

**DIVERSITY AND BASELINE FUNGICIDE SENSITIVITY OF FUSARIUM
HEAD BLIGHT IN WHEAT AND MALTING BARLEY**

by

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HEAD BLIGHT IN WHEAT AND MALTING BARLEY**

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ABSTRACT

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, is a top disease of concern to wheat and barley production, contributing to both yield losses and grain contamination across the United States (US). FHB is of particular importance to small grain production in the East Coast and Mid-Atlantic region, with widespread occurrence annually. *F. graminearum* is well adapted to the climate of the Mid-Atlantic; able to thrive during warm and wet conditions and able to infect wheat, barley, corn, beans, and other small grain crops. For many years, triazole/ demethylation inhibitor (DMI, FRAC Group 3) fungicides were the only group labeled for FHB. In 2019, Miravis Ace (Syngenta, Switzerland), was registered for use in the management of FHB, containing propiconazole (DMI) and pydiflumetofen, a Succinate Dehydrogenase Inhibitor (SDHI, FRAC Group 7). *In vitro* poison plate mycelial assays were performed on *Fusarium* isolated from wheat and malting barley to determine baseline sensitivities to pydiflumetofen. Wheat samples were submitted from 16 states and malting barley samples were submitted from three states as part of the United States Wheat and Barley Scab Initiative (USWBSI) National FHB Collection. In total, 177 *Fusarium graminearum* isolates from wheat were obtained across 2020 (n=98), 2021 (n=65), and from historic isolate collections ranging from 1991-2014 (n=14) with no exposure to pydiflumetofen. The effective concentration to reduce mycelial growth by 50% (EC₅₀) was determined for wheat isolates using potato dextrose agar (PDA) Petri dish assays amended with pydiflumetofen to final concentrations of 0, 0.01, 0.05, 0.25, 1.0, and 5.0 µg/mL. Wheat

EC50 values ranged from 0.18 to 0.73 $\mu\text{g}/\text{mL}$, averaging 0.4 $\mu\text{g}/\text{mL}$ and 0.37 $\mu\text{g}/\text{mL}$, in 2020 and 2021 respectively. The average EC50 value of historic isolates was 0.35 $\mu\text{g}/\text{mL}$. Malting barley production has been expanding across the Mid-Atlantic. From 2020-21, malting barley isolates were collected to identify species diversity and screen for fungicide baseline fungicide sensitivity to pydiflumetofen. In the preliminary set of 28 barley isolates, 79% were identified as *F. graminearum*, 7% *F. verticillioides*, 7% *F. acuminatum*, 3.5% *F. poae*, and 3.5% *F. asiaticum*. Diversity of *Fusarium* species recovered from malting barley samples merits continued exploration. *In vitro* assays were conducted on amended yeast bacto agar (YBA) plates and a subset of isolates were also tested on amended PDA to compare growth. Media plates were amended with pydiflumetofen to final concentrations of 0, 0.01, 0.05, 0.25, 1.0, and 5.0 $\mu\text{g}/\text{mL}$. Average EC50 values for barley isolates on YBA were 1.09 $\mu\text{g}/\text{mL}$ for *F. graminearum* (n=22), 1.07 $\mu\text{g}/\text{mL}$ for *F. verticillioides* (n=2), 1.51 $\mu\text{g}/\text{mL}$ for *F. acuminatum* (n=2), 1.09 $\mu\text{g}/\text{mL}$ for *F. poae* (n=1), and 1.09 $\mu\text{g}/\text{mL}$ for *F. asiaticum* (n=1). Isolates grown on YBA and PDA responded to treatment similarly, with EC50 values of 1.09 and 1.04 $\mu\text{g}/\text{mL}$, respectively. YBA-grown isolates experienced more uniform lateral growth and were more amenable to rating than PDA-grown isolates. The established baseline fungicide sensitivity levels in wheat and malting barley will aid fungicide research and allow for monitoring of sensitivity levels in *F. graminearum* populations in small grain production regions of the United States as SDHI fungicide use expands.

Chapter 1

FUSARIUM HEAD BLIGHT OF SMALL GRAIN CROPS IN DELAWARE

Introduction

In 2019, the top four grain crops grown in Delaware (DE) included corn (*Zea mays*), soybeans (*Glycine max*), winter wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) with 28.9, 7.1, 3.6, and 1.1 million bushels produced in each crop, respectively (DDA 2020). Grain produced in the state supports the region's robust broiler chicken industry, with Sussex County, DE producing more broilers than any other county in the US (USDA NASS, County Profile 2017). Each of these grain crops can be infected by the fungal pathogen *Fusarium graminearum*, resulting in seedling diseases, root rot, stalk or ear rot in corn, or Fusarium head blight (FHB) in small grains (Goswami and Kistler 2004). Within the range of diseases caused by *Fusarium* species, FHB is of particular importance to small grain production due to yield loss and mycotoxin contamination. From 2019-2021, yield losses in winter wheat due to FHB in the Mid-Atlantic states of DE, Maryland (MD), Virginia (VA), Pennsylvania (PA), and New York (NY) were estimated to be more than 13 million dollars (Crop Protection Network, 2022). *F. graminearum* is well adapted to the climate of the Mid-Atlantic; able to thrive during warm and wet conditions. With many host crops produced in the region and annual

weather conditions typically conducive to disease development, FHB is frequently at high risk for widespread epidemics in DE and surrounding states.

Fusarium Head Blight

Fusarium head blight (FHB) is the most devastating disease of wheat worldwide (McMullen et al. 1997). FHB can also impact Barley (*Hordeum*), Rye (*Secale*), Oats (*Avena*), and Triticale (*Triticale*) (Goswami and Kistler 2004; McMullen et al. 1997). Susceptible crops provide habitable residues for fungal tissue to colonize between seasons and later act as inoculum sources. Previous crop debris provide the largest source of inoculum (Champeil et al. 2004). Infected small grain kernels used as seed can also be an inoculum source, and infected seeds may experience Fusarium-related root, crown, and foot rot symptoms, seedling blight, or fail to germinate (Walter et al. 2009). Most notably, FHB affects grain development, limiting crop yields by reducing the total number of kernels produced per head or reducing kernel size and weight. Disease incidence and severity can be estimated before harvest by counting the number of infected heads with any presence of FHB (incidence) or the abundance of infected grains per head (severity). Infected grains typically die prematurely causing bleached sections of the head to be visible around 21 days after flower initiation (anthesis)



Figure 1.1: Wheat head with FHB symptoms and orange spore masses

(D'Angelo et al. 2014). Under favorable environmental conditions, orange to salmon colored spore masses may also be visible (Figure 1.1). Within the head, severely infected kernels will be shriveled and chalky in appearance (Figure 1.2) (Osborne and Stein 2007). Disease severity



Figure 1.2: Healthy (left) wheat kernels v. scabby *Fusarium* infected kernels (right)

can be assessed post-harvest by counting the percentage of *Fusarium* damaged kernels (FDK). These kernels often have high levels of mycotoxin contamination, and due to their low weights, combine setting may be adjusted to blow some of these kernels out with chaff during harvest. Damaged kernels can be easily identified, but it is important to note that infections that occur later in the season may have limited symptoms on the kernels, but still result in elevated mycotoxin concentrations (McMullen et al. 1997). The most common contaminant, deoxynivalenol (DON, vomitoxin) is toxic to humans and livestock and is closely monitored and regulated (McMullen et al. 1997). *F. graminearum* is the dominant FHB-causing species, but recent work is expanding the understanding of species involved.

Pathogens

Fusarium head blight is caused by multiple species in the fungal genus *Fusarium* (Tekauz et al. 2000; Cerón-Bustamante et al. 2018; Cowger et al. 2020a; Fulcher et al. 2021; Duffeck et al. 2022). Among all species, the majority of FHB infections are caused

by members of the *F. graminearum* species complex (FGSC) (Goswami and Kistler 2004) and in the United States and Europe, the dominant species is *F. graminearum* (O'Donnell et al. 2000; Trail 2009). Other species such as *F. armeniacum*, *F. boothii*, *F. ceralis*, *F. poae*, and *F. sporotrichioides* are classified with *F. graminearum* in the broader *F. sambucinum* species complex (FSAMSC) (Cerón-Bustamante et al. 2018). Outside of the FSAMSC there are several other FHB-causing species complexes associated with small grains, namely the *F. tricinctum* species complex (FTSC) including *F. tricinctum* and *F. avenacum*, the *F. fujikuroi* species complex (FFSC), the *F. incarnatum-equiseti* species complex (FIESC), and the *F. oxysporum* species complex (FOSC) (O'Donnell et al. 2022; Cowger et al. 2020a; Cerón-Bustamante et al. 2018; Trail 2009). These pathogens may operate independently or in concert and contribute to FHB disease progression (Osborne and Stein 2007). Understanding of *Fusarium* diversity has been a focus of recent studies in the United States and around the world (Cowger et al. 2020a; Duffeck et al. 2022; Fulcher et al. 2021; Valverde-Bogantes et al. 2019).

In 2014, a study in North Carolina (NC) collected FHB-symptomatic wheat spikes resulting in 2,197 isolates. Within this isolate set, 86.2% belonged to FSAMSC, 11.3% to FTSC, 2.2% to FFSC, and 0.3% to FIESC. Within FSAMSC, *F. graminearum* (1,878 isolates, 99.5%) was the dominant species, but *F. poae*, *F. armeniacum*, and *F. sporotrichioides* were also recovered. Members of the FTSC included *F. acuminatum*, *F. reticulatum*, *F. avenacum*, and unidentified FTSC species. The FFSC was represented by *F. proliferatum*, *F. concentratum*, and unidentified FFSC isolates. A small number of FIESC were identified as well. (Cowger et al. 2020a). In 2018 and 2019, symptomatic

heads of wheat and ‘ancient grains’ emmer (*T. dicoccum*), spelt (*T. spelta*), einkorn (*T. monococcum*), and heritage wheat (*T. aestivum*) were collected in New York (NY). The major pathogens identified included the *F. graminearum* species complex and *F. avenaceum*, followed by the *F. incarnatum-equiseti* species complex, *F. poae*, *F. sporotrichioides*, and *F. armeniacum* (Fulcher et al. 2021). Another 2018-19 study was conducted in Pennsylvania (PA), generating 461 isolates from symptomatic wheat, barley, spelt, and rye heads. The population overwhelming consisted of *F. graminearum* (90.7%). Isolates not identified as *F. graminearum* included *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and 1 FFSC (Duffeck et al. 2022).

While the diversity of *Fusarium* species has been investigated in surrounding states, the *Fusarium* species composition has not been closely analyzed in Delaware (DE) and Maryland (MD). Knowing which *Fusarium* species are active locally can inform decisions regarding disease management and crop preparation. *Fusarium* species may have varying optimal climactic conditions for germination and spread, may produce different subsets of toxins, and may differ in aggressiveness or preference to specific host plants (Trail 2009). *Fusarium* species involved in FHB infect can be geographically distinct. For instance, *Fusarium graminearum* is dominant in many parts of the world and not greatly restricted by climactic conditions but is less ubiquitous in Mexico compared to *F. avenaceum* (FTSC) and *F. boothii* (FSAMSC), which are relatively better adapted to warmer conditions (Cerón-Bustamante et al. 2018). Similarly, *F. avenaceum* is better adapted to the cooler conditions of northern Europe and Canada and *F. asiaticum* is better

adapted to the warmer conditions of China and other parts of Asia than *F. graminearum* (Goswami and Kistler 2004).

Although *Fusarium* species have been characterized to be established in different regions of the world; changing environmental conditions, evolving management strategies, establishment of novel crops, and fluctuating weather patterns open up avenues for new pathogen introduction and establishment. In Italy, Argentina, Manitoba (Canada), and the Czech Republic drier conditions have favored *Fusarium poae* over *F. graminearum*, resulting in population shifts (Valverde-Bogantes et al. 2019). The introduction of corn cultivation to wheat rotation systems in Europe coupled with gradual increases in temperature and humidity has favored the spread of *F. graminearum* over the once dominant European *F. culmorum* species (Valverde-Bogantes et al. 2019). *F. asiaticum*, the dominant FHB-causing species in Asia, has been closely associated with rice production and has been reported in Uruguay, Brazil, and Louisiana (Valverde-Bogantes et al. 2019). In addition to species shifts, shifts in mycotoxin profiles have also been observed in *F. graminearum* (Cowger et al 2020a, Lofgren et al. 2018, Valverde-Bogantes et al. 2019, Varga et al. 2015).

Mycotoxins

One of the most problematic effects of *Fusarium* head blight in cereal crops is the presence and accumulation of mycotoxins in the trichothecene and zearalenone families (Langevin et al. 2004). Mycotoxins produced by the *Fusarium* disease complex are toxic to mammals when introduced in feed and are toxic to plants as well, aiding in disease

establishment and progression. *F. graminearum* strains that do not produce trichothecene toxins fail to spread within the head of wheat, triticale, rye, and barley and show a significant reduction in ability to spread in wheat heads, suggesting that mycotoxins play a large role in determining the pathogenicity of an isolate (Langevin et al. 2004). Multiple *Fusarium* species may contribute to a single epidemic, and the different sets of mycotoxins produced act together, increasing the severity and progression of the disease.

