IDENTIFICATION OF PATHOGEN SPECIES CAUSING BROWN ROT OF PEACHES IN ILLINOIS AND EVALUATING EFFICACY OF POTENTIAL FUNGICIDES FOR MANAGING THE DISEASE

BY

HARRISON SEITZ

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois Urbana-Champaign, 2022

Urbana, Illinois

Advisers:

Professor Mohammad Babadoost, Chair
Professor Andrew Miller

ABSTRACT

Brown rot, caused by *Monilinia* spp., is one of the most important fungal diseases of peach in Illinois. To our knowledge, no research has been conducted in Illinois on the occurrence and management of brown rot disease of peach or other stone fruit crops in Illinois. The objectives of this study were to: (i) conduct orchard surveys to assess incidence of brown rot disease in commercial peach orchards in Illinois, (ii) identify fungal pathogens causing brown rot disease, (iii) evaluate variation among the pathogen isolates, and (iv) evaluate the efficacy of eleven registered potential fungicides for managing brown rot of peach in the orchard.

Orchard surveys were conducted in 2020, 2021, and 2022 to assess occurrence of blossom blight, shoot blight, and fruit rot caused by *Monilinia* spp. in commercial peach orchards in Illinois. No blossom blight or shoot blight was observed. Results showed 9 of 14, 4 of 8, and 6 of 13 orchards had symptomatic brown rot fruits in 2020, 2021, and 2022, respectively. Symptomatic fruits were collected from peach cultivars Bounty, Carolina Belle, Contender, Fayette, Loring, Redhaven, Starfire, and an unknown cultivar in eight different orchards throughout the state. No infection was detected in cultivar July Prince. Infected fruit tissues were cultured on potato dextrose agar medium, and the pathogens were isolated. Based on the cultural characteristics and sequences of the ITS region, 127 of 129 collected isolates were identified as *Monilinia fructicola* (Winter) Honey, and two isolates were identified as *M. laxa* (Aderhold & Ruhland) Honey.

Fungicide sensitivity of *Monilinia fructicola* isolates was conducted in the laboratory using eleven fungicides, including azoxystrobin (Abound 2.08SC), captan (Captan 80WDG), fenhexamid (Elevate 50WDG), trifloxystrobin (Flint Extra 4.05E), penthiopyrad (Fontelis 1.67SC), difenoconazole + cyprodinil (Inspire Super 2.82SC), fluopyram + tebuconazole (Luna Experience 3.34SC), fluopyram + trifloxystrobin (Luna Sensation 4.20SC), fluxapyroxad +

pyraclostrobin (Merivon 4.18SC), propiconazole (Tilt 3.6EC), and thiophanate-methyl (Topsin-M 70WP). The EC_{50} of Abound, Captan, Flint Extra, and Fontelis for the colony development of the isolates were significantly ($P = 0.05$) higher than the other fungicides tested. Field trials were conducted at the University of Illinois Fruit Research Farm in Urbana, IL on ‘Redhaven’ and ‘Contender’ peaches in 2021 and 2022, to evaluate efficacy of the above-mentioned fungicides for managing brown rot disease. Trees were sprayed with fungicides at 10- and 14-day intervals from petal fall until two weeks to harvest. In 2021, incidence of symptomatic fruits was significantly ($P = 0.05$) higher in untreated plots than treated plots. In 2022, only nine fruits of ‘Redhaven’ with brown rot symptoms were observed, and there was no fruit of ‘Contender’ with brown rot symptoms. Luna Experience 3.34SC, Luna Sensation 4.20SC, and Merivon 4.18SC were the most effective fungicides for management of brown rot and other summer diseases of peach.

ACKNOWLEDGMENTS

I would like to thank Dr. Mohammad Babadoost for giving me the opportunity to pursue a Master’s degree at the University of Illinois at Urbana-Champaign, and for his guidance, support, and input during all stages of the research and thesis writing process. I would like to thank Dr. Andrew Miller for allowing me to use his molecular laboratory to sequence my isolates. Finally, I would like to thank my lab-mate Festus Acheampong for help with applying fungicides during the 2021 field season, and Jason Karakehian for his instruction and countless pieces of advice during the molecular sequencing phase.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION AND OVERVIEW

Introduction

Peach The peach tree (*Prunus persica* pv. *persica*) is a deciduous plant in the Rosaceae family. Peach production originated in the Zhejiang province in eastern China in roughly 6,000 BCE (Zheng et al., 2014). Due to the chilling requirement of at least 500 hours below 10ºC each winter, peach trees only grow in a limited range of dry, temperate climates, mostly in the northern hemisphere (Lockwood and Coston, 2007). Optimal regions include the United States (US) and Mexico in North America; Brazil, Argentina, and Chile in South America; Mediterranean Europe and northern Africa; some Middle Eastern countries such as Turkey and Iran; India; and eastern Asian countries such as China, South Korea, and Japan.

Peach production Total peach production in 2020 was estimated at 27.08 million tons, with China accounting for 61% of global peach production. The US ranks 7th worldwide with 0.62 million tons (United Nations, 2022). Peach orchards account for the 5th largest acreage of non-citrus fruits in the US, with 74,000 acres in 2021. In addition, peaches are the 5th most produced non-citrus fruit by weight and the 6th most valuable by utilized production value ($625 million in 2021) (USDA, 2022). Peaches are commercially grown in 20 states as of 2017 (AgMRC, 2021), with roughly 75% of production occurring in California (USDA, 2022). There are roughly 3,000 acres of peach orchards in Illinois (USDA, 2022).

Peach diseases in Illinois Major peach diseases in Illinois include peach leaf curl, powdery mildew, perennial canker, bacterial spot, scab, and brown rot (Babadoost, 2017a; Babadoost, 2017b; University of Illinois Extension, 1984; University of Illinois Extension, 2022).

Peach leaf curl Peach leaf curl is caused by the fungus *Taphrina deformans*. While peach leaf curl rarely kills trees, it often leaves them in a weakened state, and more susceptible to other pathogens and winter damage. Infected leaves are often weakened, crispy, and severely puckered (University of Wisconsin Extension, 2021). Although the affected leaves are often replaced by a second growth in June or July, the tree is now in a weakened state. After the distorted leaves become visible, developing fungal asci break through the cuticle. These asci produce ascospores, which produce conidia by budding. Conidia are splashed, blown, or washed onto bark cracks and crevices, where they overwinter. In early spring, the spores infect swelling leaves and flowers within the buds (UC Davis Extension, 2012). Preventive measures taken before trees break dormancy in early spring can control this disease, and the trees are only susceptible to infection between swelling and opening of the buds (Babadoost, 2017a).

Powdery mildew Powdery mildew of peaches is caused by the fungus *Sphaerotheca pannosa*. The pathogen infects fruits, young shoots, and leaves, and may cause yield losses of more than 50% if not managed. It is not an especially economically important disease in Illinois (Babadoost, 2017b). *S. pannosa* overwinters as mycelia in the inner bud scales of peach and infects the leaves as they emerge from the buds. The pathogen causes secondary infection on nearby leaves in warm, humid conditions during the growing season. Conidia are produced and spread by wind, and infection is more likely when humidity is high (UC Davis Extension, 2015). Leaves may become white with mealy mycelia, and may curl and become stunted. Fruits are susceptible from the early stages of growth until pit-hardening. Circular white spots will form which may enlarge and cover the entire fruit. This may cause deformation of young fruits, and on older fruits, the lesions may become scabby and necrotic (Babadoost, 2017b).

Perennial canker of peach Perennial canker of peach is caused by the fungi *Cytospora leucostoma* and *Cytospora cincta.* Symptoms include gum or sap exudate from scaffold limbs or trunks of trees. The pathogen is incapable of infecting healthy peach tree bark, but if the bark is wounded or dead, the fungus can invade (Utah State University Extension, 2022). This causes a light amber gum to form, which gradually turns dark brown. Underneath the gum is a small black necrotic lesion, which gradually expands and causes a collapse of the inner bark tissue. The necrotic area continues to expand each year, eventually causing entire branches to collapse (Ohio State University Extension, 2016). The pathogen survives as tiny black pycnidia on cankered surfaces all year long. Pycnidiospores are spread by wind, rain, machinery, birds, and insects. They germinate during warm, moist periods, and infect tissues. New pycnidia form on cankered regions 7-8 weeks after initial infection, completing the disease cycle (University of Illinois Extension, 1984).

Bacterial spot Bacterial spot of peach, also known as bacterial shot-hole or bacterial canker, is caused by *Xanthomonas arboricola* pv. *pruni*. It is widespread on susceptible cultivars of all stone fruits east of the Rocky Mountains (Babadoost, 2017c). The pathogen infects leaves, twigs, and fruit, reducing both yield and quality. The disease defoliates the tree early in the growing season and weakens the tree. The tree is more susceptible to winter injury and perennial canker. The disease is favored by humid weather in June and July, and is especially problematic in areas with more potential for mechanical damage, such as sandy terrain (Michigan State University Extension, 2011). Numerous small spots appear on leaves, with a water-soaked appearance that gradually turns to dark brown or black before the tissue falls away completely, leaving a “shot-hole” appearance. Spots may merge to give the entire leaf a scorched or blighted appearance, or the leaf may fall away entirely. On fruit, small olive-brown to black spots may form, which enlarge to create irregular shaped lesions that may crack. Fruit infected in the early stages of development is the most malformed, while those infected near harvest-time usually have superficial blemishes (Penn State University Extension, 2017a). In Illinois, the bacteria infect peach twigs by entering through the leaf scars and overwintering on the twigs. In spring, cankers are produced from which bacterial ooze exudes; this is disseminated by insects or rain or dew droplets. If the ooze lands on healthy leaves, twigs, or green fruit, it is capable of penetrating through stomata, lenticels, and small wounds to cause infection (Babadoost, 2017c).

