., Hasing, T., Chandler, C. K., Plotto, A., and Baldwin, E. 2011. Historical trends in strawberry fruit quality revealed by a trial of University of Florida cultivars and advanced selections. HortScience 46:553-557.

Whitaker, V. M., Osorio, L. F., Hasing, T., and Gezan, S. 2012b. Estimation of genetic parameters for 12 fruit and vegetative traits in the University of Florida strawberry breeding population. J. Am. Soc. Hortic. Sci. 137: 316-324.

Whitaker, V. M., Peres, N., and Agehara, S. 2018. "Florida Brilliance" Strawberry. Publication HS1322. University of Florida IFAS Extension, Gainesville, FL. https://edis.ifas.ufl.edu/hs1322

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

Wilcox, W. F., Gubler, W. D., and Uyemoto, J. K. 2015. Compendium of grape diseases, disorders, and pests. American Phytopathological Society, St. Paul, MN.

Wilson, L. L., Madden, L. V., and Ellis, M. A. 1990. Influence of temperature and wetness duration on infection of immature and mature strawberry fruit by *Colletotrichum acutatum*. Phytopathology 80:111-116.

Yan, J.-Y., Jayawardena, M. M. R. S., Goonasekara, I. D., Wang, Y., Zhang, W., Liu, M., et al. 2015. Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Divers. 71:233-246.

Yang, X. 1990. Rain splash dispersal of *Colletotrichum acutatum* from infected strawberry fruit. Phytopathology 80:590.

Yoder, K., Chochran, A. E., Royston, W. S., Kilmer, S. W., and Kowalski, A. L. 2017. Evaluation of OMRI-approved and conventional products for disease management on three apple cultivars, 2017. Plant Dis. Manag. Rep.12:PF040. doi:10.1094/PDMR12

Yokosawa, S., Eguchi, N., Kondo, K., and Sato, T. 2017. Phylogenetic relationship and fungicide sensitivity of members of the *Colletotrichum gloeosporioides* species complex from apple. J. Gen. Plant Pathol. 83:291-298.

Yoshida, S., Tsukiboshi, T., Shinohara, H., Koitabashi, M., and Tsushima, S. 2007. Occurrence and development of *Colletotrichum acutatum* on symptomless blueberry bushes. Plant Pathol. 56:871-877.

Zaitlin, B., Zehr, E. I., and Dean, R. A. 2000. Latent infection of peach caused by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. Can. J. Plant Pathol. 22:224-228.

Zapparata, A., Da Lio, D., Sarrocco, S., Vannacci, G., and Baroncelli, R. 2017. First report of *Colletotrichum godetiae* causing grape (*Vitis vinifera*) berry rot in Italy. Plant Dis. 101:1051.

Zhang, X., Batzer, J. C., Li, X., Peres, N. A., and Gleason, M. L. 2019. Validation of a Florida strawberry anthracnose fruit rot (AFR) warning system in Iowa. Plant Dis. 103:28-33.

Zhang, X., Harrington, T. C., Batzer, J. C., Kubota, R., Peres, N. A., and Gleason, M. L. 2016. Detection of *Colletotrichum acutatum* sensu lato on strawberry by loop-mediated isothermal amplification. Plant Dis. 100:1804-1812.