FHB-causing *Fusarium* strains can be divided into chemotypes corresponding to which mycotoxins are produced. *F. graminearum* and other members of the FGSC typically produce the type B trichothecene toxins deoxynivalenol (DON) with conjugates 3-acetyl deoxynivalenol (3ADON) and 15-acetyl deoxynivalenol (15ADON) or nivalenol (NIV) and its derivatives, while non-FGSC members usually produce type A trichothecenes (Valverde-Bogantes et al. 2019). *F. graminearum* is thought to predominantly generate deoxynivalenol (DON) that facilitates infection and contaminates grain (Cowger et al. 2020a). *Fusarium* chemotypes include producers of type B toxins 15A-DON, 3A-DON, or NIV, and type A trichothecene group 3 α -acetoxy-7 α ,15-dihydroxy-12,13-epoxytrichothec-9-ene (NX-2) producers with almost identical chemical structure to the 3A-DON toxin but lacking a keto group at the C-8 point (Varga et al. 2015). The 15A-DON, 3A-DON, and NX-2 chemotypes can also be referred to as NA1, NA2, and NA3 populations respectively (Valverde-Bogantes et al. 2019). DON, 15-ADON, and 3-ADON levels in grain are commonly tested by grain producers, while NIV levels are not (Cowger et al. 2020a). *F. graminearum* may also produce zearalenone (ZEA) and other fumonisins, which act as protein synthesis inhibitors in eukaryotic cells

(Langevin et al. 2004). *F. poae*, another member of the FSAMSC, can produce type B trichothecene NIV, type A T-2 and HT-2 toxins, and depsipeptide toxins beauvericin (BEA) and a range of enniatins (ENNs) (Beccari et al. 2019).

The historically dominant *F. graminearum* chemotype in North America has been 15-ADON producers, but 3-ADON producers are growing in prevalence and a novel type A NX-2 mycotoxin profile was recently identified in Mexico (Varga et al. 2015). In 2017 after this discovery, previously characterized NY *F. graminearum* isolates with a high frequency (47.6%) of 3A-DON production were re-analyzed to identify possible NX-2 producers. Of 133 original isolates producing 3A-DON, 20% were re-characterized as NX-2 producers (Lofgren et al. 2017). Mycotoxin profiles have also been experiencing shifts in prevalence across the globe. In China, *F. asiaticum* NIV producers are being replaced by the 3A-DON chemotype, which are more toxigenic and aggressive with a higher benzimidazole resistance than their predecessors; similarly, population studies in the United States and Canada have shown growing numbers of 3A-DON producers which may grow faster and produce more toxins than the dominant 15A-DON producers (Valverde-Bogantes et al. 2019).

Members of the FTSC do not produce trichothecenes, but instead can produce the mycotoxins moniliformin (MON), enniatins (ENN), and beauvericin (BEA) (Beccari et al. 2019). *F. avenaceum* and *F. acuminatum* can produce ENNs and MON, and *F. avenaceum* is also capable of producing BEA (Beccari et al. 2019). Members of the FFSC are capable of producing a variety of mycotoxins including BEA, MON, ENNs, fumonisins, fusaric acid, and fusoproliferin (Duffeck et al. 2022). Other *Fusarium* species

are also capable of producing zearalenone (ZEA) and moniliformin (MON) (Osborne and Stein 2007). ZEA is non-lethal to animals, but is structurally similar to estrogenic compounds, binding receptors in their place and has been shown to stimulate the growth of breast cancer cells in rats (Wan et al. 2013). MON can induce cardiotoxic effects as well as reductions in feed consumption and weight gain (Fremy et al. 2019). BEA and ENN toxins can disrupt the cell cycle by inducing apoptosis, halting normal cell processes, and are cytotoxic. BEA and ENN toxins are also antiviral, antifungal, antibacterial, insecticidal, and phytotoxic compounds (Bertero et al, 2020). T-2 toxins and their de-acetylated HT-2 forms can induce vomiting, weight loss, and blistering as well as inflammation and necrosis of heart, liver, and brain tissues (Zhang et al. 2022).

There is some specificity in mycotoxin production among FHB-causing species. In a survey performed across multiple regions in Mexico, *F. boothii* isolates all produced 15A-DON and *F. cerealis* and *F. meridionale* isolates produced NIV type toxin (Cerón-Bustamante et. al 2018). Of the 1,878 *F. graminearum* isolates collected in a 2014 study in NC, 97% of isolates were of the 15A-DON chemotype, 2.3% were NIV, and 0.5% were 3A-DON (Cowger et al. 2020a). Of the 418 *F. graminearum* isolated identified in a PA survey in 2018 and 2019, 94.9% were 15A-DON producers, 4.1% were 3A-DON, 0.7% were NIV, and 0.3% were NX-2 producers (Duffeck et al. 2022). Although FHB disease agents can produce multiple mycotoxin profiles, few are regularly tested or fully understood.

Deoxynivalenol is also known as vomitoxin and was initially discovered in 1973 (Yoshizawa and Morooka 1973). In the US, DON is the most toxic and most common

mycotoxin produced by *F. graminearum* and its presence and concentration in harvested grain is closely monitored (Pestka et al. 2007). DON and its conjugates are known to cause a wide range of deleterious health effects when consumed by humans and livestock. Early studies connected the toxin to outbreaks of alimentary toxic leukemia (ATA) in the 1930's and 40's and animal experiments showed low dose, chronic exposures to induce growth abnormalities and immune suppression, while high doses induced vomiting, hemorrhaging, toxic shock, and death (Pestka 2007; Pestka 2010). Repeated exposure to low levels of DON in feed can induce developmental defects, feed refusal, and other chronic illnesses in livestock. DON is very heat-stable, withstanding temperature from 170-350° C (Sobrova et al. 2010). DON is also a water-soluble compound that can diffuse into plant tissues including the vasculature where it can then be translocated to other grains within the head, accelerating or facilitating disease progression (Langevin et al. 2004). Grain that appears healthy and disease-free may still have some DON accumulation for this reason.

Deoxynivalenol levels in grain products are monitored following stringent guidelines set by the United States Food and Drug Administration (FDA); 0.5 ppm DON is permissible in grain intended for brewing, 1 ppm DON in finished wheat products intended for human consumption, 5 ppm DON in grain and grain by-product intended for dairy cattle, pig, and all other animal feeds, and 10 ppm DON in grain and grain by-products intended for cattle and chicken feed (Food and Drug Administration, 2010; Bai and Shaner 2004). Contaminated grain is subject to dockage at grain intake stations. Growers that have grain that has been contaminated with DON may be forced to sell their

product at a discounted price, to sell marked as low-quality grain, or be rejected as unfit for sale due to risks associated with deoxynivalenol exposure (McMullen et al. 1997).

The Disease Cycle

Fusarium is endemic to many regions and can be introduced to new crops multiple ways. Contaminated seed, infected crop debris from previous seasons, and airborne spores can act as inoculum exposing fields to disease (Goswami and Kistler 2004). Once in the field, wind and rain splash can continue to move pathogenic spores up through the canopy, eventually reaching the grain head. Cereal crops are most susceptible to head infections during flowering (anthesis), when floral tissues first emerge from the protective leaf sheath and begin to senesce, to the grain filling stages (Osborne and Stein 2007).

F. graminearum can overwinter in colonized crop residues such as the stem, roots, or fallen kernels of cereal plants. Once introduced, the pathogen can act as a saprobe, decomposing and residing in crop residues for extended periods until field conditions allow for reproduction (Goswami and Kistler 2004). *F. graminearum* can reproduce through sexual or asexual means. Perithecia can develop on crop debris (wheat residue, corn fodder, etc.) and release sexual ascospores or *Fusarium* can generate asexually produced spores, conidia which are vectored to a developing grain head (Osborne and Stein, 2007). Inoculum will persist on crop residues and once winter conditions have passed *F. graminearum* sexual ascospores are released from perithecia during the time of wheat flowering (Leplat et al. 2012). Ascospore production is

restricted at soil moisture content below 30% and reaches maximum production levels above 80% (Xu 2003). Ascospores can be discharged from perithecia and are further translocated by wind, rain, and insect vectors (Goswami and Kistler 2004). Ascospores are the source of primary infection for FHB (Guenther and Trail 2005) while macroconidia are the source of secondary infections (Son et al. 2013). FHB is monocyclic due to the small window of peak vulnerability when cereal crops are flowering and most susceptible to infection (Bai and Shaner 2004). Disease severity is closely related to the timing of infection events. Wheat is most susceptible to infection at anthesis and remains susceptible for about 10 days after (Cowger and Arrelleno 2010). Infections beginning later than that 10-day window under high moisture conditions can still result in infected kernels with significant levels of DON but may display limited symptom development (Cowger and Arrelleno 2010). DON levels may also fluctuate in the grain over time, reaching high levels during early grain fill and declining towards the end of grain fill (Cowger et al. 2020b).

Once inoculum makes contact with a grain head, germination can begin. Hyphae develop, extending in search of penetrable tissues that would allow pathogenesis. Hyphae are thin filamentous structures, collectively called mycelia, through which fungi grow and expand. Hyphae can grow through open stomata, wounds, or other susceptible sites to begin the infection process (Goswami and Kistler 2004). *F. graminearum* produces cell-wall degrading enzymes that facilitate infection and overwintering processes (Leplat et al. 2012). *F. graminearum* utilizes these cell-wall degrading enzymes to degrade the epidermal cuticle and access the more susceptible palea, lemma and glume of the spikelet

(Walter et al. 2009). In wheat, anthers can also be easily colonized by the fungus (Walter et al. 2009). This pathogen is hemibiotrophic: infection begins with a short biotrophic phase with colonization of head tissues before the necrotrophic phase initiates and senescence is noticeable (Guenther and Trail 2005). Death of plant tissues results in water-soaked lesions and bleached florets (Trail 2009). Reactive oxygen species (ROS's) can induce programmed cell death and are commonly utilized by pathogens to facilitate infections. ROS production delineates the two trophic phases in *F. graminearum*; ROS production is low during intra- and intercellular growth of the biotrophic phase, and high during the necrotrophic phase (Guo et al. 2019). During the necrotrophic phase, the rate of fungal colonization rapidly increases as plant tissues become necrotic (Goswami and Kistler 2004). Mycotoxins produced by the Fusarium disease complex influence the pathogenicity and aggressiveness of the pathogen (Langevin et al. 2004). Competition over resources and the effect and presence of mycotoxins can reduce the size and quality of grains over time. *Fusarium* damaged kernels (FDK) may be smaller than normal with a shriveled, sunken exterior (Champeil et al. 2004).

Hyphae can continue to grow through the head of the cereal plant, between florets within the same spikelet, from spikelet to spikelet, and through the xylem and phloem vascular bundles within the head (Goswami and Kistler 2004). The infection can progress from a single grain to the entire head showing symptoms (Leplat et al. 2012). Infections occurring early in flowering or infections that are severe may result in empty heads with no grain fill. In some cases, the hyphal growth is visible on the exterior tissues of the head. Grains affected in this way have a thin layer of mycelia on their exterior which can

be white or pink in color. These scabby grains are also called tombstone kernels based on their appearance; with rough, sunken, and dull exteriors visually resembling tombstones (Friskop et al. 2018). Tombstone and other fusarium damaged kernels are lightweight, can have high mycotoxin concentrations, and can act as inoculum if being used for seed. Increasing combine fan speed at harvest can blow away some of the lighter, scabby seeds that would otherwise decrease harvest quality and act as inoculum if planted later (McMullen et al. 1997).

Wheat Infection

During the flowering stages of wheat, external florets are temporarily opened (Figure 1.3) and can be exposed to airborne spores that quickly initiate infection (Fernando et al. 2021). Wheat heads may also be directly penetrated by hyphal tissues growing through natural openings like stomata and through the inner tissues of the palea, lemma, and glume, or infiltrated through hardier tissue with the help of cell wall degrading enzymes and short infection hyphae (Walter et al. 2009; Khan et al. 2020). In wheat, FHB infections can spread from a single point of infection to other parts of the head, and down the rachis and stem tissues (Guenther and Trail 2005).



Figure 1.3: Wheat heads with anthers exposed

Several types of wheat are grown commercially in the United States, including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), winter and spring white wheat, and durum wheat. HRW is used for bread flour, HRS has high protein levels, and SRW is used for cookies, cakes, and crackers, white wheat is used for noodles, cereals, and breads, and durum wheat is used for pasta (USDA 2022). The Mid-Atlantic region primarily produces SRW. Wheat products intended for human consumption have an acceptable DON limit of 1 ppm, (FDA 2010). In the case of wheat being used for animal feed, the acceptable limits are 10 ppm DON in cattle and chicken feed, 5 ppm in swine, dairy cattle, and all other animals (FDA 2010).

Barley Infection

Barley cultivars grown in North America commonly flower while encapsulated by a protective leaf sheath so they are somewhat protected from infection during flowering, but once the self-pollinated spike emerges, other floral tissues can be susceptible to infection (Fernando et al. 2021). Barley symptoms generally begin as discolored kernels scattered throughout the head that appear tan, orange, or brown compared to adjacent healthy green tissue (Tekauz et al. 2000). Infections in barley tend to stay restrained to the first kernel infected, unlike in wheat with infections commonly spreading from the first kernel infected to other parts of the head (Hoheneder 2022). On the grain head,



Figure 1.4: Barley head with FHB symptoms and necrotic lesions

affected glumes may appear thin or empty and at harvest fusarium damaged kernels (FDK) can have visible mycelium, orange-pink growth, and black spots (Figure 1.4). FDK in barley are not as small or shriveled compared to FDK in wheat (Tekauz et al. 2000).