Peach scab Peach scab is caused by the fungus *Cladosporium carpophilum.* The pathogen mainly causes losses by spotting of the fruit epidermis. It also causes minor damage from twig dieback and premature defoliation (Babadoost, 2017d). Warm, wet conditions in spring and early summer after petal fall are necessary for a large infection. Modern fungicides can control the scab adequately, except in orchards with poor sanitation and/or continually wet terrain. Scab often forms as olive-green spots on fruit 6-7 weeks after petal fall (Babadoost, 2017d). The infected fruit may fail to ripen properly and do not store well if they are harvested, and oftentimes either drop prematurely or crack, leaving the fruit open to other infections. Pale green spots may form on the underside of leaves, and infected twigs may develop raised yellowish-brown blotches. The fungus overwinters on twig lesions, and during the growing season, large numbers of conidia form on the surface of the lesion. During periods of high humidity, the conidia are spread to developing fruit, twigs, and leaves by rain and mist (Texas A&M University Extension, 2014). Peach fruits are most susceptible at shuck split, and the period of latent infection on fruit is about 42-77 days (Penn State University Extension, 2017b).

Brown rot Brown rot is one of the most common and serious diseases affecting peach fruits (HGIC, 2021; Hu et al., 2011). Worldwide annual yield losses by brown rot were estimated to be up to 1.7 billion Euros (Martini and Mari, 2014), and up to $170 million in the US (RosBREED, 2018). It is one of the most common and destructive diseases of peaches in Illinois (Babadoost, 2017e), with fruit losses of up to 26% on Illinois orchards in recent years (Seitz et al., 2021). The pathogen causes blossom blight, shoot blight, shoot canker, and fruit rot, and is found in all peach growing areas in the world (Oliveira Lino et al., 2016).

Brown rot of peaches is caused by species of the fungal genus *Monilinia*. Three species are economically significant pathogens of peaches, including *M. fructicola* (Winter) Honey, *M. laxa* (Aderhold & Ruhland) Honey, and *M. fructigena* (Aderhold & Ruhland) Honey (Van Leeuwen and Van Kesteren, 1998). *M. fructicola* has a worldwide distribution, while *M. laxa* and *M. fructigena* have historically been confined to Europe (Oliveira Lino et al., 2016). However, in the past few decades, *M. laxa* has been documented on stone fruit in the US (Snyder and Jones, 1999; Villani and Cox, 2010).

Monilinia spp. are ascomycetous fungi, and their sexual reproduction involves producing ascospores in tubular sacs called asci on the upper surface of a cup-like fruiting body called an apothecium. These apothecia are 5-20 mm in diameter and usually sprout out of mummified fruit on the ground in spring. *Monilinia* apothecia are rarely found in the wild (Ritchie, 2000).

Monilinia spp. reproduce asexually much more often than sexually. Asexual spores called conidia are produced on tufts of conidiophores. The conidia are colorless, lemon- or football-shaped, and are produced in moniloid fashion (resembling a string of beads with the ends pinched). Conidia can germinate in 3-5 hours in optimal conditions (Ritchie, 2000).

The pathogen overwinters in shriveled fruits called mummies, as well as infected peduncles and cankers. The first stage of infection, blossom blight, occurs at bloom time in the spring, as susceptible blossom tissue emerges. The primary inoculum, conidia from the overwintering sources, may spread to the blossoms by insects, wind, or rain, and cause blossom blight. Blossom blight symptoms include yellow, wilting leaves on branches and twigs; and symptoms may appear a few days to two weeks after infection. Infection is highly dependent on both temperature and duration of wetness: at 10ºC, 18 hours of wetness are necessary to cause infection, while at 25ºC, only 5 hours of wetness are needed (Ritchie, 2000).

As the summer progresses, the peach fruit grow, ripen, and increase in sugar content, which makes them susceptible to infection. Inoculum sources for fruit infection include the previous year's mummies, blighted blossoms, cankers, and other diseased fruit in the tree or on the orchard floor. Although infection can occur on unwounded fruit, it is facilitated by wounds in the peach epidermis caused by birds, insects, weather, or mechanical damage. Warm, wet or humid weather in the 2- to 3-week period prior to harvest increase both inoculum and the infection success rate, leading to increased disease severity. Symptoms include ash-gray-brown (*M. fructicola*) or white *(M. laxa*) circular, fuzzy masses of conidia on ripe fruit in humid conditions. Roughly a week post-infection, fruits shrivel into brown, hardened spore masses called mummies, which may hang on the tree or fall to the ground, where they overwinter and provide primary inoculum the following spring (Ritchie, 2000).

Macroscopic and microscopic differentiation between the *Monilinia* species is possible both in the orchard and laboratory. The mean conidia size for *M. fructicola* is 18 µm x 12.5 µm, while the mean conidia size for *M. laxa* is 11.5 µm x 8 µm (Byrde and Willets, 1977). On potato dextrose agar medium (PDA), *M. fructicola* grew 80% faster than *M. laxa* (10.8 mm/day vs. 6.0 mm/day, respectively) (Hu et al., 2011). Margins of *M. laxa* colonies grown on PDA are typically lobed, while *M. fructicola* margins are entire (Rungjindamai et al., 2014).

Identification of *Monilinia* isolates has traditionally been based on morphological and physiological characteristics, such as colony shape and color, conidia shape, growth rate, and mycelial margins (Byrde and Willets, 1977). However, this method is subject to human error, and phenotypic variability has been demonstrated even within species (Byrde and Willets, 1977). Molecular methods are therefore widely used to accurately identify species, and the combination of molecular and morphological methods can provide the most accurate results. The most common method involves amplifying the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA), a technique that is used to identify ascomycetous fungi (Larena et al., 1999; Nilsson et al., 2009; Scoch et al., 2012). For this method, the primers ITS1F and ITS4A may be used for a polymerase chain reaction (PCR) (Larena et al., 1999). Gell et al. (2007) developed primers for species-specific *Monilinia* diagnosis using PCR, by amplifying the rDNA region using ITS5 and ITS4A primers. They developed the oligonucleotide ITS primers *IMon3-1* (GCTCGCCAGAGAATAAYY) and *IMon-2* (AGACTCAATACCAAGCTGT) for identification and differentiation of *Monilinia* spp.

While brown rot of stone fruit is one of the most important peach diseases worldwide, little successful work has been done on developing resistant cultivars of peaches because any amount of brown rot fruit infection will lead to complete loss of that fruit, and fungicides have thus far reliably controlled the disease (Byrne et al., 2012). While resistant cultivars have been found in neotropical locations in the Americas such as Mexico, Brazil, and Florida, these cultivars have only low- to moderate resistance (Byrne et al., 2012). Some cultivars in Brazil such as ‘Bolinha’ have quantitative disease resistance to *M. fructicola*; however, the resistance is only in the epidermis, so any amount of bird, insect, or weather damage will leave the fruit vulnerable to infection (Byrne et al., 2012).

Modern brown rot management relies on effective fungicide application, but this may be compromised by developed fungicide resistance of the pathogen (Cox et al., 2009). Fungicides inhibit or reduce disease development in plants by affecting pathogen cell membranes, inactivating enzymes or proteins needed for growth and reproduction, interfering with key life processes such as respiration, affecting metabolic pathways such as sterol and chitin production, or by triggering host plant immunity responses (McGrath, 2004). Site-specific fungicides, such as the methyl benzimidazole carbamates (MBCs), which target β-tubulin, and sterol demethylation inhibitors (DMIs), which target 14α-demethylase, have been used since the 1970s and 1980s, respectively, for the control of *M. fructicola* (Ma et al., 2003; Schnabel et al., 2004). However, frequent use of site-specific fungicides carries an increased risk of development of fungicide resistance (Chen et al., 2013). MBC resistance has been reported in the US since the 1970s (Ma et al. 2003), while DMI resistance was first reported in Georgia in 2004 (Schnabel et al., 2004). Both MBC and DMI resistance have since been reported in the Midwest peach orchards (Babadoost, 2019; Luo et al., 2008). Knowledge of the evolution of pathogen fungicide resistance is essential for developing effective strategies to increase the efficacy of fungicides and reduce the cost of disease management.

The overall goal of this research was to identify and characterize species of pathogens causing brown rot disease of peaches in Illinois, and to determine effective fungicides for managing the disease. The specific objectives of the study were:

1. Identify the species of pathogens causing brown rot in different areas of Illinois.

2. Evaluate efficacy of different fungicides in the laboratory on colony development, and test the fungicides on managing disease development in an orchard with a history of brown rot occurrence.

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CHAPTER 2: ASSESSING THE OCCURRENCE OF BROWN ROT DISEASE IN ILLINOIS PEACH ORCHARDS AND CHARACTERIZATION OF THE CAUSAL AGENTS

Abstract

Brown rot, caused by *Monilinia* spp., is one of the most important fungal diseases of peach in Illinois. Orchard surveys were conducted in 2020, 2021, and 2022 to assess occurrence of brown rot blossom blight, shoot blight, and fruit rot in commercial peach orchards in Illinois. No blossom blight or shoot blight was observed. Results showed 9 of 14, 4 of 8, and 6 of 13 orchards with symptomatic brown rot fruits in 2020, 2021, and 2022 respectively. Nine trees of each cultivar and 60 fruits per tree (five fruits in each of upper, middle, and lower canopies of northern, eastern, southern, and western sides) were inspected for brown rot symptoms. Incidence of symptomatic fruits ranged from 2.8 to 26.2% (mean 18%) in 2021 and 0.1 to 13.5% (mean 3%) in 2022. Symptomatic fruits were collected from peach cultivars Bounty, Carolina Belle, Contender, Fayette, Loring, Redhaven, Starfire, and an unknown cultivar in eight different orchards throughout the state. No infection was detected in cultivar July Prince. Infected tissues were cultured on potato dextrose agar medium, and the pathogens were isolated. Based on the cultural characteristics and sequences of the ITS region, 127 of 129 collected isolates were identified as *Monilinia fructicola*, and two isolates were identified as *M. laxa*.

Introduction

Brown rot, caused by *Monilinia* spp., is one of the most common and destructive diseases of stone fruits in Illinois (Babadoost, 2017). *Monilinia* spp. cause blossom blight, shoot blight, and fruit rot. In unsprayed peach orchards, fruit losses up to 50% occur in Illinois (Babadoost, 2017).

Brown rot is also one of the most common and economically important diseases of peaches worldwide (HGIC, 2021). The three economically important species of *Monilinia* are *M. fructicola, M. fructigena* and *M. laxa* (Van Leeuwen and Van Kesteren, 1998). Reports from China, Serbia, and Australia indicate that *M. fructicola* grows faster than *M. laxa* on potato dextrose agar Petri dishes at 22-25°C, by rates of 80%, 17%, and 117%, respectively (Hu et al., 2011; Hrustić et al., 2018; Tran et al., 2020). Lesion growth rates on inoculated stone fruit at 22-25°C had mixed results between the three studies; *M. fructicola* lesions grew 63% faster than *M. laxa* lesions in Australia, but *M. laxa* lesions grew 22% and 23% faster than *M. fructicola* lesions in Serbia and China, respectively (Tran et al., 2020; Hrustić et al., 2018; Hu et al., 2011). Finally, *M. laxa* isolates in Serbia grew 55% and 384% faster than *M. fructicola* on PDA Petri plates amended with the DMI fungicides propiconazole and fluopyram + trifloxystrobin, respectively (Hrustić et al., 2018).

As the fruit approaches maturity, one or several small light brown spots form on the skin (Ritchie, 2000). Within 2-3 days, the entire fruit may become somewhat watery, light brown, and rotten. The disease spreads most rapidly in warm, moist conditions (Ritchie, 2000). Several tan or gray spore tufts may break through the skin, giving the fruit a powdery appearance. Spore tufts may be scattered or arranged in concentric rings around a skin wound such as an insect injury (Babadoost, 2017). In 3-7 days, the entire fruit is consumed by tan to gray spore tufts of conidiophores and conidia of the brown rot fungus (Ritchie, 2000). Shortly after, the rotten fruit will become dehydrated and shrivel into a stiff, corrugated mass called a mummy. Infected fruit continue to rot after harvest, and the mycelium from infected fruit continue to infect any healthy fruit it contacts. Most rotten fruit fall off the tree; however, some mummies will continue to cling to the tree branches, where they become firm, black, and encrusted (Babadoost, 2017; Ogawa, 1995).

Due to the wide range of morphological variation within *Monilinia* species (Byrde and Willets, 1977), molecular methods are widely used to identify the species of *Monilinia* isolates (Gell et al., 2007; Hrustić et al., 2018; Hu et al., 2011). The most common method involves amplifying the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA), which is a technique that can be used to identify most ascomycete fungi (Larena et al., 1999; Nilsson et al., 2009; Scoch et al., 2012). For this method, the primers ITS1F and ITS4A may be used for a polymerase chain reaction (PCR). Gell et al. (2007) developed ITS5 and ITS4A primers for identifying the *Monilinia* genus, which amplify the rDNA region. They also developed the oligonucleotide primers *IMon3-1* (GCTCGCCAGAGAATAAYY) and *IMon-2* (AGACTCAATACCAAGCTGT) for differentiation of *Monilinia* spp. The ITS1F primer (CTTGGTCATTTAGAGGAAGTAA) is less species-specific and can be used for more general fungal isolate identification (Gardes and Bruns, 1993).

The objectives of this study were: (i) to conduct orchard surveys to assess incidence of brown rot disease in commercial peach orchards in Illinois, (ii) to identify fungal pathogens causing brown rot disease, and (iii) to evaluate genetic variation among the pathogen isolates. To our knowledge, no research on identification of species of brown rot pathogens has been conducted in Illinois.

Materials and methods

Field surveys In 2020, 2021, and 2022, orchard surveys were conducted to assess the incidence of brown rot blossom blight, shoot blight, and fruit rot disease of peaches in commercial orchards in Illinois. Surveys included 14, 8, and 13 orchards throughout the state in 2020, 2021, and 2022, respectively. For these purposes, an “orchard” was defined as an entity of peach trees of the same cultivar and location, meaning there could be multiple “orchards” on a single farm. Nine different cultivars, including ‘Bounty’, ‘Carolina Belle’, ‘Contender’, ‘Fayette’, ‘July Prince’, ‘Loring’, ‘Redhaven’, ‘Starfire’, and an unknown cultivar were evaluated for the presence of brown rot symptoms on blossoms, shoots, and fruits. For blossom blight, in each tree, all blossoms were inspected for blight, and four blossoms per tree were swabbed with a moist Q-Tip, which was transported back to the laboratory. The Q-Tips were swabbed onto potato dextrose agar (PDA) in Petri plates to determine presence of the brown rot pathogen. For shoot blight, the orchards were visited in early summer and nine trees per orchard were visually inspected for shoot blight.

For fruit rot, in each orchard, nine trees of each peach cultivar were selected, and 60 fruits (five fruits from each of north, east, south, and west sides, from the upper, middle, and lower canopies) were evaluated for presence of brown rot symptoms. Symptomatic fruits were collected for isolation of the pathogen. The collected fruits were placed in a cooler, stored at 4ºC, and the pathogen was isolated within 72 hours of collection.

Isolation and maintenance of pathogen isolates The fruit skin was surface sterilized by wiping the skin with a Kim-Wipe soaked with 80% ethanol. Using a sterile scalpel, small cubes of fruit skin and pulp (10 mm on each side) were cut from the outer margins of each lesion of each fruit. These surface-sterilized pieces were placed onto PDA medium in Petri plates (100 mm x 15 mm). These plates were incubated at 24ºC for 3 days in the dark. Subsequent colonies were obtained by cutting 5 mm x 5 mm pieces of agar from the edges of the developed fungal colonies and transferring them onto a fresh PDA plate. Resulting colonies were maintained on PDA plates at 24ºC for morphological characterization, DNA extraction, and pathogenicity tests.

Identification of the pathogen species Isolated fungal colonies were screened for morphological appearance. Colonies were divided into eight different morphotypes based on colony texture, color, and margin appearance after 14 days.

All collected isolates were used to identify the pathogen species using morphological and molecular characteristics. Species of the 129 isolates were identified using the following molecular methods. All isolates were grown on PDA for 10-21 days and genomic DNA was extracted using an E.Z.N.A. MicroElute Genomic DNA kit (Omega Bio Tek, Norcross, GA, USA) according to the manufacturer's protocol. The ITS1F-ITS4 primer combination was used to amplify the ITS region of the fungal rRNA operon (Gardes and Bruns, 1993). A 25 µL reaction was set up using the Go Taq Green Kit (Promega Corporation, Madison, WI, USA), including 5 µL of genomic DNA, 12.5 µL of Green Kit Master Mix, 5.5 µL of DNA-free water, and 1 µL each of 10 µM ITS1F and ITS4 primers. PCR amplification was performed using a C1000 Touch thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA), with initial denaturation at 94°C for 2 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, 55°C for 45 seconds and 72°C for 1 minute, and a final elongation step of 72°C for 10 minutes. From each PCR product, 3 µL was examined in 1% (w/v) agarose gels stained with EtBr in 1x TBE buffer electrophoresed at 95 V for 30 min. PCR product purification was done using a Wizard SV Gel and PCR Clean-Up kit (Promega Corporation, Madison, WI, USA). A Big Dyes kit was used to attach fluorescently-labeled nucleotides to the sequences and sent for Sanger sequencing in one direction using the ITS1F primer at the Sanger DNA Services Lab, Roy J. Carver Biotechnology Center, University of Illinois, Champaign, IL. Sequences were identified using the BLASTn aligner in the 'nr' database from the National Center for Biotechnology Information (NCBI).

Phylogenetic analysis Alignments of the ITS region for all sampled isolates were assembled using MUSCLE as implemented in Sequencher 5.4. The best-fit model was determined to be the general time reversible (GTR) model (Rodriguez et al., 1990) by jModeltest (Darriba et al., 2012; Guindon and Gascuel, 2003) based on the Akaike information criterion (AIC) (Posada and Buckley, 2004). A maximum likelihood (ML) analysis with 100 bootstrap replicates was performed using PhyML (Guindon and Gascuel, 2003; Guindon et al., 2010) as implemented in SeaView 4.7 (Gouy et al., 2010), with all parameters optimized in the GTR model. Clades with bootstrap values (BV) ≥ 70% were considered significant and strongly supported (Hillis and Bull, 1993). Two *Monilinia laxa* isolates from Urbana, Illinois, were included in the phylogenetic analysis for genetic variation comparison.

Pathogenicity test Identified *Monilinia fructicola* isolates were tested for pathogenicity in a laboratory on 'Redhaven' and 'Contender' peach fruits grown in an orchard in Urbana, Illinois. Peach fruits used for this experiment were harvested, stored in the dark at 4°C, and utilized in the experiment within 72 hours of harvest. The Hong et al. (1998) method for surface disinfestation was followed, by first soaking in 0.5% NaClO and then in 80% ethanol, followed by washing in sterile distilled water (SDW) for twice, each for 1 minute. The process was repeated twice. Surface-disinfested fruits were air dried on sterile paper towels on lab benches at room temperature (23°C). Twelve fruits of each cultivar were inoculated with each of four *Monilinia fructicola* isolates: three isolated from western Illinois and one from central Illinois. Fruits were wounded using syringe needles to produce five punctures, each 0.5 mm deep. Wounded fruits were inoculated by placing a 3 mm x 3 mm piece of PDA containing the mycelium onto each wounded spot. The piece of

PDA was cut from the edge of 7-14 day old growing cultures of each isolate. Pieces of plain PDA were placed onto control fruits. The fruits were placed in translucent plastic boxes and incubated at room temperature (23°C) on a 12 hour light/12 hour dark cycle. Diameters of developing lesions were measured daily for 5 days.

Results

Incidence of blossom blight and shoot blight in the orchards No blossom blight or shoot blight was observed in the orchards. Culturing the swabbed Q-Tip on PDA medium did not result in developing colonies of *Monilinia* spp.

Incidence of brown rot in orchards The incidence of brown rot in peach orchards ranged from 0 to 25% in 2020, 0 to 26.2% in 2021, and 0 to 13.5% in 2022. Brown rot incidence was recorded in 9 of 14 orchards visited in 2020 (Table 2.1 and Figure 2.1), 4 of 8 in 2021 (Table 2.1 and Figure 2.2), and 6 of 13 in 2022 (Table 2.1 and Figure 2.3). During the three years of surveys, fruits with brown rot were observed in 19 of 35 (54.2%) of visited commercial peach orchards (Tables 2.1 and 2.2). Overall, 66.7%, 28.6%, and 83.3% of visited peach orchards in western, southern, and central Illinois, respectively, had fruits with brown rot symptoms (Table 2.2).