Barley can be used as animal feed, in human food products, and is a major component of the brewing and distilling industries (Drakoupoulos et al. 2021). Barley is often designated 2-row or 6-row based on kernel arrangement on the head. Barley that is 6-row is most commonly used for feed. Malting barley can include both 2-row and 6-row barley, but 2-row is generally preferred by brewers due to lower protein and enzyme contents (Figure 1.4). Local brewing industries have expanded rapidly in recent years. A malting facility was opened in Laurel, DE in 2018 creating new markets for malting barley production in the Mid-Atlantic. In both feed grade and malting barley, FDK and DON contamination lower the quality of the grain and can result in price discounts or rejection (McMullen et al. 1997). Malting barley intended for brewing is under even stricter limits, with an acceptable limit of <0.5 ppm DON contamination (Bai and Shaner 2004) compared to 1 ppm DON limit in for all other human consumption.

Environment and Risk Modeling

The role of environmental conditions on *F. graminearum* has been widely studied (Anderson 1948; Tschanz et al. 1976; Sung and Cook 1981; Parry et al. 1995; Xu 2003; De Wolf et al. 2003; Cowger et al. 2009; Cowger and Arrelleno 2010; Kriss et al. 2010; Shah et al. 2019). Infection is favored by temperatures ranging from 15 to 30 C and

relative humidity >90% for extended 48 to 72 h periods (Anderson 1948). When favorable weather conditions overlap with the flowering period of cereals epidemics can develop (Parry et al. 1995). Inoculum is produced in the fall after an infected crop is harvested and debris left in the field can become infested with *F. graminearum* which can live saprophytically, decomposing crop residues (Leplat et al. 2012). Although tillage can reduce the inoculum load by burying infested debris underground where soil-borne decomposers have more physical access and better growing conditions, reduced and no-tillage practices have become wide-spread (Xu 2003).

Sporulation studies of *F. graminearum*, *F. avenaceum*, *F. culmorum*, and *Microdochium nivale* have shown optimal temperature for production of asexual macroconidia to be 32 C for *F. graminearum* and *F. culmorum*, 28 C for *F. avenaceum*, and 26 C for *M. nivale* (Xu 2003). Spore density on wheat spikes coincides greatly with high relative humidity and rain intensity and moderate temperatures within ~60-30 days pre-anthesis (Kriss et al. 2010). Development of sexual perithecia is influenced by moisture and favored between 15 to 29 C, becoming limited above 30°C (Tschantz et al. 1976; De Wolf et al. 2003). Water potential also influences spore production. Macroconidia and perithecia production of *F. graminearum*, *F. culmorum*, and *F. avenaceum* was shown to be highest around -15 bars and to cease between -50 and -60 bars; in humid climates plant surfaces are often wet with water potentials at 0 bar (Sung and Cook 1981). For *F. graminearum*, soil moisture must exceed 30% before ascospore production becomes possible and reaches maximums at 80% soil moisture with subsequent germination peaking at 90% relative humidity (atmospheric) (Xu 2003; Kriss

et al. 2010). Discontinuous rainfall events may result in reduced infection efficiency (Bai and Shaner 1991), and even if pre-anthesis conditions are not conducive to wide-spread infection and disease progression, post-anthesis conditions may still favor disease progression and mycotoxin production (Cowger and Arrelleno 2010; Shah et. al 2019). In an inoculated wheat study, FHB, FDK and DON increased under conditions with post-anthesis mist-irrigation (Cowger et al. 2009). Wheat plots were inoculated with *Fusarium* at mid-anthesis, when 50% of heads were flowering, and mist-irrigated for 0, 10, 20, and 30-days post-inoculation. FHB disease incidence and severity were rated at 20 days post-inoculation demonstrating that symptom development following 10 and 20 days of irrigation was significantly higher than 0 days. FDK ratings did not differ between 0 and 10 days of irrigation and were significantly lower than both 20- and 30-days irrigation. DON levels also significantly increased with irrigation level, increasing from 0- to 10-days, and 10- to 20-days (Cowger et al. 2009).

The role of environmental conditions on development of disease severity has led to a robust history of developing risk assessment models to predict FHB severity (De Wolf et al. 2003; Kriss et al. 2010; Shah et al. 2019). There are multiple FHB risk modeling programs available to growers and researchers, most of which relate FHB risk to weather data including relative humidity, rainfall, and temperature leading up to and following crop anthesis. The one most commonly used in eastern and central U.S. small grain production regions is the Fusarium Head Blight Prediction Center and Risk Assessment Tool (<https://www.wheatcab.psu.edu/>). This tool can predict FHB epidemics (field severity greater than 10%) with up to 75% accuracy based on local weather

conditions in the seven days prior to flowering. The United States Wheat and Barley Scab Initiative (USWBSI) has a number of other useful tools for predicting and managing FHB across multiple production regions as well (<https://scabusa.org/tools>). Prediction and forecasting tools can give insight to growers when fungicide applications are necessary or most cost-effective (Shah et al. 2019).

Fusarium Head Blight Management

Crop Rotation

F. graminearum is adapted to infect heads of wheat and barley plants, but can also colonize corn, soybeans, and other rotational crops common in the Mid-Atlantic. In addition to other hosts, the fungus can infect multiple tissue types causing crown rots, foot rots, and seedling blights in wheat and barley (Goswami and Kistler 2004) as well as in corn and bean crops (Leplat et al. 2012). Until recently, Fusarium root rot of dry beans (*Phaseolus vulgaris* L.) was thought to only be caused by *Fusarium solani* f. sp. *phaseoli*, but *F. graminearum* can also cause root rot diseases in black beans, pinto beans, and kidney beans, among others (Bilgi et al. 2011). In soybean (*Glycine max*), *F. graminearum* can induce pre- and post-emergence diseases inducing lesions on the seeds, roots, and shoots (Broders et al. 2007). *F. graminearum* (Teleomorph: *Gibberella zeae*) has also been isolated from potato and sugar beet crops, causing Fusarium dry rot in potato, and wilting and discoloration of the tap root in sugar beet (Burkaloti et al. 2008).

In the Mid-Atlantic, small grains are most commonly planted after corn. In a corn/wheat rotation, field corn is planted in the summer and harvested in early fall. Wheat

or barley can then be planted in the fall to overwinter, breaking dormancy in the spring and eventually be harvested in the early summer. Corn, wheat, and barley are all hosts to *Fusarium* head blight pathogens and growing these crops in rotation can lead to increased levels of inoculum borne in the soil or on plant debris (Parry et al. 1995). *Fusarium* can infect corn at all growth stages, causing seedling blight, root and stalk rot, and ear rot (Broders et al. 2007). At harvest, corn stubble is often left on the field as chopped up



Figure 1.5: Corn debris left in small grain field

stalks and plant bases are left at the soil surface (Figure 1.5) as no-till or low tillage practices have become widespread following conservation legislation in the 1990's (Dill-Macky and Jones 2000). *Fusarium* can colonize leftover corn debris and live saprophytically or as a soil organism between growing seasons until a new host plant is introduced (Broders et al. 2007). Corn/wheat rotations can lead to increasing levels of inoculum conducive to FHB or other *Fusarium* disease epidemics (Parry et al. 1995).

Another rotation system common in the Mid-Atlantic is double cropping soybeans. In this cropping system small grains like wheat and barley are planted in the fall and harvested early the following summer, with soybeans planted after for late fall harvest. A corn crop would typically be planted in the spring of the next season. In full season crops, soybeans are often harvested later in the fall than corn which can push back

the planting date of small grains in rotation, cutting down the already limited growth window of small grains before winter dormancy takes effect. Ideally, inoculum levels would decrease during the soybean growing season, but *Fusarium graminearum* can cause root rot of beans and *F. graminearum* and other species can colonize soybean debris as well (Bilgi et al. 2011; Broders et al. 2007). If trying to plant small grains behind soybeans, early planting dates and maturity groups that allow for early fall harvest can enable timely small grain planting.

Genetic Resistance

Genetic resistance is one of the most economically effective options to manage plant diseases. Within wheat there are two main types of partial resistance to FHB termed Type I and Type II resistance. Type I resistance reduces initial infection and Type II resistance reduces spread of disease within the grain head (Bai and Shaner 2004). Barley head infections are usually restrained to the initially infected kernel and do not spread within the head (Hoheneder 2022), so efforts in resistance breeding focus on Type I resistance (Bai and Shaner 2004). Aside from types I and II, there are also grain quality resistance types III, IV, V, and VI. Type III is resistance to kernel infection, type IV is tolerance to yield loss, type V is resistance to mycotoxin accumulation, and type VI is resistance to changes in the grain makeup (Martin et al. 2018). Type V resistance, in which DON levels are low in grain, can be achieved by detoxification, suppression of DON-production, or some mechanism of barring mycotoxin entry (Bai and Shaner 2004).

Although wheat and barley have multiple forms of resistance, no commercially available cultivars are immune to infection (Friskop et al. 2018). Sumai 3 is an FHB-resistant cultivar discovered in China in the '70s that is commonly used as parent stock in breeding programs and has been the source of several resistance genes (Fernando et al. 2021). Resistance to FHB can be found on many quantitative trait loci (QTLs), numerous genes in the wheat and barley germplasms that can confer a degree of resistance to infection (Buerstmayr et al. 2021). QTLs derived from Sumai 3 include *Fhb1*, *Fhb2*, and *Fhb5* have been cloned and disseminated to resistance breeding programs all over the world (Fernando et al. 2021).

Chemical Management

Use of fungicides has become increasingly common for management of FHB since 1998. Coordinated uniform fungicide trials (UFT's) have been carried out in numerous locations across the country testing product efficacy, application rates, and optimal application timing. Analysis of these collective data sets have aided in recommendations for best management practices to reduce FHB incidence and severity, as well as DON contamination (Paul et al. 2008; Willyerd et al. 2012; Paul et al. 2018; Paul et al. 2019). Small grain crops are most susceptible to infection during anthesis when anthers become exposed to environmental inoculum (Parry et al. 1995). Anthesis, or flowering, is divided into three main phases: early, mid, and late. In early anthesis (Feekes 10.5.1) of wheat flowers in the middle portion of the head are exposed, in mid anthesis (Feekes 10.5.2) flowers emerge from the tip portion of the head, and in late

anthesis (Feekes 10.5.3)
(Broske et al. 2021)
flowers emerge from the
bottom of the head.
Fungicide applications at
or closely following
anthesis have been shown
to be most effective in



Figure 1.6: From left to right Feekes 10.3, Anthesis/Feekes 10.5.1 (yellow anthers beginning flowering), 4 days after anthesis (white anthers post flowering)

controlling disease incidence and severity and reducing DON contamination and are more effective than heading applications when the grain head has emerged but has yet to flower (Paul et al. 2018) (Figure 1.6).

Triazole-based Demethylation Inhibitor (FRAC code 3) fungicides have historically been the dominant fungicide group labeled for management of FHB in the United States (Paul et al. 2008). DMI fungicides function by inhibiting sterol biosynthesis leading to accumulation of ergosterol precursors and fatty acids which affect normal fungal growth (Anderson et al. 2020). Triazole fungicides are site specific and partially systemic. When applied before anthesis, triazoles are more preventative in action, making contact with the grain head before spores can initiate infection, whereas application at or after anthesis is more curative in action, affecting spores that may have already initiated infection events (Paul et al. 2019). DMI fungicides are most effective applied at early anthesis (Feekes 10.5.1) or later, as FHB can continue to initiate infection in the days following anthesis (Willyerd et al. 2012; Cowger et al. 2016; Paul et al. 2018).

DMI fungicides that have been labelled for control of FHB include metconazole (Caramba 90 SL; BASF Agricultural Products, Research Triangle Park, NC), propiconazole (Tilt 3.6 F EC; Syngenta Crop Protection Inc., Greensboro, NC), prothioconazole (Proline 480 SC; Bayer Cropscience, Research Triangle Park, NC), tebuconazole (Folicur 3.6 F; Bayer Cropscience), and combination product tebuconazole and prothioconazole (Prosaro 421 SC; Bayer Cropscience) (Paul et al. 2008). These products are commonly mixed with the nonionic surfactant, Induce (Helena Chemical Company, Collierville, TN) at a rate of 0.125% or 0.25% v/v (Paul et al. 2008; Singh et al. 2020). Prothioconazole (Proline) was the first triazole officially labelled for wheat in 2006, but during severe FHB epidemics occurring between 1997 to 2008, a special Section 18 Crisis Exemption (Federal Insecticide, Fungicide, and Rodenticide Act) allowed the use of tebuconazole on wheat from 1998 until its official registration in 2008. Propiconazole (Tilt) was first labelled for FHB suppression in 2007, metconazole (Caramba) and tebuconazole (Folicur) in 2008, and combination product tebuconazole plus prothioconazole (Prosaro) was labelled for use in 2009 (United States Environmental Protection Agency).