Identification of pathogen species Using the species-specific ITS5 and ITS4 primers was unsuccessful, so the general ITS1F and ITS4 primers were used. ITS sequences of 435 bp confirmed that all isolates tested were *Monilinia* species. Sequence similarity searches using ITS indicated that 127 of 129 isolates were *M. fructicola,* with two isolates identified as *M. laxa*. Molecular phylogenetic analyses, based on final sequence alignment of ITS regions using MUSCLE in Sequencher software (version 5.4), and subsequent maximum likelihood analyses using PhyML in SeaView 4.7, grouped all isolates into two clades (Figure 2.6). The two isolates of *Monilinia laxa* originated from the same orchard in Urbana, in the central region. Only 2 of 27 (7.4%) collected isolates from this orchard were *M. laxa*.

Pathogenicity test All four isolates of *Monilinia* species tested were pathogenic and produced typical brown rot symptoms on inoculated peach fruits of ‘Redhaven’ and ‘Contender’. The symptoms exhibited fuzzy tan circular lesions (Figure 2.7). Pathogens were re-isolated from the lesions and re-identified as *Monilinia fructicola* using morphology and molecular sequencing.

Discussion

This is the first report of *Monilinia laxa* in Illinois peach orchards. The species had previously been identified in the Midwest on cherries in Michigan in 1999 (Snyder and Jones, 1999). Hrustić et al. (2018) found *M. laxa* isolates from Serbia grew faster than *M. fructicola* on PDA Petri plates amended with the DMI fungicides propiconazole and fluopyram + trifloxystrobin, demonstrating that different *Monilinia* species vary in their sensitivity to fungicides. Since brown rot of peaches is largely managed by fungicide applications, accurate identification of the pathogenic species is important for effectively managing brown rot disease. Results of our studies showed that, across years, the prevalence of brown rot of peaches is higher in orchards in western Illinois, where many commercial peach orchards are located, but we did not discover any *Monilinia laxa* isolates in the western or southern regions of the state; only the central region where few commercial peach orchards exist. These results indicate there is not enough evidence of *M. laxa* occurrence in major peach-growing regions of Illinois to change any management strategies. Additional research is needed to determine susceptibility of peach cultivars in Illinois to the brown rot pathogen; however, in Ontario, Canada, Biggs and Northover

(1985) found that early-maturing peach cultivars inoculated in laboratory conditions tended to have greater numbers of *M. fructicola* conidia than late-maturing cultivars.

As there was a large degree of morphological variation among *Monilinia* isolates (Byrde and Willetts, 1977), we relied on molecular methods to identify species of *Monilinia* causing brown rot of peaches in Illinois orchards. Our use of the ITS5 and ITS4 species-specific primers designed by Gell et al. (2007) was unsuccessful, as a gel electrophoresis test showed our PCR did not successfully amplify the ITS region. Hu et al. (2011) was able to successfully differentiate *M. fructicola* from *M. laxa*, using these primers, so a possible reason for our failure was improper storage of our primers (after receipt in the mail, they were stored unopened at room temperature on a desk for eight months until usage). While in our case, amplifying the ITS5 and ITS4 regions with custom primers was unsuccessful, amplifying the ITS region using the ITS1F and ITS4 primers described by Larena et al. (1999) was an effective method for species identification.

The results of our phylogenetic analysis indicate the presence of *M. laxa* in at least one peach orchard in central Illinois. Further research is needed to assess whether *M. laxa* occurs in more Illinois orchards. If this were the case, farmers could potentially see a slight reduction in the efficacy of DMI fungicides, according to results of Hrustić et al. (2018).

As no blossom blight or shoot blight was observed in any Illinois peach orchard visited in 2021 or 2022, this suggests either pathogen inoculum may not be present at bloom time or weather conditions are not conducive for blossom infection in Illinois. Further surveys can provide more information in this regard.

Tables and figures

<u>Table 2.1</u> Commercial peach orchards in Illinois surveyed for the incidence of brown rot of fruits in 2020, 2021, and 2022, sorted by year.

Year	Area	Orchards surveyed (no)	Incidence [no (%)]
2020	Western	6	5 (83.3%)
	Southern	6	2 (33.3%)
	Central	2	2 (100%)
	Total	14	9 (64.2%)
2021	Western	4	2 (50.0%)
	Southern	2	0 (0.0%)
	Central	2	2 (100%)
	Total	8	4 (50.0%)
2022	Western	5	3 (60.0%)
	Southern	6	2 (33.3%)
	Central	2	1 (50.0%)
	Total	13	6 (46.2%)
Grand Total		35	19 (54.2%)

Table 2.2 Commercial peach orchards in Illinois surveyed for the incidence of brown rot of fruits in 2020, 2021, and 2022, sorted by area.

Area	**Year**	**Orchards surveyed (no)**	**Incidence [no (%)]**
Western	2020	6	5 (83.3%)
	2021	4	2 (50.0%)
	2022	5	3 (60.0%)
	Total	15	10 (66.7%)
Southern	2020	6	2 (33.3%)
	2021	2	0 (0.0%)
	2022	6	2 (33.3%)
	Total	14	4 (28.6%)
Central	2020	2	2 (100%)
	2021	2	2 (100%)
	2022	2	1 (50.0%)
	Total	6	5 (83.3%)
Grand Total		35	19 (54.2%)

Table 2.3 Peach cultivars in Illinois commercial orchards surveyed for the incidence of brown rot disease in 2020, 2021, and 2022.

Cultivar	**Orchard surveyed (no)**									**Total orch-ards**
	2020			**2021**			**2022**			
	West	**South**	**Central**	**West**	**South**	**Central**	**West**	**South**	**Central**	
Bounty		2 (0)[z]		1 (0)				1 (1)		4 (1)[z]
Carolina Belle	1 (1)									1 (1)
Contender			1 (1)			1 (1)			1 (0)	3 (2)
Fayette								1 (1)		1 (1)
July Prince		1 (0)								1 (0)
Loring	1 (1)						1 (1)			2 (2)
Redhaven	3 (2)	1 (1)	1 (1)	2 (2)	1 (0)	1 (1)	2 (2)	1 (0)	1 (1)	13 (10)
Starfire	1 (1)			1 (0)			1 (0)			3 (1)
Unknown		2 (1)			1 (0)		1 (0)	3 (0)		7 (1)
Total orchards	6 (5)	6 (2)	2 (2)	4 (2)	2 (0)	2 (2)	5 (3)	6 (2)	2 (1)	35 (19)

[z] Number outside parenthesis indicate number of orchards surveyed, and number inside parenthesis shows number of orchards with brown rot disease.

Table 2.4 Mean lesion diameter of *Monilinia fructicola* isolates on two peach cultivars (Redhaven, Contender), 48 hours post inoculation in the laboratory.

Isolate	Mean lesion diameter (mm) on fruits[z]	
	Redhaven	Contender
Isolate from Jerseyville (Jersey County, West)	8.4	23.6
Isolate from Golden Eagle (Calhoun County, West)	12.0	12.3
Isolate from Marine (Madison County, West)	6.5	7.0
Isolate from Urbana (Champaign County, Central)	8.9	15.1

[z] Mean diameter of 12 inoculated fruits.

Figure 2.1 Locations of 14 surveyed commercial peach orchards in Illinois in 2020.

Figure 2.2 Locations of 8 surveyed commercial peach orchards in Illinois in 2021.

Figure 2.3 Locations of 13 surveyed commercial peach orchards in Illinois in 2022.

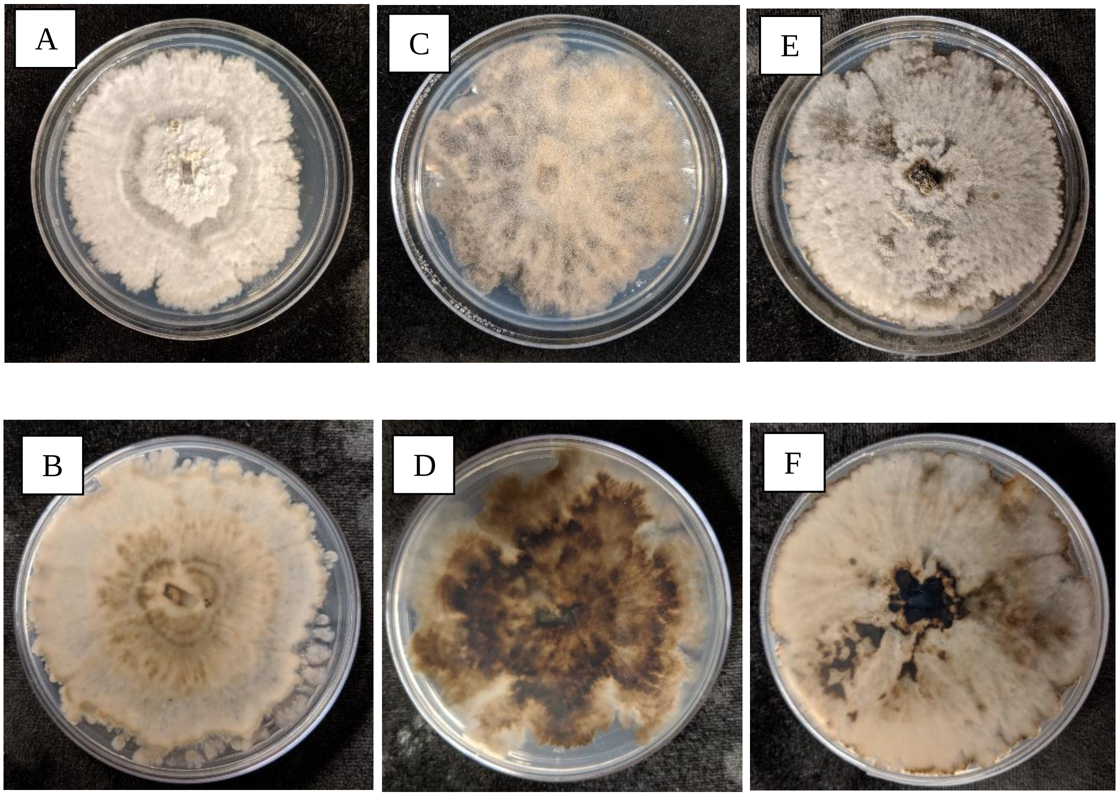


Figure 2.4 Colonies of *Monilinia fructicola* isolates from commercial peach orchards in Illinois during 2020-2022. Cultures were 10 days old on potato dextrose agar in Petri plates. Images A and B are from isolate D7 from Jerseyville (Jersey County, West), C and D from isolate F19 from Union County (South), and E and F are from isolate N13 from Urbana (Champaign County, Central). Images A, C, and E are from tops of colonies and images B, D, and F are from bottoms of colonies in Petri plates.

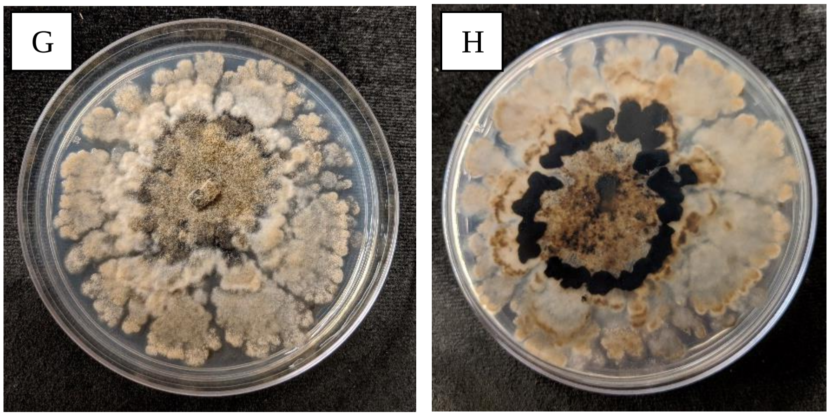


Figure 2.5 Colony of *Monilinia laxa* isolate from Urbana (Champaign County, Central) peach orchard in Illinois in 2022. Culture was 16 days old on potato dextrose agar in Petri plate. Image G is from top of colony and image H is from bottom of colony in Petri plate.

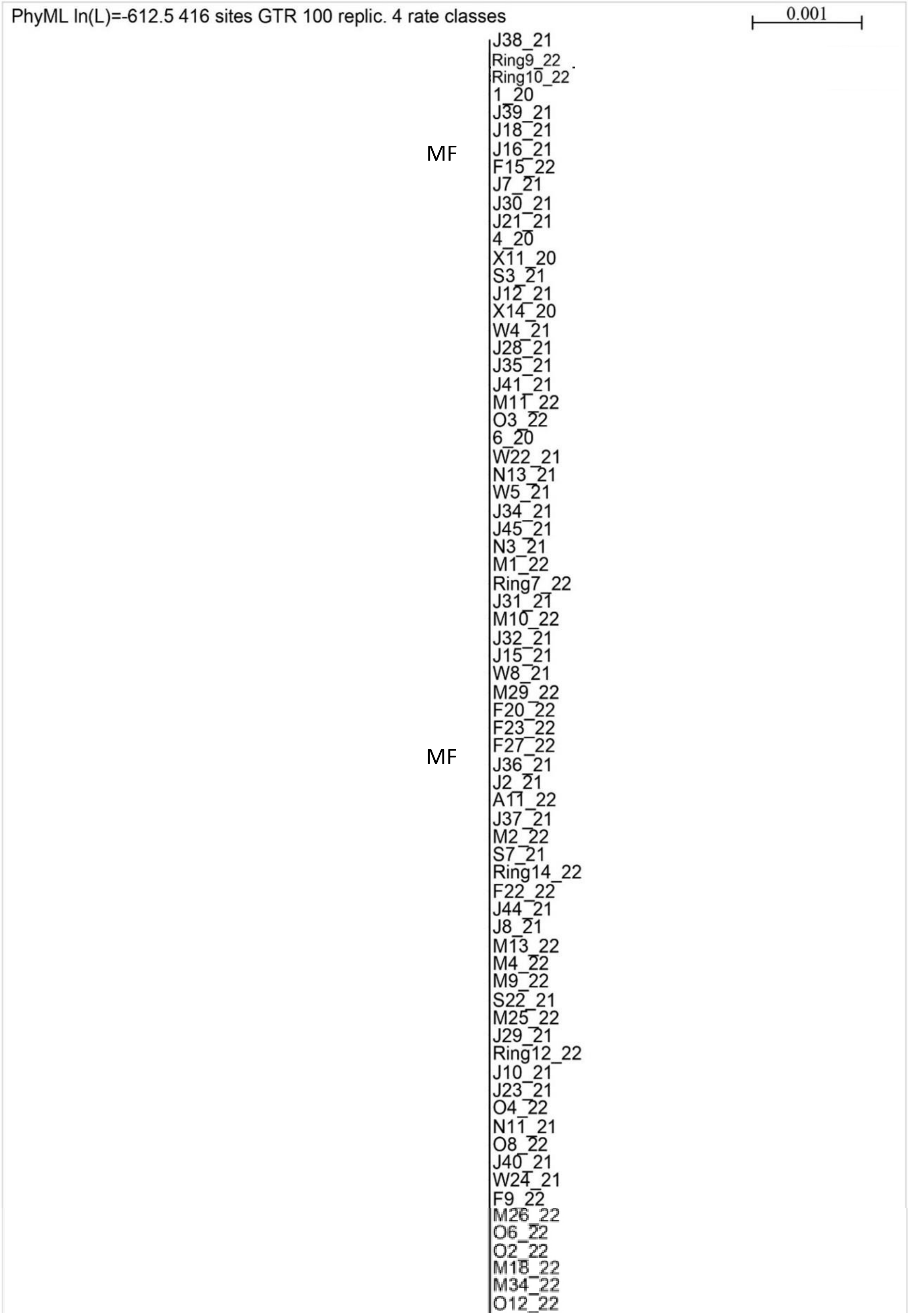
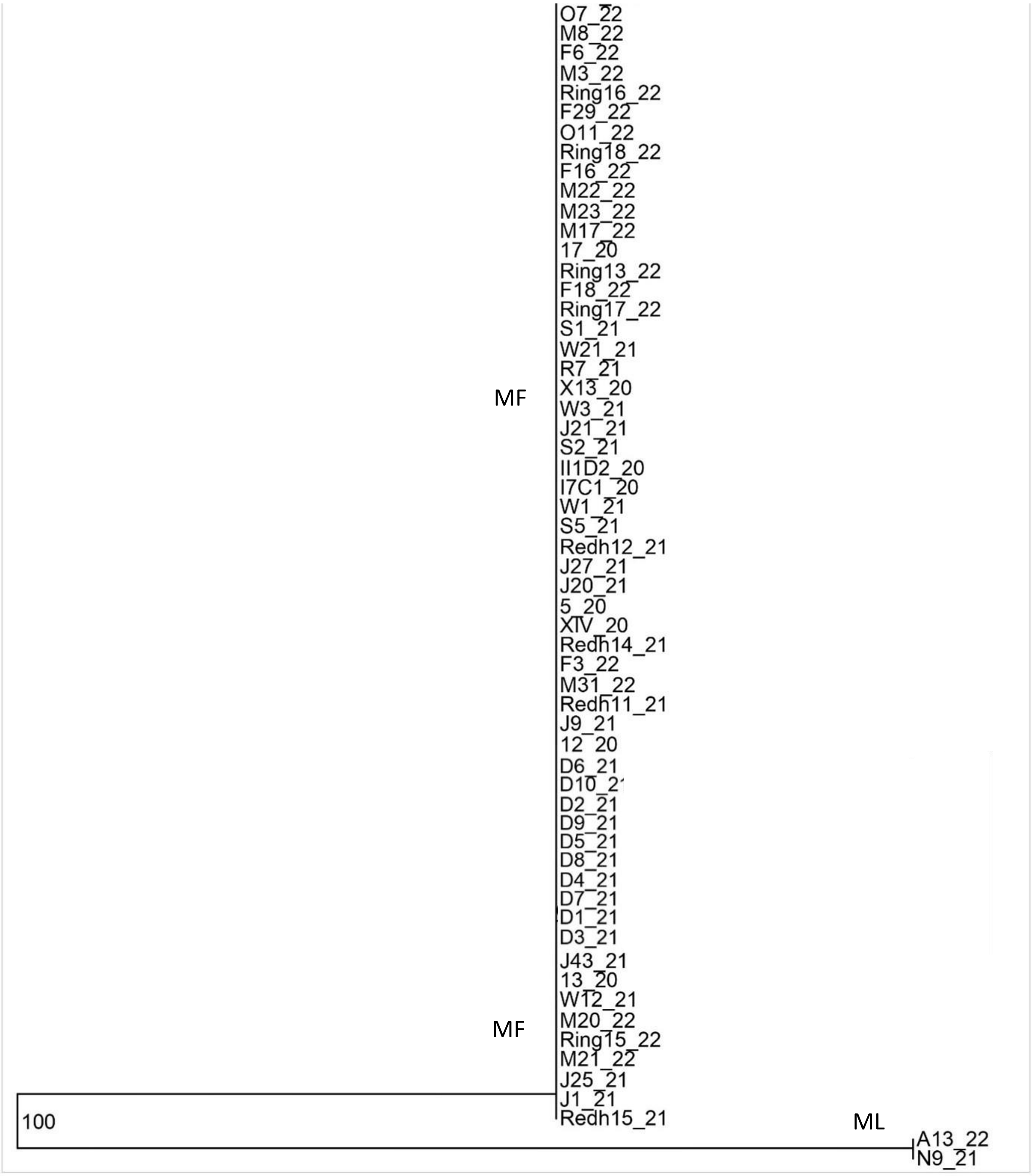


Figure 2.6 (cont.)



<u>Figure 2.6</u> ITS-based phylogenetic tree of *Monilinia* species identified in Illinois. The phylogeny is rooted with two *M. laxa* isolates. Bootstrap support values ≥ 70 are shown above the branches. Tree was produced using PhyML maximum likelihood analysis as implemented in SeaView ver. 5.0.4. MF indicates *M. fructicola* specimens, while ML indicates *M. laxa*.