There have been reports of fungicide resistance to specific triazole fungicides, as well as reports of cross-resistance to the triazole class of fungicides in *F. graminearum* developing which is especially troubling due to the widespread use of DMI fungicides to control FHB (Anderson et al. 2020). The first *F. graminearum* isolate resistant to tebuconazole was recovered in 2014 (Spolti et al. 2014). In Europe, analysis of *F. graminearum* isolates collected between 1987 and 2004 were assessed for fungicide

sensitivity to a range of triazole products, and efficacy of epoxiconazole was shown to decrease by 0.22% every year on average (Klix et al. 2007). In a study of 159 *Fusarium asiaticum* and *F. graminearum* isolates from China, 2 *F. asiaticum* isolates and 1 *F. graminearum* isolate showed resistance to the DMI tebuconazole as well as DMI prochloraz (Yin et al. 2009). Due to this, there was interest in introducing diverse modes of action to manage FHB disease development. The first commercially released Succinate Dehydrogenase Inhibitor (SDHI) + DMI combination product, Miravis Ace (Syngenta Crop Protection Inc., Greensboro, NC) containing active ingredients pydiflumetofen (FRAC code 7, SDHI) and propiconazole (DMI) was labeled for use in 2020. SDHI's function by inhibiting cellular respiration in fungal cells, impeding energy production. This product, with its dual mode of action, has been shown to be as (or more) effective compared to traditional fungicides when applied at anthesis. This product is also labeled for earlier application timing at 50% head emergence (Feekes 10.3, before flower emergence) (Singh et al. 2020; Singh et al. 2021). With the use of a new mode of action, there is opportunity to document current baseline fungicide sensitivity values to monitor for resistance development as use becomes more widespread.

A second SDHI/DMI combination Prosaro Pro (Bayer Cropscience) was registered in 2021. Prosaro Pro combines DMI's prothioconazole and tebuconazole along with the SDHI active ingredient fluopyram. In 2021, Sphaerex (BASF Agricultural Products) was also registered combining two DMI active ingredients metconazole and prothioconazole. Quinone Outside Inhibitors (QoI's) in FRAC group 11 are not

recommended for treatment of FHB as they can increase DON contamination, even compared to non-treated checks, when applied alone (Paul et al. 2018).

Fusarium head blight is a highly destructive disease to small grain production globally and is a major concern to wheat and barley production in the United States East Coast. While primarily associated with *F. graminearum*, multiple species can cause FHB. There is a need to better understand the species composition of *Fusarium* in the Mid-Atlantic region. The most economically damaging aspects of *Fusarium* are reductions in small grain yields through diminished test weight and grain fill and degradation of grain quality through physical and chemical contamination. Integrated approaches to management consist of cultural controls like crop rotation and tillage, host genetic resistance, and use of fungicides. These strategies, even if used in concert, cannot guarantee complete control as *Fusarium* has a wide host range and tillage in the region is limited, additionally, there are no totally immune small grain hosts, and fungicides do not provide complete control of disease. Fungicide efficacy can be diminished if *Fusarium* develops resistances to chemical management compounds. With the release of new products containing SDHI modes of action, there is an opportunity to document baseline fungicide sensitivities to monitor for development of fungicide resistance.

REFERENCES

- Andersen, A. L. (1948). The development of *Gibberella zeae* head blight of wheat. *Phytopathology*, 38:595-611.
- Anderson, N. R., Freije, A. N., Bergstrom, G. C., Bradley, C. A., Cowger, C., Faske, T., Hollier, C., Kleczewski, N., Padgett, G. B., Paul, P., Price, T., & Wise, K. A. (2020). Sensitivity of *Fusarium graminearum* to metconazole and tebuconazole fungicides before and after widespread use in wheat in the United States. *Plant Health Progress*, 21(2), 85–90. <https://doi.org/10.1094/php-11-19-0083-rs>
- Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to fusarium head blight. *Annual Review of Phytopathology*, 42(1), 135–161. <https://doi.org/10.1146/annurev.phyto.42.040803.140340>
- Bai, G.-H., and Shaner, G. (1991). Effect of moist period on response of wheat cultivars to infection by *Fusarium graminearum*. (Abstr.) *Phytopathology*, 81:1145.
- Beccari, G., Arellano, C., Covarelli, L., Tini, F., Sulyok, M., & Cowger, C. (2019). Effect of wheat infection timing on fusarium head blight causal agents and secondary metabolites in grain. *International Journal of Food Microbiology*, 290, 214–225. <https://doi.org/10.1016/j.ijfoodmicro.2018.10.014>
- Bertero, A., Fossati, P., Tedesco, D. E., & Caloni, F. (2020). Beauvericin and Enniatins: In vitro intestinal effects. *Toxins*, 12(11), 686. <https://doi.org/10.3390/toxins12110686>
- Bilgi, V. N., Bradley, C. A., Mathew, F. M., Ali, S., & Rasmussen, J. B. (2011). Root rot of dry edible bean caused by *Fusarium graminearum*. *Plant Health Progress*, 12(1). <https://doi.org/10.1094/php-2011-0425-01-rs>
- Broders, K. D., Lipps, P. E., Paul, P. A., & Dorrance, A. E. (2007). Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. *Plant Disease*, 91(9), 1155–1160. <https://doi.org/10.1094/pdis-91-9-1155>
- Broeske, M., Gaska, J., & Roth, A. (2021). *A Visual Guide to Winter Wheat Development and Growth Staging*. Cool Bean. Retrieved November 8, 2022, from https://www.coolbean.info/wp-content/uploads/sites/3/2021/08/UW_WheatGrowthStages.pdf

- Buerstmayr, M., Wagner, C., Nosenko, T., Omony, J., Steiner, B., Nussbaumer, T., Mayer, K. F., & Buerstmayr, H. (2021). Fusarium head blight resistance in European winter wheat: Insights from genome-wide transcriptome analysis. *BMC Genomics*, 22(1). <https://doi.org/10.1186/s12864-021-07800-1>
- Burkaloti, R. R., Ali, S., Secor, G. A., Neate, S. M., McMullen, M. P., & Adhikari, T. B. (2008). Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato, and sugar beet in the Upper Midwest of the United States. *Phytopathology*, 98(9), 969–976. <https://doi.org/10.1094/phyto-98-9-0969>
- Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., Leyva-Mir, S. G., Villaseñor-Mir, H. E., Ayala-Escobar, V., & Nava-Díaz, C. (2018). Regional differences in the composition of fusarium head blight pathogens and mycotoxins associated with wheat in Mexico. *International Journal of Food Microbiology*, 273, 11–19. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.003>
- Champeil, A., Doré, T., & Fourbet, J. F. (2004). Fusarium head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by fusarium in wheat grains. *Plant Science*, 166(6), 1389–1415. <https://doi.org/10.1016/j.plantsci.2004.02.004>
- Crop Protection Network. 2022. Estimates of corn, soybean, and wheat yield losses due to diseases and invertebrate pests: an online tool. <https://loss.cropprotectionnetwork.org/> <https://doi.org/10.31274/cpn-20191121-0>
- Cowger, C., Ward, T. J., Nilsson, K., Arellano, C., McCormick, S. P., & Busman, M. (2020a). Regional and field-specific differences in *Fusarium* species and mycotoxins associated with blighted North Carolina Wheat. *International Journal of Food Microbiology*, 323, 108594. <https://doi.org/10.1016/j.ijfoodmicro.2020.108594>
- Cowger, C., Beccari, G., & Dong, Y. (2020b). Timing of susceptibility to fusarium head blight in winter wheat. *Plant Disease*, 104(11), 2928–2939. <https://doi.org/10.1094/pdis-03-20-0527-re>
- Cowger, C., Weisz, R., Arellano, C., & Murphy, P. (2016). Profitability of integrated management of fusarium head blight in North Carolina winter wheat. *Phytopathology*, 106(8), 814–823. <https://doi.org/10.1094/phyto-10-15-0263-r>
- Cowger, C., & Arrellano, C. (2010). Plump kernels with high deoxynivalenol linked to late *Gibberella zeae* infection and marginal disease conditions in winter wheat. *Phytopathology*, 100(7), 719–728. <https://doi.org/10.1094/phyto-100-7-0719>

- Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., & Perugini, L. (2009). Post-anthesis moisture increased fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. *Phytopathology*, 99(4), 320–327. <https://doi.org/10.1094/phyto-99-4-0320>
- D'Angelo, D. L., et al. Efficacy of fungicide applications during and after anthesis against Fusarium head blight and deoxynivalenol in soft red winter wheat. *Plant Disease* 98.10 (2014): 1387-1397.
- De Wolf, E. (n.d.). *Fusarium risk tool*. Fusarium Risk Tool. Retrieved October 25, 2022, from <https://www.wheatcab.psu.edu/>
- De Wolf, E. D., Madden, L. V., & Lipps, P. E. (2003). Risk assessment models for wheat fusarium head blight epidemics based on within-season weather data. *Phytopathology*, 93(4), 428–435. <https://doi.org/10.1094/phyto.2003.93.4.428>
- Dill-Macky, R., & Jones, R. K. (2000). The effect of previous crop residues and tillage on fusarium head blight of wheat. *Plant Disease*, 84(1), 71–76. <https://doi.org/10.1094/pdis.2000.84.1.71>
- Drakopoulos, D., Sulyok, M., Jenny, E., Kägi, A., Bänziger, I., Logrieco, A. F., Krska, R., & Vogelgsang, S. (2021). Fusarium head blight and associated mycotoxins in grains and straw of barley: Influence of agricultural practices. *Agronomy*, 11(4). <https://doi.org/10.3390/agronomy11040801>
- Duffeck, M. R., Bandara, A. Y., Weerasooriya, D. K., Collins, A. A., Jensen, P. J., Kuldau, G. A., Del Ponte, E. M., & Esker, P. D. (2022). Fusarium head blight of small grains in Pennsylvania: Unravelling species diversity, toxin types, growth, and triazole sensitivity. *Phytopathology*, 112(4), 794–802. <https://doi.org/10.1094/phyto-02-21-0070-r>
- FDA. (2010). *Guidance on advisory levels for deoxynivalenol (Don) in finished wheat*. U.S. Food and Drug Administration. Retrieved October 25, 2022, from <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-fda-advisory-levels-deoxynivalenol-don-finished-wheat-products-human>
- Fernando, W. G. D., Oghenekaro, A. O., Tucker, J. R., & Badea, A. (2021). Building on a foundation: Advances in epidemiology, resistance breeding, and forecasting research for reducing the impact of fusarium head blight in wheat and barley. *Canadian Journal of Plant Pathology*, 43(4), 495–526. <https://doi.org/10.1080/07060661.2020.1861102>

- Fremy, J.-M., Alassane-Kpembi, I., Oswald, I. P., Cottrill, B., & Van Egmond, H. P. (2019). A review on combined effects of Moniliformin and co-occurring *Fusarium* toxins in farm animals. *World Mycotoxin Journal*, *12*(3), 281–291. <https://doi.org/10.3920/wmj2018.2405>
- Friskop, A., Zhong, S., & Brueggeman, R. (2022, September 19). Fusarium head blight (scab) of small grains. NDSU Agriculture and Extension. Retrieved October 25, 2022, from <https://www.ndsu.edu/agriculture/ag-hub/publications/fusarium-head-blight-scab-small-grains>
- Fulcher, M. R., Winans, J. B., Benscher, D., Sorrells, M. E., & Bergstrom, G. C. (2021). *Triticum* varieties grown as ‘ancient grains’ in New York differ in susceptibility to fusarium head blight and harbor diverse fusarium flora. *European Journal of Plant Pathology*, *159*(3), 693–699. <https://doi.org/10.1007/s10658-020-02183-7>
- Goswami, R., & Kistler, H.C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, *5*(6), 515–525. <https://doi.org/10.1111/j.1364-3703.2004.00252.x>
- Guenther, J. C., & Trail, F. (2005). The development and differentiation of *Gibberella zea* (anamorph: *Fusarium graminearum*) during colonization of wheat. *Mycologia*, *97*(1), 229–237. <https://doi.org/10.1080/15572536.2006.11832856>
- Guo, Y., Yao, S., Yuan, T., Wang, Y., Zhang, D., & Tang, W. (2019). The spatiotemporal control of Katg2 catalase-peroxidase contributes to the invasiveness of *Fusarium graminearum* in host plants. *Molecular Plant Pathology*, *20*(5), 685–700. <https://doi.org/10.1111/mpp.12785>
- Hoheneder, F., Biehl, E. M., Hofer, K., Petermeier, J., Groth, J., Herz, M., Rychlik, M., Heß, M., & Hückelhoven, R. (2022). Host genotype and weather effects on fusarium head blight severity and mycotoxin load in spring barley. *Toxins*, *14*(2), 125. <https://doi.org/10.3390/toxins14020125>
- Khan, M. K., Pandey, A., Athar, T., Choudhary, S., Deval, R., Gezgin, S., Hamurcu, M., Topal, A., Atmaca, E., Santos, P. A., Omay, M. R., Suslu, H., Gulcan, K., Inanc, M., Akkaya, M. S., Kahraman, A., & Thomas, G. (2020). Fusarium head blight in wheat: Contemporary Status and Molecular Approaches. *3 Biotech*, *10*(4). <https://doi.org/10.1007/s13205-020-2158-x>
- Klix, M. B., Verreet, J.-A., & Beyer, M. (2007). Comparison of the declining triazole sensitivity of *Gibberella zea* and increased sensitivity achieved by advances in triazole fungicide development. *Crop Protection*, *26*(4), 683–690. <https://doi.org/10.1016/j.cropro.2006.06.006>