Figure 2.7 Symptoms of brown rot disease 96 hours after inoculation of peach fruits of cultivar Contender with *Monilinia fructicola* isolates in the laboratory. The isolates for the fruit inoculations were from left to right: Jerseyville (Jersey County, West); Golden Eagle (Calhoun County, West); Marine (Madison County, West); Urbana (Champaign County, Central).

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CHAPTER 3: EFFECTIVENESS OF SELECTED FUNGICIDES FOR MANAGEMENT OF BROWN ROT DISEASE OF PEACH

Abstract

Laboratory and field studies were conducted to evaluate the effectiveness of registered and commonly used fungicides for managing brown rot disease of peaches in commercial orchards. Fungicide sensitivity of *Monilinia fructicola* isolates was conducted in the laboratory using eleven fungicides, including azoxystrobin (Abound 2.08SC), captan (Captan 80WDG), fenhexamid (Elevate 50WDG), trifloxystrobin (Flint Extra 4.05E); penthiopyrad (Fontelis 1.67SC), difenoconazole + cyprodinil (Inspire Super 2.82SC); fluopyram + tebuconazole (Luna Experience 3.34SC), fluopyram + trifloxystrobin (Luna Sensation 4.20SC), fluxapyroxad + pyraclostrobin (Merivon 4.18SC), propiconazole (Tilt 3.6EC), and thiophanate-methyl (Topsin-M 70WP). The EC_{50} of Abound, Captan, Flint Extra, and Fontelis for the colony development of the isolates was significantly ($P = 0.05$) higher than the other fungicides tested. Field trials were conducted at the University of Illinois Fruit Research Farm in Urbana, IL on ‘Redhaven’ and ‘Contender’ peaches in 2021 and 2022, to evaluate efficacy of the above-mentioned fungicides for managing brown rot disease. Using a motorized backpack sprayer, trees were sprayed with fungicides at 10- and 14-day intervals from petal fall until two weeks to harvest. In 2021, incidence of symptomatic fruits was significantly ($P = 0.05$) higher in untreated plots than treated plots. In 2022, only a few fruits of ‘Redhaven’ had brown rot and no fruit of ‘Contender’ developed brown rot. Luna Experience 3.34SC, Luna Sensation 4.20SC, and Merivon 4.18SC were the most effective fungicides for management of brown rot and other summer peach diseases. Incidence of brown rot was the lowest in plots sprayed with Merivon 4.18SC plus Captan 80WDG.

Introduction

Brown rot, caused by *Monilinia* spp., is one of the most important diseases of peaches in Illinois (Babadoost, 2017; Seitz et al., 2021). Fungicides are used to control brown rot in commercial peach orchards. However, the fungicides have varying efficacy depending on the nature of inoculum sources, the ontogeny of host species, pathogen species, the mode of infection of fungicide and organ attacked, local climate, and economic factors (Martini and Mari, 2014). Management of brown rot disease of peach is a challenging task, due to factors such as the lengthy period for infection, susceptibility of cultivars, and fungicide efficacy (Oliveira Lino et al., 2016). Without effective management strategies, yield losses up to 100% may occur (Babadoost, 2017).

Benzimidazole fungicides (MBC, FRAC 1), such as thiophanate-methyl, were one of the first fungicides to be labeled for control of brown rot in the 1970s, and for a time, two sprays of an MBC fungicide during bloom were sufficient to control brown rot of peaches for the entire season (Martini and Mari, 2014). However, by the late 1970s, MBC-resistant *Monilinia* isolates began to appear in California (Ma et al., 2003). High levels of resistance to MBC fungicides have also been reported in South Carolina (Zhu et al., 2010) and Michigan (Koenraadt et al., 1992). Ma et al. (2003) and Schnabel et al. (2011) defined high levels of resistance to MBC fungicides as an isolate's half maximal effective concentration (EC_{50}) being greater than 50 mg/L. As MBC resistance has been reported in Midwest orchards for 30 years, there is a high probability of its spread to Illinois.

Although fungicides from many Fungicide Resistance Action Committee (FRAC) groups have been approved for use against brown rot of stone fruit trees, FRAC groups 3, 7, 9, and 11 are reported to be most effective against this disease (Peter, 2018). These fungicide groups are demethylation inhibitors (DMI, FRAC 3); succinate dehyrogenase inhibitors (SDHI, FRAC 7);

anilinopyrimidines (AP, FRAC 9); and quinone-outside inhibitors (QoI, FRAC 11). However, due to their highly specific mechanism of action, a high propensity of DMI and QoI (strobilurin) resistance in *Monilinia fructicola* has been reported (Cox et al., 2009; Parker et al. 2006; Schnabel et al., 2004; Zehr et al., 1999). Reduced sensitivity of *M. fructicola* to DMI fungicides was first reported in South Carolina in 1999 (Zehr et al., 1999), and in the early 2000s was reported in other Eastern states of United States of America (US), such as Georgia in 2004 (Schnabel et al., 2004) and New York in 2006 (Parker et al. 2006). QoI and SDHI fungicides belong to a class called respiration inhibitor (RI) fungicides. QoI fungicides inhibit fungal mitochondrial respiration by targeting the cytochrome b (*cytb*) gene, and SDHI fungicides inhibit the fungal mitochondrial electron transport chain by targeting the succinate dehydrogenase reductase enzyme complex II (Amiri et al., 2010). By 2008, some reduced sensitivity to QoI and SDHI fungicides appeared in South Carolina peach orchards (Amiri et al., 2010).

Single-site mode of action fungicides run the risk of developing resistance. Therefore, it is generally recommended to alternate between different FRAC groups for sprays, such as rotating QoIs with DMIs (Cox et al., 2009) or QoIs and SDHIs (Amiri et al., 2010).

The goal of our study was to evaluate the effectiveness of potential fungicides for management of brown rot disease of peaches in Illinois. The objectives of the research were: (i) to determine the half maximal effective concentration (EC_{50}) of the potential fungicides on *Monilinia* species identified in the commercial peach orchards in Illinois; and (ii) to assess the efficacy of the fungicides for managing brown rot of peach in the orchard.

Materials and methods

In-vitro evaluation of the effects of fungicides on colony development of Monilinia fructicola isolates Sensitivity of *Monilinia fructicola* isolates to eleven fungicides was evaluated. Six isolates from western Illinois, and three isolates from central Illinois, were selected for laboratory tests based on morphological and geographical diversity (Table 3.1). Isolates J8, J18, J25, D1, D7, and D10 were from western Illinois; and isolates N13, R15, and W4 were from central Illinois (Table 3.1). Fungicides used included azoxystrobin (Abound 2.08SC, Syngenta Inc., Greensboro, NC); captan (Captan 80WDG, Albaugh LLC, Ankeny, IA); fenhexamid (Elevate 50WDG, Arysta LifeScience North America, Cary, NC); trifloxystrobin (Flint Extra 4.05E, Bayer CropScience, St. Louis, MO); penthiopyrad (Fontelis 1.67SC, Corteva Inc., Indianapolis, IN); difenoconazole + cyprodinil (Inspire Super 2.82SC, Syngenta Inc., Greensboro, NC); tebuconazole + fluopyram (Luna Experience 3.34SC, Bayer CropScience LP, Chesterfield, MO); fluopyram + trifloxystrobin (Luna Sensation 4.20SC, Bayer CropScience LP, Chesterfield, MO); fluxapyroxad + pyraclostrobin (Merivon 4.18SC, BASF Corp., Research Park Triangle, NC); propiconazole (Tilt 3.6EC, Syngenta Inc., Greensboro, NC); and thiophanate-methyl (Topsin-M 70WP, UPI, King of Prussia, PA).

Isolates were grown on potato dextrose agar (PDA) medium in Petri plates at 24ºC for 7-14 days. A 4 mm x 4 mm piece of agar was cut from the actively growing edge of each colony and transferred onto PDA in Petri plates amended with fungicide, including Abound 2.08SC at concentrations of 10, 100, 250, 1000, and 10000 mg/L (recommended label rate: 80-100 mg/L); Captan 80WDG at 16, 40, 80, 160, 800, and 1600 mg/L (recommended label rate: 500-1000 mg/L); Elevate 50WDG at 0.1, 0.25, 1, 5, 10, and 25 mg/L (recommended label rate: 200-300 mg/L); Flint Extra 4.05E at 100, 1000, 2500, 5000, and 10000 mg/L (recommended label rate: 31.5-48.0 mg/L);

Fontelis 1.67SC at 0.1, 0.5, 1, 2.5, 5, 10, 25, 500, 1000, and 10000 mg/L (recommended label rate: 72.8-104.0 mg/L); Inspire Super 2.82SC at 0.01, 0.025, 0.1, 0.25, 0.3, 0.5, 1, 5, 10, and 50 mg/L (recommended label rate: 140.6-175.8 mg/L); Luna Experience 3.34SC at 0.01, 0.05, 0.1, 0.2, 1, 2, and 5 mg/L (recommended label rate: 62.4-104.0 mg/L); Luna Sensation 4.20SC at 0.01, 0.1, 0.25, 0.4, 0.5, 1, 5, 50, 100, 200, and 500 mg/L (recommended label rate: 65.0-98.5 mg/L); Merivon 4.18SC at 0.01, 0.05, 0.1, 0.25, 0.5, 1, 25, 50, 100, 200, and 500 mg/L (recommended label rate: 52.0-87.0 mg/L); Tilt 3.6EC at 0.005, 0.01, 0.05, 0.1, 0.2, 0.25, 0.5, 1, and 2.5 mg/L (recommended label rate: 44.7 mg/L); and Topsin-M 70WP at 0.2, 0.5, 1, 2, 5, 10, and 50 mg/L (recommended label rate: 280-420 mg/L). Control PDA plates were without any fungicide. Four plates were included in each fungicide test and the tests were performed in triplicate. To calculate EC_{50} values of the fungicides, mean colony diameter of isolates in control plates and test plates were measured 7 days post inoculation (dpi) of plates. Percent inhibition of the colony was calculated as:

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

where C = colony diameter (in mm) in control PDA plate and F = colony diameter (in mm) in PDA plate amended with fungicide (Schnabel et al., 2011).

Screening of isolates for potential resistance to MBC fungicide thiophanate-methyl

Sensitivity of 123 *Monilinia* isolates from Illinois peach orchards to the MBC fungicide thiophanate-methyl (Topsin-M 70WP) was evaluated. Isolates were grown on potato dextrose agar (PDA) medium in Petri plates at 24ºC for 7-14 days. A 4 mm x 4 mm piece of agar was cut from the actively growing edge of each colony and transferred onto PDA in Petri plates amended with thiophanate-methyl at 5 and 50 mg/L. Control PDA plates were without any fungicide. Four plates were included in each fungicide test. To calculate EC_{50} values of the fungicides, mean colony diameter of isolates in control plates and test plates were measured 7 days post inoculation (dpi) of plates. Percent inhibition of the colony was calculated as:

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

where C = colony diameter (in mm) in control PDA plate and F = colony diameter (in mm) in PDA plate amended with fungicide (Schnabel et al., 2011).

Field trials In 2021 and 2022, orchard trials were conducted to evaluate the effectiveness of 11 fungicides tested in the laboratory (Table 3.1) for managing brown rot and other diseases of 'Redhaven' and 'Contender' peaches at the University of Illinois Fruit Research Farm in Urbana, IL (N: 40º 04.91'; W: 088º 21.40; Elevation: 740 m). 'Redhaven' and 'Contender' are two widely grown peach cultivars in Illinois. Trees of each cultivar were in separate blocks. The trees were 10 years old, planted in 12 rows, each with 4 trees of each cultivar. For each cultivar, the trial was performed in a randomized complete block design with four replications, each with one tree. During the season, weeds were controlled by mowing. To control early-season diseases (blossom and shoot blight phases of brown rot, leaf curl, powdery mildew, bacterial spot, scab), all trees were sprayed from the pink growth stage until the shuck split stage with Bravo Weather Stik plus Inspire Super plus Kocide-3000 alternated with Bravo Weather Stik plus Fontelis plus Kocide-3000 at 7-day intervals.

To prevent branch snapping under the weight of fruits, peaches were thinned using a stick and by hand in June of both years. Applications of the fungicides for managing brown rot and other peach diseases in 2021 began at petal fall on 11 May, at 10- and 14-day intervals, and ended on 30 July for ‘Redhaven’ trees and 10 August for ‘Contender’. Applications of the same fungicides in 2022 began at petal fall on 13 May, at 10- and 14-day intervals, and ended on 5 August (21 days before harvesting fruits). To manage bacterial spot of leaves and fruits, Calcium Oxytetracycline bactericide (Mycoshield at 12 oz/acre) was mixed with fungicide sprays from shuck-split stage until 21 days to harvest. To control insect pests, insecticide Assail 70WP (at 3.4 oz/acre) and Asana XL (at 14.5 fl oz/acre) were alternatively mixed with fungicide sprays. Fungicides were spray-applied with a motorized backpack sprayer using 2 L of spray suspension per tree. Inside and outside tree canopies were thoroughly covered with the spray.

Each year, during bloom, nine trees of each cultivar were inspected for blossom blight. Blossoms from upper, middle, and lower canopies on each of the northern, eastern, southern, and western sides were inspected and cultured for *Monilinia* spp. In addition, four weeks after petal fall, new shoots on upper, middle, and lower canopies on each of the northern, eastern, southern, and western sides of trees were inspected for shoot blight.

Beginning the first week of July, experimental trees were inspected weekly for incidence of brown rot. Percent of symptomatic fruits in each tree was determined by examining 60 fruits, including five fruits from each of upper, middle, and lower canopies on each of the northern, eastern, southern, and western sides.

In 2021, average high and low temperatures (°F) were 72/51, 85/64, 84/66, and 86/65 in May, Jun, Jul, and during 1-24 Aug, respectively. Recorded precipitation was 6 days (3.51 in.) in May, 9 days (7.24 in.) in Jun, 9 days (4.54 in.) in Jul, and 2 days (2.09 in.) during 1-24 Aug. In 2022, average high and low temperatures (°F) were 76/55, 87/63, 87/67, and 88/66 in May, Jun, Jul, and during 1-12 Aug, respectively. Recorded precipitation was 6 days (1.85 in.) in May, 3 days (0.77 in.) in June, 5 days (2.54 in.) in July, and 4 days (2.26 in.) during 1-12 Aug.

Results

In-vitro evaluation of the effects of the fungicides on the colony development of Monilinia fructicola isolates EC_{50} and 100% inhibition concentration of the fungicides are presented in Table 3.1. For Abound 2.08SC, the EC_{50} ranged from 10 to 3190 mg/L, and the 100% inhibition concentration ranged from 10,000 to >10,000 mg/L. For Captan 80WDG, the EC_{50} ranged from 16 to 57.6 mg/L, and the 100% inhibition concentration ranged from 80 to >8,000 mg/L. For Elevate 50WDG, the EC_{50} ranged from 0.10 to 0.90 mg/L, and the 100% inhibition concentration ranged from 5 to 25 mg/L. For Flint Extra 4.05E, the EC_{50} ranged from 100 to >10,000 mg/L, and the 100% inhibition concentration was >10,000 mg/L. For Fontelis 1.67SC, the EC_{50} ranged from 0.05 to 500 mg/L, and the 100% inhibition concentration ranged from 10,000 to >10,000 mg/L. For Inspire Super 2.82SC, the EC_{50} ranged from 0.01 to 0.3 mg/L, and the 100% inhibition concentration ranged from 5 to 50 mg/L. For Luna Experience 3.34SC, the EC_{50} ranged from 0.01 to 0.2 mg/L, and the 100% inhibition concentration ranged from 2 to 5 mg/L. For Luna Sensation 4.20SC, the EC_{50} ranged from 0.04 to 0.38 mg/L, and the 100% inhibition concentration ranged from 100 to >1,000 mg/L. For Merivon 4.18SC, the EC_{50} ranged from 0.01 to 0.46 mg/L, and the 100% inhibition concentration ranged from 100 to >1000 mg/L. For Tilt 3.6EC, the EC_{50} ranged from 0.005 to 0.1 mg/L, and the 100% inhibition concentration ranged from 0.5 to 2.5 mg/L. For Topsin-M 70WP, the EC_{50} ranged from 0.20 to >50 mg/L, and the 100% inhibition concentration ranged from 5 to >50 mg/L.

Screening of isolates for potential resistance to MBC fungicide thiophanate-methyl

Results of whether *Monilinia* isolates had an $EC_{50} > 50$ mg/L for thiophanate-methyl are presented in Table 3.2. 83.3%, 1.8%, and 28.6% of collected isolates from 2020, 2021, and 2022, respectively, had an $EC_{50} > 50$ mg/L for thiophanate-methyl. 27.1%, 0.0%, and 28.6% of collected isolates from the west, central, and south regions of Illinois, respectively, had an $EC_{50} > 50$ mg/L for thiophanate-methyl.

2021 field trial No leaf curl, scab, powdery mildew, blossom blight, shoot blight, or insect damage developed in the experimental trees. Negligible bacterial spot developed on leaves, but fruits were bacterial spot-free. Fruit brown rot disease was the only summer disease of peaches that developed in the orchard. The disease was first observed on 6 August. Luna Experience 3.34SC, Luna Sensation 4.20SC, and Merivon 4.18SC were the most effective fungicides in preventing development of brown rot in fruits across both 'Redhaven' and 'Contender' cultivars (Table 3.3). Topsin-M 70WP alternated with Abound 2.08SC was the least effective at preventing development of brown rot in fruits across both cultivars. Incidence of brown rot of fruits was significantly ($P = 0.01$) higher in untreated plots compared to treated plots.

2022 field trial No leaf curl, scab, powdery mildew, blossom blight, shoot blight, bacterial spot or insect damage developed in the experimental trees. Only nine fruits with brown rot symptoms were observed in the 'Redhaven' plots. There was no fruit with brown rot symptoms in 'Contender' plots (Table 3.4).

Discussion

After apple, peach is the second most important economical fruit crop in Illinois (USDA, 2022). Brown rot is the most important fruit rot of peaches in the state (Babadoost, 2017). Thus, developing and implementing effective management of brown rot in Illinois peach orchards is vital for peach production, peach growing communities, and Illinois agriculture economy.

Since species of brown rot pathogens vary in their sensitivity to fungicides (Abate et al., 2018), accurate identification of the pathogen species (*Monilinia* spp.) causing the disease at any location is essential for developing strategies for effective management of brown rot (Babadoost, 2017). Our studies identified *Monilinia fructicola* was the main pathogen causing brown rot of peach fruits in Illinois orchards. For determining effective fungicides for managing brown rot, we conducted laboratory and field trials, which resulted in reliable information for management of the disease.

EC_{50} concentrations were significantly ($P = 0.05$) lower for Elevate 50WDG, Inspire Super 2.82SC, Luna Experience 3.34SC, Luna Sensation 4.20SC, Merivon 4.18SC, Tilt 3.6EC, and Topsin-M 70WP than those of Abound 2.08SC, Captan 80WDG, Flint Extra 4.05E, and Fontelis 1.67SC. Results of our laboratory studies were similar to reports by Abate et al. (2018), in that demethylation inhibitor (DMI; FRAC 3), succinate dehydrogenase inhibitor (SHDI; FRAC 7), and quinone outside inhibitor (QoI; FRAC 11) fungicides remain the most effective for managing brown rot disease. While DMI, SDHI, and QoI fungicides are well-studied for use against brown rot, our results indicated high effectiveness of the reduced-risk fungicide fenhexamid (hydroxyanilides; FRAC 17), which is consistent with results of Förster et al. (2007).

Although we identified two isolates of *Monilinia laxa*, we did not have time to incorporate them in our fungicide efficacy studies. Hrustić et al., (2018) found that *M. laxa* isolates in Serbia were more tolerant of DMI fungicides than *M. fructicola* isolates. Further research could be conducted to determine if this is the case in the United States. However, this omission shouldn't change Illinois brown rot management strategies, because only 2 of 129 isolates were *M. laxa,* a rather insignificant percentage.

Results from our orchard trials showed that Luna Experience 3.34SC, Luna Sensation 4.20SC, and Merivon 4.18SC were highly effective for managing brown rot diseases on both 'Redhaven' and 'Contender' peaches. We did not have access to other cultivars for orchard testing. The field trials cannot be conducted in commercial orchards because control trees (non-fungicide treated trees) can provide inoculum for infection of other trees.