- Kriss, A. B., Paul, P. A., & Madden, L. V. (2010). Relationship between yearly fluctuations in fusarium head blight intensity and environmental variables: A window-pane analysis. *Phytopathology*, *100*(8), 784–797. <https://doi.org/10.1094/phyto-100-8-0784>
- Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during fusarium head blight development in six cereal species. *European Journal of Plant Pathology*, *110*(7), 735–746. <https://doi.org/10.1023/b:ejpp.0000041568.31778.ad>
- Leplat, J., Friberg, H., Abid, M., & Steinberg, C. (2012). Survival of *Fusarium graminearum*, the causal agent of fusarium head blight. A Review. *Agronomy for Sustainable Development*, *33*(1), 97–111. <https://doi.org/10.1007/s13593-012-0098-5>
- Lofgren, L., Riddle, J., Dong, Y., Kuhnem, P. R., Cummings, J. A., Del Ponte, E. M., Bergstrom, G. C., & Kistler, H. C. (2017). A high proportion of NX-2 genotype strains are found among *Fusarium graminearum* isolates from northeastern New York State. *European Journal of Plant Pathology*, *150*(3), 791–796. <https://doi.org/10.1007/s10658-017-1314-6>
- Martin, C., Schöneberg, T., Vogelgsang, S., Morisoli, R., Bertossa, M., Mauch-Mani, B., & Mascher, F. (2018). Resistance against *Fusarium graminearum* and the relationship to β -glucan content in barley grains. *European Journal of Plant Pathology*, *152*(3), 621–634. <https://doi.org/10.1007/s10658-018-1506-8>
- McMullen, M., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease*, *81*(12), 1340–1348. <https://doi.org/10.1094/pdis.1997.81.12.1340>
- O'Donnell, K., Kistler, H. C., Tacke, B. K., & Casper, H. H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences*, *97*(14), 7905–7910. <https://doi.org/10.1073/pnas.130193297>
- Osborne, L. E., & Stein, J. M. (2007). Epidemiology of fusarium head blight on small-grain cereals. *International Journal of Food Microbiology*, *119*(1-2), 103–108. <https://doi.org/10.1016/j.ijfoodmicro.2007.07.032>
- Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (SCAB) in small grain cereals—a review. *Plant Pathology*, *44*(2), 207–238. <https://doi.org/10.1111/j.1365-3059.1995.tb02773.x>

- Paul, P. A., Salgado, J. D., Bergstrom, G., Bradley, C. A., Byamukama, E., Byrne, A. M., Chapara, V., Cummings, J. A., Chilvers, M. I., Dill-Macky, R., Friskop, A., Kleczewski, N., Madden, L. V., Nagelkirk, M., Stevens, J., Smith, M., Wegulo, S. N., Wise, K., & Yabwalo, D. (2019). Integrated effects of genetic resistance and prothioconazole + tebuconazole application timing on fusarium head blight in wheat. *Plant Disease*, *103*(2), 223–237. <https://doi.org/10.1094/pdis-04-18-0565-re>
- Paul, P. A., Bradley, C. A., Madden, L. V., Dalla Lana, F., Bergstrom, G. C., Dill-Macky, R., Wise, K. A., Esker, P. D., McMullen, M., Grybauskas, A., Kirk, W. W., Milus, E., & Ruden, K. (2018). Effects of pre- and postanthesis applications of demethylation inhibitor fungicides on fusarium head blight and deoxynivalenol in spring and winter wheat. *Plant Disease*, *102*(12), 2500–2510. <https://doi.org/10.1094/pdis-03-18-0466-re>
- Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., & Madden, L. V. (2008). Efficacy of triazole-based fungicides for fusarium head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. *Phytopathology*, *98*(9), 999–1011. <https://doi.org/10.1094/phyto-98-9-0999>
- Pestka, J. J. (2010). Deoxynivalenol: Mechanisms of action, human exposure, and toxicological relevance. *Archives of Toxicology*, *84*(9), 663–679. <https://doi.org/10.1007/s00204-010-0579-8>
- Pestka, J. J. (2007). Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Animal Feed Science and Technology*, *137*(3-4), 283–298. <https://doi.org/10.1016/j.anifeedsci.2007.06.006>
- Shah, D. A., De Wolf, E. D., Paul, P. A., & Madden, L. V. (2019). Functional data analysis of weather variables linked to fusarium head blight epidemics in the United States. *Phytopathology*, *109*(1), 96–110. <https://doi.org/10.1094/phyto-11-17-0386-r>
- Singh, L., Schulden, T., Wight, J. P., Crank, J., Thorne, L., Erwin, J. E., Dong, Y., & Rawat, N. (2021). Evaluation of application timing of Miravis Ace for control of fusarium head blight in wheat. *Plant Health Progress*, *22*(2), 94–100. <https://doi.org/10.1094/php-01-21-0007-rs>
- Singh, L., Wight, J. P., Crank, J., Thorne, L., Dong, Y., & Rawat, N. (2020). Efficacy assessment of a new fungicide, Miravis Ace, for control of fusarium head blight in wheat. *Plant Health Progress*, *21*(4), 365–368. <https://doi.org/10.1094/php-06-20-0050-rs>

- Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology*, 3(3). <https://doi.org/10.2478/v10102-010-0019-x>
- Son, H., Kim, M.-G., Min, K., Seo, Y.-S., Lim, J. Y., Choi, G. J., Kim, J.-C., Chae, S.-K., & Lee, Y.-W. (2013). AbaA regulates conidiogenesis in the ascomycete fungus *Fusarium graminearum*. *PLoS ONE*, 8(9). <https://doi.org/10.1371/journal.pone.0072915>
- Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., & Bergstrom, G. C. (2014). Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York Wheat and competitiveness of a tebuconazole-resistant isolate. *Plant Disease*, 98(5), 607–613. <https://doi.org/10.1094/pdis-10-13-1051-re>
- Sung, J.-M., & Cook, R. J. (1981). Effect of water potential on reproduction and spore germination by *Fusarium roseum* 'graminearum,' 'culmorum,' and 'avenaceum'. *Phytopathology*, 71(5), 499. <https://doi.org/10.1094/phyto-71-499>
- Tekauz, A., McCallum, B., & Gilbert, J. (2000). Review: Fusarium head blight of Barley in western Canada. *Canadian Journal of Plant Pathology*, 22(1), 9–16. <https://doi.org/10.1080/07060660009501156>
- Trail, F. (2009). For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. *Plant Physiology*, 149(1), 103–110. <https://doi.org/10.1104/pp.108.129684>
- Tschanz, A. T., Horst, R. K., & Nelson, P. E. (1976). The effect of environment on sexual reproduction of *Gibberella zeae*. *Mycologia*, 68(2), 327–340. <https://doi.org/10.1080/00275514.1976.12019914>
- United States Department of Agriculture. (2017). *2017 Census of Agriculture, Sussex County Delaware*. 2017 State and County Profiles | 2017 Census of Agriculture | USDA/NASS. Retrieved October 21, 2022, from https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/County_Profiles/index.php
- United States Wheat and Barley Scab Initiative. (2022). *Grower and industry tools*. Home Page. Retrieved October 25, 2022, from <https://scabusa.org/tools>
- Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., & Hallen-Adams, H. E. (2019). Recent population changes of fusarium head blight pathogens: Drivers and implications. *Canadian Journal of Plant Pathology*, 42(3), 315–329. <https://doi.org/10.1080/07060661.2019.1680442>

- Varga, E., Wiesenberger, G., Hametner, C., Ward, T. J., Dong, Y., Schöfbeck, D., McCormick, S., Broz, K., Stückler, R., Schuhmacher, R., Krska, R., Kistler, H. C., Berthiller, F., & Adam, G. (2015). New tricks of an old enemy: Isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. *Environmental Microbiology*, *17*(8), 2588–2600. <https://doi.org/10.1111/1462-2920.12718>
- Walter, S., Nicholson, P., & Doohan, F. M. (2009). Action and reaction of host and pathogen during fusarium head blight disease. *New Phytologist*, *185*(1), 54–66. <https://doi.org/10.1111/j.1469-8137.2009.03041.x>
- Wan, L. Y., Turner, P. C., & El-Nezami, H. (2013). Individual and combined cytotoxic effects of *Fusarium* toxins (deoxynivalenol, nivalenol, Zearalenone and Fumonisin B1) on swine jejunal epithelial cells. *Food and Chemical Toxicology*, *57*, 276–283. <https://doi.org/10.1016/j.fct.2013.03.034>
- Wheat sector at a glance*. USDA ERS - Wheat Sector at a Glance. (2022, September 29). Retrieved October 2, 2022, from <https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/>
- Willyerd, K. T., Li, C., Madden, L. V., Bradley, C. A., Bergstrom, G. C., Sweets, L. E., McMullen, M., Ransom, J. K., Grybauskas, A., Osborne, L., Wegulo, S. N., Hershman, D. E., Wise, K., Bockus, W. W., Groth, D., Dill-Macky, R., Milus, E., Esker, P. D., Waxman, K. D., ... Paul, P. A. (2012). Efficacy and stability of integrating fungicide and cultivar resistance to manage fusarium head blight and deoxynivalenol in wheat. *Plant Disease*, *96*(7), 957–967. <https://doi.org/10.1094/pdis-09-11-0763>
- Xu, X. (2003). Effects of environmental conditions on the development of fusarium ear blight. *Epidemiology of Mycotoxin Producing Fungi*, 683–689. https://doi.org/10.1007/978-94-017-1452-5_3
- Yin, Y., Liu, X., Li, B., & Ma, Z. (2009). Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. *Phytopathology*®, *99*(5), 487–497. <https://doi.org/10.1094/phyto-99-5-0487>
- Yoshizawa, T., & Morooka, N. (1973). Deoxynivalenol and its monoacetate: New mycotoxins from *Fusarium roseum* and moldy barley. *Agricultural and Biological Chemistry*, *37*(12), 2933–2934. <https://doi.org/10.1080/00021369.1973.10861103>
- Zhang J, Liu X, Su Y, Li T. An update on T2-toxins: metabolism, immunotoxicity mechanism and human assessment exposure of intestinal microbiota. *Heliyon*. 2022 Jul 20;8(8):e10012. doi: 10.1016/j.heliyon.2022.e10012. PMID: 35928103; PMCID: PMC9344027.

Chapter 2

ESTABLISHMENT OF *FUSARIUM GRAMINEARUM* BASELINE FUNGICIDE SENSITIVITY LEVELS TO PYDIFLUMETOFEN FROM WHEAT IN THE UNITED STATES

Abstract

Fusarium Head Blight (FHB), primarily caused by *Fusarium graminearum*, is a top disease of concern across United States (US) wheat production, contributing to both yield losses and grain contamination. For many years, triazole/demethylation inhibitor (DMI, FRAC Group 3) fungicides were the only group labeled for FHB. In 2019, Miravis Ace (Syngenta, Switzerland), was registered for use in the management of FHB, containing propiconazole (DMI) and pydiflumetofen, a Succinate Dehydrogenase Inhibitor (SDHI, FRAC Group 7). *In vitro* poison plate mycelial assays were performed to determine baseline fungicide sensitivity levels of *Fusarium* isolated from wheat to pydiflumetofen. Samples were submitted from 16 states as part of the United States Wheat and Barley Scab Initiative (USWBSI) National FHB Collection. In total, 177 isolates were screened for fungicide sensitivity, including 98 isolates obtained in 2020, 65 in 2021, and 14 historic isolates collected from 1991-2014 with no exposure to pydiflumetofen. The effective concentration to reduce mycelial growth by 50% (EC₅₀) was determined for each isolate using fungicide-amended PDA plates at 0, 0.01, 0.05, 0.25, 1.0, and 5.0 µg/mL. Mycelial growth EC₅₀ values averaged by state ranged from 0.18 to 0.73 µg/mL, cumulatively averaging 0.4 µg/mL and 0.37 µg/mL, in 2020 and 2021 respectively. The

average EC50 value of historic isolates was 0.35 µg/mL. The established baseline fungicide sensitivity levels will aid fungicide research and allow for monitoring of sensitivity levels in *F. graminearum* populations as use of SDHI active ingredients expands across small grain production in the United States.

Introduction

Fusarium Head Blight, primarily caused by *Fusarium graminearum*, is one of the most important small grain crop diseases in the world and can cause severe yield and quality losses (McMullen et al. 1997). Small grain crops are most susceptible to developing FHB in the weeks leading up to and following anthesis (Osborne and Stein 2007). The pathogen has a short disease cycle: spores can germinate on susceptible floral or other head tissues and quickly spread from a single kernel to the entire grain head resulting in significant epidemics under favorable environmental conditions (McMullen et al. 1997; Goswami and Kistler 2004). Economic losses to FHB are two-fold. Under favorable conditions, yield can be reduced by more than 50% (Parry et al. 1995) and *Fusarium* species can produce several mycotoxins during infection and colonization (Osborne and Stein 2007; Varga et al. 2015). Mycotoxins produced by *F. graminearum* can have both food safety and economic impacts. Deoxynivalenol (DON) producers are the most prevalent chemotype in *F. graminearum* and can be further separated to 15-acetyldeoxynivalenol (15A-DON) and 3-acetyldeoxynivalenol (3A-DON) producers (Cowger et al. 2020). The 15A-DON chemotype has historically dominated the United States *Fusarium* population, but the 3A-DON profile has been increasing in prevalence, along with nivalenol (NIV), and 3 α -acetoxy-7 α ,15-dihydroxy-12,13-epoxytrichothec-9-ene (NX-2) profiles (Cowger et al. 2020; Duffeck et al. 2021).