Commercial growers have been more successful in managing summer diseases of peaches when they collected infected mummies, removed prunes and neglected trees from the orchards, and applied fungicides timely at 14-day intervals from petal fall until two weeks to harvest (Babadoost, 2017). With the different chemistry and modes of action, any combination of demethylation inhibitor (DMI; FRAC 3), succinate dehydrogenase inhibitor (SHDI; FRAC 7), and quinone outside inhibitor (QoI; FRAC 11) fungicides could provide an effective means of chemical management of brown rot of peaches with minimal risk of fungicide resistance development in the *Monilinia* pathogens (Amiri et al., 2010; Cox et al., 2009). Fungicides that were highly effective in both field and laboratory that fall into these categories include difenoconazole + cyprodinil (Inspire Super 2.82SC; FRAC 3, 9); fluopyram + tebuconazole (Luna Experience 3.34SC; FRAC 3, 7), fluopyram + trifloxystrobin (Luna Sensation 4.20SC; FRAC 7, 11), fluxapyroxad + pyraclostrobin (Merivon 4.18SC; FRAC 7, 11), and propiconazole (Tilt 3.6EC; FRAC 3). While

Monilinia resistance to DMI fungicides has been reported in several eastern states of US, such as Georgia, New Jersey, New York, Ohio, Pennsylvania, and South Carolina (Chen et al., 2013), our research indicates the resistance has not yet developed/spread to Illinois peach orchards, so at present DMI fungicides are safe to use in Illinois. However, we suggest additional tests in the future years to assess DMI resistance of *Monilinia* spp. in Illinois commercial orchards.

Ma et al. (2003) and Schnabel et al. (2011) reported that *Monilinia* isolates with $EC_{50} > 50$ mg/L of thiophanate-methyl resistance (Topsin-M) had high levels of resistance to this fungicide. In our study, we found 27 of 123 isolates of *Monilinia* isolates collected from Illinois peach orchards in 2020, 2021, and 2022 had an EC_{50} greater than 50 mg/L. Therefore, further testing of *Monilinia* isolates from Illinois will be needed to determine effectiveness of this fungicide for managing brown rot disease of peaches in Illinois.

Our laboratory results indicate fenhexamid (Elevate 50WDG) as highly effective against all *Monilinia fructicola* isolates from Illinois. However, we did not have enough trees in our orchard to conduct a fungicide treatment where we could solely spray with Elevate and Captan (without it alternating with Topsin-M). Additional research is needed to evaluate the efficacy of Elevate for managing brown rot of peaches.

Brown rot disease conidial development is highly dependent on moisture (Tamm and Flückiger, 1993). The recorded rainfalls during June-July were 11.78 and 3.31 in. in 2021 and 2022, respectively. Thus, the period of June-July in 2022 was much drier than the same period of 2021. We hypothesize that dry weather conditions in 2022 resulted in low infection of peach fruit in our experimental site.

Tables and figures

Table 3.1 Colony development of *Monilinia fructicola* isolates from peach orchards in Illinois on PDA culture medium amended with fungicides.

Monil.* isolate	**Fungicide concentration (mg/L) for EC_{50} and 100% inhibition (Inh) of colony growth**											
	Abound		**Captan**		**Elevate**		**Flint Extra**		**Fontelis**		**Inspire Sup**	
	EC_{50}	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**
N13	493	>10k	54.2	80	0.25	10	2500	>10k	2.5	>10k	0.1	50
R15	3190	>10k	26.4	80	0.25	10	>10k	>10k	500	>10k	0.3	50
W4	908	10k	40	80	0.1	10	>10k	>10k	5	10k	0.25	5
J8	584	>10k	16	1600	0.1	5	5000	>10k	10.6	>10k	0.25	10
J18	1000	>10k	40	800	0.45	5	10k	>10k	2.4	>10k	0.25	50
J25	1157	10k	40	80	0.77	10	7557	>10k	500	>10k	0.25	5
D1	10	>10k	52.2	160	0.1	5	100	>10k	5	>10k	0.01	5
D7	100	>10k	54.6	>8k	0.90	25	>10k	>10k	6.8	>10k	0.1	5
D10	10	>10k	57.6	1600	0.25	10	100	>10k	0.05	10k	0.025	5

Monilinia* isolate	**Fungicide concentration (mg/L) for EC_{50} and 100% inhibition (Inh) of colony growth**									
	Luna Experience		**Luna Sensation**		**Merivon**		**Tilt**		**Topsin-M**	
	EC_{50}	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**	**EC_{50}**	**Inh**
N13	0.05	5	0.25	500	0.16	500	0.007	2.5	0.31	10
R15	0.2	5	0.38	500	0.1	200	0.005	2.5	0.2	5
W4	0.2	5	0.25	500	0.1	500	0.1	0.5	0.32	5
J8	0.1	5	0.1	1000	0.01	>1000	0.1	2.5	0.71	10
J18	0.2	5	0.25	100	0.25	200	0.1	2.5	>50	>50
J25	0.2	2	0.4	200	0.25	100	0.05	2.5	0.34	5
D1	0.01	5	0.05	500	0.25	500	0.007	2.5	0.33	10
D7	0.05	2	0.1	>1000	0.46	>1000	0.05	0.5	0.31	10
D10	0.05	5	0.04	500	0.01	500	0.01	0.5	0.75	10

***** Isolates N13, R15, and W4 were from central Illinois; and isolates J8, J18, J25, D1, D7, and D10 were from western Illinois.

Table 3.2 Percentage of isolates collected from peach orchards in various locations in Illinois that had an EC_{50} > 50 mg/L for thiophanate-methyl (Topsin-M 70WP), indicating high levels of resistance to thiophanate-methyl.

	2020	**2021**	**2022**	**Total**
West	90.9% (10/11)	2.9% (1/35)	30.8% (12/39)	27.1% (23/85)
Central	0.0% (0/1)	0.0% (0/20)	0.0% (0/3)	0.0% (0/24)
South	n/a	n/a	28.6% (4/14)	28.6% (4/14)
Total	83.3% (10/12)	1.8% (1/55)	28.6% (16/56)	22.0% (27/123)

Table 3.3 Efficacy of selected fungicides for management of brown rot of ‘Redhaven’ and ‘Contender’ peaches in 2021.

Redhaven cultivar			Contender cultivar		
Treatment and rate/A (application timing)^{wx}	Spray interval (days)	Incidence fruit w/ brown rot on 6 Aug (%)^{y}	Treatment and rate/A (application timing)^{wx}	Spray interval (days)	Incidence fruit w/ brown rot on 22 Aug (%)^{y}
Untreated check	n/a	11.11 a^{z}	Untreated check	n/a	22.09 a^{z}
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,4,7,9,11) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,2,4,6,7,8,9,10,11)	10	1.67 bc	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,4,7,9,11) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,2,4,6,7,8,9,10,11,12)	10	2.50 b
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	3.75 b	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10,12) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	3.34 b
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10) *alt* Elevate, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	1.67 bc	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10,12) *alt* Elevate, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	2.94 b
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	2.50 bc	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	2.50 b
Flint Extra, 3.8 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	2.08 bc	Flint Extra, 3.8 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	2.08 b
Fontelis, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	0.84 bc	Fontelis, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	1.67 b
Inspire Super, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	1.67 bc	Inspire Super, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	2.08 b
Merivon, 6.7 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	0.00 c	Merivon, 6.7 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	0.00 b
Tilt, 4 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	0.42 c	Tilt, 4 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	3.75 b

Table 3.3 (cont.)

Luna Experience, 10 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	0.00 c	Luna Experience, 10 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	0.00 b
Luna Sensation, 7.6 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11)	14	0.00 c	Luna Sensation, 7.6 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,8,9,10,11,12)	14	0.42 b
LSD (*P* = 0.05)		3.16			6.93

[w] Application date: 1 = 11 May, 2 = 21 May, 3 = 25 May, 4 = 1 June, 5 = 8 June, 6 = 11 June, 7 = 22 June, 8 = 2 July, 9 = 13 July, 10 = 20 July, 11 = 30 July, and 12 = 10 August.

[x] Mycoshield was not included in the spray on 10 August.

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

[z] Values within each column followed with the same letter are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test (Williams and Abdi, 2010).

Table 3.4 Efficacy of selected fungicides for management of brown rot of ‘Redhaven’ and ‘Contender’ peaches in 2022.

Redhaven cultivar			Contender cultivar		
Treatment and rate/A (application timing)^{vw}	Spray interval (days)	Incidence fruit w/ brown rot on 29 Jul (%)^{x}	Treatment and rate/A (application timing)^{vw}	Spray interval (days)	Incidence fruit w/ brown rot on 12 Aug (%)^{x}
Untreated check	n/a	1.67 a^{y}	Untreated check	n/a	0.00
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,4,7,9,11) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,2,4,6,7,8,10,12)	10	0.42 ab	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,4,7,9,11) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,2,4,6,7,8,10,12,13)	10	0.00
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10,12) *alt* Abound, 15.5 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10) *alt* Elevate, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.83 ab	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,5,8,10,12) *alt* Elevate, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.42 ab	Topsin-M, 1.5 lb + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Flint Extra, 3.8 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Flint Extra, 3.8 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Fontelis, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Fontelis, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Inspire Super, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Inspire Super, 20 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Merivon, 6.7 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Merivon, 6.7 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Tilt, 4 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Tilt, 4 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00

Table 3.4 (cont.)

Luna Experience, 10 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.42 ab	Luna Experience, 10 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
Luna Sensation, 7.6 fl oz + captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11)	14	0.00 b	Luna Sensation, 7.6 fl oz+ captan 80WDG, 5 lb + Mycoshield, 12 oz (1,3,5,7,9,11,13)	14	0.00
LSD (*P* = 0.05)		1.34			NS[z]

[v] Application date: 1 = 13 May, 2 = 24 May, 3 = 28 May, 4 = 2 June, 5 = 9 June, 6 = 13 June, 7 = 23 June, 8 = 3 July, 9 = 9 July, 10 = 14 July, 11 = 22 July, 12 = 25 July, and 13 = 5 August.

[w] Mycoshield was not included in the sprays after 9 July.

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

[y] Values within each column followed with the same letter are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test (Williams and Abdi, 2010).

[z] Not significant.

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