Fusarium Head Blight can spread quickly between plants and among fields, rapidly diminishing yields. The growth of the fungus and the drain on plant resources

may lead to the formation of tombstone kernels; shriveled, sunken seeds that are visibly coated with mycelium. Tombstone kernels can act as an inoculum source the following season and may contain increased levels of DON. Other than causing head blight of wheat and barley, *Fusarium* species can infect multiple tissue types causing crown rots, foot rots, and seedling blights in wheat and barley (Goswami and Kistler 2004) and infect corn, soybeans, and other rotational crops (Leplat et al. 2012). Integrated pest management (IPM) techniques for FHB management include cultural practices, host resistance, and use of fungicides. There are many partially resistant varieties of wheat that decrease the risk of *Fusarium* infection during the growing season, but complete genetic resistance to FHB is not available at this time (Willyerd et al. 2012; Buerstmayr et al. 2021). Combined use of available resistance and properly timed fungicide application is a common approach to management.

Triazole/demethylation inhibitor (DMI) fungicides (FRAC Group 3) fungicides have been used since 1998 to control FHB. DMIs inhibit the proper biosynthesis of fungal membranes and by 2008, full federal registration was obtained for four DMI fungicides on wheat. Products including DMI active ingredients tebuconazole, prothioconazole, metconazole, and propiconazole have been the major source of chemical management of FHB, and instances of fungicide resistance have been reported (Anderson et al. 2020). In 2014, a New York *F. graminearum* isolate was identified as the first tebuconazole-resistant isolate (Spolti et al. 2014), in Europe, *F. graminearum* isolates collected between 1987 and 2004 exhibited a yearly 0.22% reduction in epoxiconazole sensitivity (Klix et al. 2007), and in China two *F. asiaticum* isolates and one *F.*

graminearum isolate showed resistance to the DMI products tebuconazole and prochloraz (Yin et al. 2009). Concerns of DMI fungicide resistance becoming widespread led to investigation and implementation of other chemical modes of action for control of FHB. In 2019, Miravis Ace (Syngenta Crop Protection Inc., Switzerland) became the first dual mode of action product registered for FHB management in wheat with a succinate dehydrogenase inhibitor (SDHI) active ingredient, pydiflumetofen (FRAC code 7) and DMI propiconazole. Succinate Dehydrogenase Inhibitors function by inhibiting respiration and preventing energy production in fungal cells. The pydiflumetofen trademark name is Adepidyn™ with Miravis™ Ace labeled for wheat and Miravis™ Neo labeled for *Fusarium* ear molds in corn.

The fungicide resistance action committee (FRAC) classifies pydiflumetofen as medium to high risk for resistance development (FRAC code list 2022, www.frac.info/publications). Resistance to pydiflumetofen has not been documented to date, but resistance to SDHI fungicides in other pathosystems has been widely documented (FRAC 2020, Peng et al. 2021). Three of the four subunits of the SDH enzyme (subunits B, C, and D) are associated with fungicide binding and mutations can confer resistance (Peng et al. 2021). Baseline *in vitro* sensitivities to pydiflumetofen were first screened in *F. asiaticum* (Hou et al. 2017), *Cercospora zea-maydis* (Neves and Bradley 2019), *Venturia inaequalis* (Ayer et al. 2019), *F. oxysporum* f. sp. *niveum* (Miller et al. 2020), *Cercospora sojina* and *Corynespora cassicola* (Neves and Bradley 2021). The first sensitivity data of *F. graminearum* to pydiflumetofen was conducted on a subset of 94 isolates originating from 2011-2017 from wheat, corn, dry bean, and soybean

surveys in Michigan (Breunig and Chilvers 2021). Prior to widespread use of the chemistry, there is need to expand observations on baseline sensitivities to encompass grain production regions across the US. The objectives of this study were i) to identify baseline fungicide sensitivities of *F. graminearum* isolates to pydiflumetofen, ii) to identify if regional differences in baseline fungicide sensitivities exist based on state of origin, and iii) to compare sensitivities of recently collected isolates to historic isolates with no exposure to pydiflumetofen.

Materials and Methods

Isolate Collection

Isolates used in this study were collected from wheat fields across the United States. The collection included pre-isolated pure culture samples that were mailed on Petri dishes or freeze-dried filter paper discs and grain samples that were mailed in bulk and single spore isolations were obtained once received. All isolates were maintained on full-strength potato dextrose agar (PDA; Difco) amended with 0.05 mg Penicillin G (100 ug ml⁻¹) and Streptomycin sulfate (100 ug ml⁻¹) prior to assays. For long term storage, a colonized hyphal plug was transferred to a 9 cm diam Petri dish containing PDA, and four pieces of sterile 2.5 cm diameter filter paper were placed around the plug (Whatman, Global Life Sciences Solutions, USA). Fully colonized discs were removed and placed into coin envelopes for storage at -80 C. Where possible, meta-data for each isolate

included collection date, state, and county of collection, host cultivar, and fungicide treatment if applicable.

Molecular Confirmation of Isolates

Fungal isolates used in this study were selected based on morphological growth features characteristic of *F. graminearum* and species identity was confirmed by polymerase chain reaction (PCR) amplification and sequencing of translation elongation factor 1- α (*TEF1*) (O'Donnell et al. 1998). Two 6.75 mm plugs from each isolate were transferred into 60 x 15 mm Petri dishes containing 8 ml of potato dextrose broth (PDB) (Difco) and stored at 23°C until fully colonized. Isolates showing no contamination were vacuum-aspirated through autoclave-sterilized filter paper in a Buchner funnel. Dried tissue was separated into 1.5 ml microcentrifuge tubes and stored at -20°C for DNA extraction.

Genomic DNA from dried, frozen tissue was extracted by macerating tissue with 400 μ l of cell lysis solution (Qiagen, Hilden, Germany) using autoclave-sterilized plastic pestles. Following the addition of 3 μ l Puregene proteinase K (Qiagen) to each sample, tubes were vortexed for 20 s, and incubated at 55°C for 1 h. The supernatant of microcentrifuged samples was treated with RNase (3 μ l; Qiagen) and incubated at 37°C for 30 min followed by the addition of 200 μ l of protein precipitation solution (Qiagen) and 1 h incubation on ice. Tubes were then centrifuged for 5 min and supernatant was pipetted into microcentrifuge tubes containing 400 μ l isopropanol to precipitate DNA. Tubes were centrifuged for 3 min, the supernatant was poured off, and the DNA pellet was washed

with 200 μl ethanol. After centrifuging, the supernatant was poured off and samples were allowed to dry on a heating block before being rehydrated with 50 μl of sterile, nuclease-free water. DNA concentration and purity were measured by spectrophotometry and standardized to a concentration of 20 ng ml^{-1} before storage at -20°C .

For each isolate, the elongation factor 1 α gene region was amplified using 50 μl PCR reactions that consisted of 10 X DreamTaq Master Mix, 10 μM of forward and reverse primer: EF1 (5'-ATG GGT AAG GAG GAC AAG AC-3') and EF2 (5'-GGA AGT ACC AGT GAT CAT G-3') (Cerón-Bustamante et al. 2018) and 20 ng of genomic DNA. Thermal cycling parameters were set as: 95°C for 3 min followed by 35 cycles of DNA denaturation for 40 s at 95°C ; primer annealing for 30 s at 55°C ; and primer extension for 1 min at 72°C . A final extension step of 72°C for 10 min completed the cycle.

Amplification was verified using gel electrophoresis, at 90 V for 40 min. Aliquots of amplified products were cleaned with ExoSAP-IT (USB products Affymetrix, Inc. ThermoFisher Scientific, Waltham Massachusetts). Samples were sequenced at Eurofins Genomics (Louisville, KY). Analysis of sequence data was completed with CLC Main Workbench 20.0.4 (CLC Bio: a Qiagen Company, Germany) and copied into the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) for comparison against published, identified specimens (Table 2.1).

Mycelial Growth Assay

Poison plate mycelial assays were performed on selected isolates to determine EC50 values, the effective concentration at which mycelial growth is reduced by 50 percent. Isolates were grown on PDA for seven days at 22 C prior to assay plating. A 7 mm diameter cork borer was used to cut plugs from the leading edge of the colonies. A single 7 mm plug was transferred to the center of each amended Petri dish. Amended plates were generated by autoclaving PDA and cooling to approximately 55 C before addition of pydiflumetofen stock solutions. Fungicide stock solutions were prepared by adding formulated pydiflumetofen (Miravis, Syngenta Crop Protection Inc.) to sterile deionized water and making serial dilutions for concentrations of 5000, 1000, 250, 50, and 10 $\mu\text{g/mL}$ pydiflumetofen. Stock solutions were added to cooled PDA in a 1:1000 mL ratio to produce PDA poison plates with final pydiflumetofen concentrations of 5.0, 1.0, 0.25, 0.05, 0.01, and 0 $\mu\text{g/mL}$ (Breunig and Chilvers 2021; Hou et al. 2017). The non-treated control was amended with 1 mL sterile DI water. Each 60 x 15 mm Petri dish (Fischerbrand, Fischer Scientific USA) was filled with 8 mL of fungicide-amended liquid media aliquoted using a media pump (Digital Mini Pump Dispensing Systems, Argos Technologies, Radnor, Pennsylvania).

Once transferred, Petri dishes were incubated in the dark for 5 days at 24 C. Three experiments were performed with 55-63 isolates per experiment, totaling 177 isolates. Each trial included three Petri dish replicates of each concentration by isolate combination and was conducted twice. Mycelial growth was measured at the widest point and transverse to the widest point going through the original plug with a digital caliper

(iGaging ABSOLUTE ORIGIN 0-6” Digital Electronic Caliper, iGaging, San Clemente, California). The two measurements were averaged and the width of the original plug (7 mm) was subtracted to give the total growth. The percent growth inhibition was calculated using the formula

$$\% \text{ Inhibition} = \left(1 - \frac{\text{diameter of control} - \text{diameter of treatment}}{\text{diameter of control}} \right) * 100.$$

Data Analysis

JMP Statistical Software was used to determine the mycelial EC50 values for each isolate. Percent inhibition was graphed against log treatment concentration and a line of best fit was generated. The line equation was set to Y=50 to determine the effective concentration to inhibit mycelial growth by 50%. Absolute EC50 values were estimated separately for each run and mean of the two runs presented. Data from the replicated experiments were combined and sorted by state and collection year. EC50 values were calculated for each state by linear regression of the percent inhibition on log10-transformed fungicide concentration in SAS 9.4 (SAS Institute, Cary, North Carolina).

Results

Molecular Confirmation of Isolates

Isolates used in this study were contributed from 16 states in the US (Figure 2.1). In 2020, samples were submitted from 13 states including Arkansas, Delaware, Illinois, Kansas, Kentucky, Louisiana, Maryland, Michigan, Nebraska, New York, North

Carolina, Ohio, South Dakota, and Wisconsin. In 2021, isolates were submitted from 5 states; Illinois, Indiana, Kansas, Kentucky, and North Dakota. In addition to the 2020-21 samples, historic samples were submitted from Kansas and New York. These samples were collected from 1991-2014 with no prior exposure to pydiflumetofen. Following molecular identification, 177 isolates were selected for mycelial growth assays (Table 2.1).

Sensitivity of mycelial growth to pydiflumetofen by year

The isolate set screened included isolates submitted in 2020 (n=98), 2021 (n=65) and historical isolates (n = 14). Split up by year; the EC50 mean value of historic isolates was 0.353 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.044 to 1.645 $\mu\text{g}/\text{mL}$. The mean EC50 value of isolates submitted in 2020 was 0.395 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.006 to 1.789 $\mu\text{g}/\text{mL}$. The mean EC50 value of isolates submitted in 2021 was 0.365 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.01 ppm to 1.45 $\mu\text{g}/\text{mL}$ (Figure 2.2).

Sensitivity of mycelial growth to pydiflumetofen by state

Mean EC50 values were calculated for each state (Table 2.2). Kansas and New York were the only two states with historic isolates (Fig 2.3). Split up by state, the mean EC50 value of Kansas historic isolates (n=5) was 0.19 $\mu\text{g}/\text{mL}$ and individual values ranged from 0.044 to 0.35 $\mu\text{g}/\text{mL}$. The mean Kansas 2020 EC50 (n=7) value was 0.277 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.077 to 0.659 $\mu\text{g}/\text{mL}$. The mean 2021

Kansas EC50 (n=8) value was 0.351 $\mu\text{g}/\text{mL}$ and isolate EC50 values ranged from 0.191 to 0.757 $\mu\text{g}/\text{mL}$. The historic New York mean EC50 value (n=9) was 0.443 $\mu\text{g}/\text{mL}$ and isolates ranged from 0.079 to 1.645 $\mu\text{g}/\text{mL}$. New York 2020 isolates (n=2) had the lowest mean EC50 value for the 2020 and 2021 time period, with an average EC50 value of 0.182 $\mu\text{g}/\text{mL}$. New York 2020 isolate mean EC50 values ranged from 0.125 to 0.238 $\mu\text{g}/\text{mL}$.

States represented by both 2020 and 2021 time periods included Kentucky and Illinois. Illinois isolates collected in 2020 had a mean EC50 value of 0.309 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.156 $\mu\text{g}/\text{mL}$ to 0.52 $\mu\text{g}/\text{mL}$. In 2021, the mean EC50 value for Illinois was 0.344 $\mu\text{g}/\text{mL}$ and isolate EC50 values ranged from 0.087 $\mu\text{g}/\text{mL}$ to 0.913 $\mu\text{g}/\text{mL}$. In 2020, the Kentucky mean EC50 value was 0.491 $\mu\text{g}/\text{mL}$ and individual isolate EC50 values ranged from 0.216 to 1.3 $\mu\text{g}/\text{mL}$. The Kentucky 2021 isolates mean EC50 values was 0.39 $\mu\text{g}/\text{mL}$ and individual isolates ranged from 0.042 to 1.04 $\mu\text{g}/\text{mL}$.

States represented in 2020 included Arkansas, Delaware, Illinois, Kentucky, Louisiana, Maryland, Michigan, Nebraska, North Carolina, Ohio, South Dakota, and Wisconsin (Figure 2.4). The Arkansas mean EC50 value was 0.479 $\mu\text{g}/\text{mL}$ and isolates ranged from 0.083 to 1.303 $\mu\text{g}/\text{mL}$. In Delaware the mean EC50 value was 0.479 $\mu\text{g}/\text{mL}$ and individual isolate EC50 values ranged from 0.192 to 1.085 $\mu\text{g}/\text{mL}$. The mean EC50 value for Louisiana in 2020 was the highest mean EC50 value in the 2020 and 2021 time period and was 0.732 $\mu\text{g}/\text{mL}$. Louisiana isolates ranged from 0.344 $\mu\text{g}/\text{mL}$ to 1.121 $\mu\text{g}/\text{mL}$. The Maryland mean EC50 value was 0.429 $\mu\text{g}/\text{mL}$ and individual EC50 values ranged from 0.235 $\mu\text{g}/\text{mL}$ to 0.622 $\mu\text{g}/\text{mL}$. In Michigan isolates, the mean EC50 was

0.279 $\mu\text{g/mL}$ and isolate EC50 values ranged from 0.088 to 0.802 $\mu\text{g/mL}$. The mean EC50 value of Nebraska isolates was 0.439 $\mu\text{g/mL}$ and isolate EC50 values ranged from 0.133 $\mu\text{g/mL}$ to 1.789 $\mu\text{g/mL}$, which was also the highest individual isolate EC50 in 2020. The North Carolina mean EC50 value was 0.199 $\mu\text{g/mL}$ and isolate EC50 values ranged from 0.02 to 0.502 $\mu\text{g/mL}$. The Ohio mean EC50 value was 0.569 $\mu\text{g/mL}$ and individual isolate mean EC50 values ranged from 0.222 to 1.481 $\mu\text{g/mL}$. The South Dakota mean EC50 value was 0.535 $\mu\text{g/mL}$ and individual EC50 values ranged from 0.307 $\mu\text{g/mL}$ to 0.764 $\mu\text{g/mL}$. In Wisconsin isolates the mean EC50 value was 0.275 and isolate EC50 values ranged from 0.006 $\mu\text{g/mL}$, the lowest individual EC50 value in 2020, to 0.58 $\mu\text{g/mL}$.

States represented in 2021 included Indiana and North Dakota. Kentucky, Kansas, and Illinois (Figure 2.5). The Indiana 2021 mean EC50 value was 0.395 $\mu\text{g/mL}$ and individual values ranged from 0.01 $\mu\text{g/mL}$ to 1.453 $\mu\text{g/mL}$, the lowest and highest individual EC50 values in 2021 isolates, respectively. In North Dakota, the 2021 mean EC50 value was 0.301 $\mu\text{g/mL}$ and individual isolate EC50 values ranged from 0.218 to 0.460 $\mu\text{g/mL}$.

Historic state mean EC50 values ranged from 0.192 (Kansas) to 0.443 (New York) $\mu\text{g/mL}$. Individual historic isolate EC50 values ranged from 0.079 (New York) to 1.645 $\mu\text{g/mL}$ (New York). 2020 isolate state mean EC50 values ranged from 0.182 (New York) to 0.732 $\mu\text{g/mL}$ (Louisiana). EC50 values for individual 2020 isolates ranged from 0.006 (Wisconsin) to 1.789 $\mu\text{g/mL}$ (Nebraska). 2021 state mean EC50 values ranged

from 0.301 (North Dakota) to 0.395 $\mu\text{g}/\text{mL}$ (Indiana). Individual EC50 values for 2021 isolates ranged from 0.01 (Indiana) to 1.453 $\mu\text{g}/\text{mL}$ (Indianan).

Discussion

Fungicides are an important component of *Fusarium* management in small grains to reduce yield loss and mycotoxin contamination. In this study, baseline sensitivities of *F. graminearum* isolates from across the United States to the SDHI active ingredient pydiflumetofen were established. All isolates tested in the mycelial assay study appear to be sensitive to pydiflumetofen. EC50 values varied by state and by year, but all means were below 0.732 $\mu\text{g}/\text{mL}$. This is consistent with previous studies where *F. graminearum* mycelial EC50 values ranged from 0.008 to 0.263 $\mu\text{g}/\text{mL}$ Michigan isolates (Breunig and Chilvers 2021). Closely related *F. asiaticum* isolates were also found to be sensitive to pydiflumetofen with mycelial EC50 values of 0.208 $\mu\text{g}/\text{mL}$ or below (Hou et al. 2017).

Products containing pydiflumetofen were first registered in 2019. This active ingredient is also present in numerous field and vegetable crop products that were registered in 2018. With the prevalence of corn-small grain rotations, it is possible that isolates collected in 2020 and 2021, may have been exposed to pydiflumetofen. Isolates from Delaware, Ohio, Illinois, and Kentucky were from fields that received an anthesis application of Miravis Ace.

DMI products have been the major source of FHB chemical control since they were first registered in 2006, and reliance on a single fungicide class mode of action poses significant risk to food safety and security in the case of fungicide resistance

developing (Morton and Staub 2008). Fungicides of the same mode of action function similarly, increasing the risk of cross-resistance, wherein a pathogen that becomes resistant to a single product may have reduced sensitivity to related products in the same fungicide class. By combining SDHI and DMI modes of action, the threat of efficacy loss is reduced as pathogens must become resistant to multiple fungicide modes of action. Resistance development can also be slowed by rotating fungicides of the same chemical class being applied. Even with fungicides of the same chemical class the actual target, or method of inhibition, can vary. Alternating between SDHI products as well as other DMI products can help decrease the risk of fungicide resistance developing. Prosaro Pro (Bayer CropScience, Research Triangle Park, NC) is another recently released fungicide that combines the SDHI fluopyram with DMI's prothioconazole and tebuconazole. Future studies should investigate the baseline sensitivity of *Fusarium* to fluopyram while comparing to pydiflumetofen baseline sensitivities to track the development of resistance and cross-resistance.

Spore germination assays were attempted using a single isolate representative of each state, but ascospores failed to generate sufficient macroconidia using carboxymethylcellulose (CMC) broth (Breunig and Chilvers 2021; Hou et al. 2017). In Michigan isolates, the spore germination EC₅₀ value was 0.583 µg/mL, significantly higher than the mycelial growth EC₅₀ value was 0.263 µg/mL (Breunig and Chilvers, 2021). In China, *F. asiaticum* macroconidia germination EC₅₀ values ranged from 0.058 to 0.424 µg/mL and mycelial growth EC₅₀ values ranged from 0.019 to 0.208 µg/mL (Hou et al. 2017). In both of these studies, inhibition of conidial germination EC₅₀ values

were higher than inhibition of mycelial growth EC50 values, and there was no correlation between mycelial and spore germination assays. No indication of resistance was identified in either of these studies.

The current study is the first to examine *F. graminearum* isolates across a broad geographic scope of the US for sensitivity to pydiflumetofen. No resistant isolates were detected in this study. It is possible that resistance present at low levels was not able to be detected due to the sample size of isolates collected. The results of this study serve as a baseline that resistance is not currently present and continued testing across the US can build from these findings. Although no *Fusarium graminearum* isolates have been identified as resistant to pydiflumetofen, other pathogen species resistant to SDHI products have been identified; including *Blumeriella jaapii* (Cherry leaf spot) resistance to boscalid, fluopyram, and fluxapyroxad; and SDHI resistance has also been achieved in *S. sclerotiorum* in mutation studies (FRAC 2020, Peng et al. 2021, Outwater et al. 2019). Future work may look to identifying discriminatory dose screening to enable high throughput screening of larger isolate volumes to better capture state distributions. Pydiflumetofen remains to be a viable treatment option in the management of FHB and has been shown to be as or more effective than previously available fungicide options (Singh et al. 2021). This study provides baseline ED50 values for researchers to track product efficacy in the field and to monitor for changes in fungicide sensitivity in their region.

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REFERENCES

- Andersen, A. L. (1948). The development of *Gibberella zeae* head blight of wheat. *Phytopathology*, 38:595-611
- Ayer, K. M., Villani, S. M., Choi, M.-W., & Cox, K. D. (2019). Characterization of the *Visdhc* and *Visdhd* genes in *Venturia inaequalis*, and sensitivity to Fluxapyroxad, Pydiflumetofen, Inpyrfluxam, and Benzovindiflupyr. *Plant Disease*, 103(6), 1092–1100. <https://doi.org/10.1094/pdis-07-18-1225-re>
- Breunig, M., & Chilvers, M. I. (2021). Baseline sensitivity of *Fusarium graminearum* from wheat, corn, dry bean and soybean to Pydiflumetofen in Michigan, USA. *Crop Protection*, 140, 105419. <https://doi.org/10.1016/j.cropro.2020.105419>
- Buerstmayr, M., Wagner, C., Nosenko, T., Omony, J., Steiner, B., Nussbaumer, T., Mayer, K. F., & Buerstmayr, H. (2021). Fusarium head blight resistance in European winter wheat: Insights from genome-wide transcriptome analysis. *BMC Genomics*, 22(1). <https://doi.org/10.1186/s12864-021-07800-1>
- Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., Leyva-Mir, S. G., Villaseñor-Mir, H. E., Ayala-Escobar, V., & Nava-Díaz, C. (2018). Regional differences in the composition of fusarium head blight pathogens and mycotoxins associated with wheat in Mexico. *International Journal of Food Microbiology*, 273, 11–19. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.003>
- Cowger, C., Ward, T. J., Nilsson, K., Arellano, C., McCormick, S. P., & Busman, M. (2020). Regional and field-specific differences in *Fusarium* species and mycotoxins associated with blighted North Carolina Wheat. *International Journal of Food Microbiology*, 323, 108594. <https://doi.org/10.1016/j.ijfoodmicro.2020.108594>
- Duffeck, M. R., Bandara, A. Y., Weerasooriya, D. K., Collins, A. A., Jensen, P. J., Kuldau, G. A., Del Ponte, E. M., & Esker, P. D. (2022). Fusarium head blight of small grains in Pennsylvania: Unravelling species diversity, toxin types, growth, and triazole sensitivity. *Phytopathology*, 112(4), 794–802. <https://doi.org/10.1094/phyto-02-21-0070-r>
- FRAC. 2020. *List of first confirmed cases of plant pathogenic organisms resistant to disease control agents*. https://www.frac.info/docs/default-source/publications/list-of-resistant-plant-pathogens/list-of-first-confirmed-cases-of-plant-pathogenic-organisms-resistant-to-disease-control-agents_05_2020.pdf?sfvrsn=7073499a_2

- Goswami RS, Kistler HC. Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*. 2004 Nov 1;5(6):515-25. Doi: 10.1111/j.1364-3703.2004.00252.x. PMID: 20565626.
- Hou, Y.-P., Mao, X.-W., Wang, J.-X., Zhan, S.-W., & Zhou, M.-G. (2017). Sensitivity of *Fusarium asiaticum* to a novel succinate dehydrogenase inhibitor fungicide pydiflumetofen. *Crop Protection*, 96, 237–244. <https://doi.org/10.1016/j.cropro.2017.02.011>
- Klix, M. B., Verreet, J.-A., & Beyer, M. (2007). Comparison of the declining triazole sensitivity of *Gibberella zea* and increased sensitivity achieved by advances in triazole fungicide development. *Crop Protection*, 26(4), 683–690. <https://doi.org/10.1016/j.cropro.2006.06.006>
- Leplat, Johann & Friberg, Hanna & Abid, Muhammad & Steinberg, Christian. (2013). Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. *Agronomy for Sustainable Development*. 33. 97-111. 10.1007/s13593-012-0098-5.
- Miller, N. F., Standish, J. R., & Quesada-Ocampo, L. M. (2020). Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and pydiflumetofen in vitro and efficacy for fusarium wilt management in watermelon. *Plant Health Progress*, 21(1), 13–18. <https://doi.org/10.1094/php-08-19-0056-rs>
- McMullen M, Jones R, Gallenberg D. Scab of Wheat and Barley: A Re-emerging Disease of Devastating Impact. *Plant Disease*, 1997 Dec;81(12):1340-1348. Doi: 10.1094/PDIS.1997.81.12.1340. PMID: 30861784.
- Morton, V. and Staub, T. 2008 A Short History of Fungicides. Online, APSnet Features. Doi: 10.1094/APSnetFeature-2008-0308.
- Neves, D. L., & Bradley, C. A. (2021). Baseline sensitivity of *Cercospora Sojina* and *Corynespora cassiicola* to pydiflumetofen. *Crop Protection*, 147, 105461. <https://doi.org/10.1016/j.cropro.2020.105461>
- Neves, D. L., & Bradley, C. A. (2019). Baseline sensitivity of *Cercospora zea-maydis* to pydiflumetofen, a new succinate dehydrogenase inhibitor fungicide. *Crop Protection*, 119, 177–179. <https://doi.org/10.1016/j.cropro.2019.01.021>

- O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., Broders, K., Kim, H.-S., McCormick, S. P., Busman, M., Aoki, T., Torres-Cruz, T. J., & Geiser, D. M. (2022). DNA sequence-based identification of *Fusarium*: A work in progress. *Plant Disease*, *106*(6), 1597–1609. <https://doi.org/10.1094/pdis-09-21-2035-sr>
- Osborne LE, Stein JM. Epidemiology of *Fusarium* head blight on small-grain cereals. *International Journal of Food Microbiology*, 2007 Oct 20;119(1-2):103-8. Doi: 10.1016/j.ijfoodmicro.2007.07.032. Epub 2007 Jul 31. PMID: 17716761.
- Outwater, C. A., Proffer, T. J., Rothwell, N. L., Peng, J., & Sundin, G. W. (2019). Boscalid resistance in *Blumeriella jaapii*: Distribution, effect on field efficacy, and molecular characterization. *Plant Disease*, *103*(6), 1112–1118. <https://doi.org/10.1094/pdis-09-18-1555-re>
- Parry, D. W., Jenkinson, P., & McLeod, L. (1995). *Fusarium* ear blight (SCAB) in Small Grain Cereals- a Review. *Plant Pathology*, *44*(2), 207–238. <https://doi.org/10.1111/j.1365-3059.1995.tb02773.x>
- Peng, J., Sang, H., Proffer, T. J., Gleason, J., Outwater, C. A., Jung, G., & Sundin, G. W. (2021). A method for the examination of SDHI fungicide resistance mechanisms in phytopathogenic fungi using a heterologous expression system in *Sclerotinia sclerotiorum*. *Phytopathology*®, *111*(5), 819–830. <https://doi.org/10.1094/phyto-09-20-0421-r>
- Singh, L., Schulden, T., Wight, J. P., Crank, J., Thorne, L., Erwin, J. E., Dong, Y., & Rawat, N. (2021). Evaluation of application timing of Miravis Ace for control of fusarium head blight in wheat. *Plant Health Progress*, *22*(2), 94–100. <https://doi.org/10.1094/php-01-21-0007-rs>
- Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., & Bergstrom, G. C. (2014). Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. *Plant Disease*, *98*(5), 607–613. <https://doi.org/10.1094/pdis-10-13-1051-re>
- Varga, E., Wiesenberger, G., Hametner, C., Ward, T. J., Dong, Y., Schöfbeck, D., McCormick, S., Broz, K., Stücker, R., Schuhmacher, R., Krska, R., Kistler, H. C., Berthiller, F., & Adam, G. (2015). New tricks of an old enemy: Isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. *Environmental Microbiology*, *17*(8), 2588–2600. <https://doi.org/10.1111/1462-2920.12718>

- Willyerd, K. T., Li, C., Madden, L. V., Bradley, C. A., Bergstrom, G. C., Sweets, L. E., McMullen, M., Ransom, J. K., Grybauskas, A., Osborne, L., Wegulo, S. N., Hershman, D. E., Wise, K., Bockus, W. W., Groth, D., Dill-Macky, R., Milus, E., Esker, P. D., Waxman, K. D., ... Paul, P. A. (2012). Efficacy and stability of integrating fungicide and cultivar resistance to manage fusarium head blight and deoxynivalenol in wheat. *Plant Disease*, 96(7), 957–967.
<https://doi.org/10.1094/pdis-09-11-0763>
- Yin, Y., Liu, X., Li, B., & Ma, Z. (2009). Characterization of sterol demethylation inhibitor resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. *Phytopathology*, 99(5), 487–497.
<https://doi.org/10.1094/phyto-99-5-0487>

Table 2.1: List of *Fusarium graminearum* isolates collected from wheat used in pydiflumetofen baseline sensitivity assay, including assay ID, year and state of collection.

Isolate ID	Assay ID	Year Collected	State	Species
WF20_5	1	2020	Delaware	<i>F. graminearum</i>
WF20_13	3	2020	Delaware	<i>F. graminearum</i>
WF20_14	4	2020	Delaware	<i>F. graminearum</i>
WF20_15	5	2020	Delaware	<i>F. graminearum</i>
WF20_25	6	2020	Delaware	<i>F. graminearum</i>
WF20_59	7	2020	Delaware	<i>F. graminearum</i>
WF20_117	9	2020	Delaware	<i>F. graminearum</i>
US.FHB.Wheat.NE.14.1.2020	10	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.15.1.2020	11	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.18.1.2020	12	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.26.1.2020	13	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.OH.39.1.2020	14	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.OH.44.1.2020	15	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.OH.45.1.2020	16	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.OH.48.1.2020	17	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.LA.51.1	18	2020	Louisiana	<i>F. graminearum</i>
US.FHB.Wheat.LA.52.1	19	2020	Louisiana	<i>F. graminearum</i>
US.FHB.Wheat.WI.53.1	20	2020	Wisconsin	<i>F. graminearum</i>
US.FHB.Wheat.WI.54.4	21	2020	Wisconsin	<i>F. graminearum</i>
US.FHB.Wheat.WI.55.4	22	2020	Wisconsin	<i>F. graminearum</i>
US.FHB.Wheat.NC.56.1	23	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.NC.60.1	24	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.NC.62.1	25	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.NC.64.1	26	2020	North Carolina	<i>F. graminearum</i>
US.FHB.SD.71.1.2020	27	2020	South Dakota	<i>F. graminearum</i>
US.FHB.SD.72.1.2020	28	2020	South Dakota	<i>F. graminearum</i>
US.FHB.Wheat.KY.76.1.2020	29	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.80.1.2020	30	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.83.2.2020	31	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.84.1.2020	32	2020	Kentucky	<i>F. graminearum</i>
US.FHB.MD.88.1.2020	33	2020	Maryland	<i>F. graminearum</i>
US.FHB.MD.89.1.2020	34	2020	Maryland	<i>F. graminearum</i>
US.FHB.MI.129	35	2020	Michigan	<i>F. graminearum</i>
US.FHB.MI.133	36	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.136.1.2020	37	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.139.1.2020	38	2020	Michigan	<i>F. graminearum</i>
US.FHB.KS.140.1991	39	1991	Kansas	<i>F. graminearum</i>

US.FHB.KS.141.2004	40	2004	Kansas	<i>F. graminearum</i>
US.FHB.KS.143.2009	41	2009	Kansas	<i>F. graminearum</i>
US.FHB.KS.146.2020	42	2020	Kansas	<i>F. graminearum</i>
US.FHB.KS.149.2020	43	2020	Kansas	<i>F. graminearum</i>
US.FHB.Wheat.KS.150.2020	44	2020	Kansas	<i>F. graminearum</i>
US.FHB.Wheat.KS.151.2020	45	2020	Kansas	<i>F. graminearum</i>
US.FHB.KS.152	46	2020	Kansas	<i>F. graminearum</i>
US.FHB.Wheat.NY.161.2008	47	2008	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.170.2013	48	2013	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.171.2013	49	2013	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.175	50	2020	New York	<i>F. graminearum</i>
US.FHB.Wheat.ND.3.1	51	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.66.1.2020	52	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.69.1.2020	53	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.102.1	54	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.116.1	55	2020	Illinois	<i>F. graminearum</i>
AR.Wheat.JK.02.2020	56	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.JK.04.1.2020	57	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.SW.10.2020	58	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.SW.11.1.2020	59	2020	Arkansas	<i>F. graminearum</i>
ND.Wheat.2.1.2021	60	2021	North Dakota	<i>F. graminearum</i>
ND.Wheat.2.2.2021	61	2021	North Dakota	<i>F. graminearum</i>
IN.SS.01.2021	62	2021	Indiana	<i>F. graminearum</i>
IN.SS.03.2021	63	2021	Indiana	<i>F. graminearum</i>
IN.Wheat,SS.1.2021	64	2021	Indiana	<i>F. graminearum</i>
IN.Wheat,SS.7.1.2021	65	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.NE.10.1.2020	66	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.12.1.2020	67	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.17.1.2020	68	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.25.1.2020	69	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.27.1.2020	70	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.29.1.2020	71	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.33.1.2020	72	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.NE.34.3.2020	73	2020	Nebraska	<i>F. graminearum</i>
US.FHB.Wheat.OH.40.1.2020	74	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.OH.42.1.2020	75	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.OH.47.2.2020	76	2020	Ohio	<i>F. graminearum</i>
US.FHB.Wheat.NC.57.2	78	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.NC.58.1	79	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.NC.59.3	80	2020	North Carolina	<i>F. graminearum</i>
US.FHB.Wheat.KY.72.1.2020	84	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.73.1.2020	85	2020	Kentucky	<i>F. graminearum</i>

US.FHB.Wheat.KY.75.1.2020	86	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.77.1.2020	87	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.78.1.2020	88	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.81.2.2020	89	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.82.1.2020	90	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.86.1.2020	91	2020	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.MI.127	92	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.128	93	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.130	94	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.131	95	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.132	96	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.134	97	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.135	98	2020	Michigan	<i>F. graminearum</i>
US.FHB.Wheat.MI.137.1.2020	99	2020	Michigan	<i>F. graminearum</i>
US.FHB.KS.142.2009	100	2009	Kansas	<i>F. graminearum</i>
US.FHB.KS.144.2009	101	2009	Kansas	<i>F. graminearum</i>
US.FHB.KS.147.2020	102	2020	Kansas	<i>F. graminearum</i>
US.FHB.KS.148.2020	103	2020	Kansas	<i>F. graminearum</i>
US.FHB.Wheat.NY.155.1996	104	1996	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.158.2007	105	2007	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.162.2008	107	2008	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.163.2011	108	2011	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.173.2014	109	2014	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.174.2014	110	2014	New York	<i>F. graminearum</i>
US.FHB.Wheat.NY.176	111	2020	New York	<i>F. graminearum</i>
US.FHB.Wheat.IL.67.1.2020	112	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.93.1	113	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.95.1	114	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.100.1	115	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.101.1	116	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.117.1	118	2020	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.120.1	119	2020	Illinois	<i>F. graminearum</i>
AK.Wheat.JK.01.2020	120	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.JK.05.2020	122	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.JK.06.2020	123	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.JK.07.1.2020	124	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.SW.08.1.2020	125	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.SW.09.2020	126	2020	Arkansas	<i>F. graminearum</i>
AR.Wheat.SW.11.2.2020	127	2020	Arkansas	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.1.2021	128	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.2.2021	129	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.3.2021	130	2021	Kentucky	<i>F. graminearum</i>

US.FHB.Wheat.KY.CB.6.2021	131	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.7.2021	132	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.9.2021	133	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.12.2021	134	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.13.2021	135	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.14.2021	136	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.21.2021	137	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.23.2021	138	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.24.2021	139	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.27.2021	140	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.28.2021	141	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.29.2021	142	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.30.2021	143	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.32.2021	144	2021	Kentucky	<i>F. graminearum</i>
US.FHB.Wheat.KY.CB.33.2021	145	2021	Kentucky	<i>F. graminearum</i>
US.FHB.KS.GH.1.2021	146	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.WS.2.2021	147	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.WS.4.2021	148	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.WH.9.2021	149	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.GE.5.2021	150	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.TH.6.2021	152	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.TH.7.2021	153	2021	Kansas	<i>F. graminearum</i>
US.FHB.KS.TH.8.2021	154	2021	Kansas	<i>F. graminearum</i>
US.FHB.IL.SX.1.2021	156	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.3.2021	157	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.4.2021	158	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.5.2021	159	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.7.2021	160	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.9.2021	161	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.10.2021	162	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.11.2021	163	2021	Illinois	<i>F. graminearum</i>
US.FHB.IL.SX.12.2021	164	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.2.1.2021	165	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.3.2021	166	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.4.1.2021	167	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.4.2.2021	168	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.6.2021	169	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.7.2021	170	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.9.2021	171	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.11.2021	172	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.13.2021	173	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.15.2021	174	2021	Illinois	<i>F. graminearum</i>

US.FHB.Wheat.IL.NK.17.1.2021	175	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.17.2.2021	176	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.18.2021	177	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.19.2021	178	2021	Illinois	<i>F. graminearum</i>
US.FHB.Wheat.ND.2.3.2021	179	2021	North Dakota	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.4.1.2021	182	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.2.3.2021	183	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IL.NK.16.2021	184	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.2.2021	185	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.3.1.2021	186	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.3.2.2021	187	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.4.2.2021	188	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.7.1.2021	189	2021	Indiana	<i>F. graminearum</i>
US.FHB.Wheat.IN.SS.7.2.2021	190	2021	Indiana	<i>F. graminearum</i>

Table 2.2: Mean EC50 Values of *F. graminearum* isolates to pydiflumetofen split by year and state

State	Mean EC50 Values ($\mu\text{g/mL}$)		
	Historic	2020	2021
Arkansas		0.479	
Delaware		0.479	
Illinois		0.309	0.344
Indiana			0.395
Kansas	0.192	0.277	0.351
Kentucky		0.491	0.390
Louisiana		0.732	
Maryland		0.429	
Michigan		0.279	
Nebraska		0.439	
New York	0.443	0.182	
North Carolina		0.199	
North Dakota			0.301
Ohio		0.569	
South Dakota		0.535	
Wisconsin		0.275	

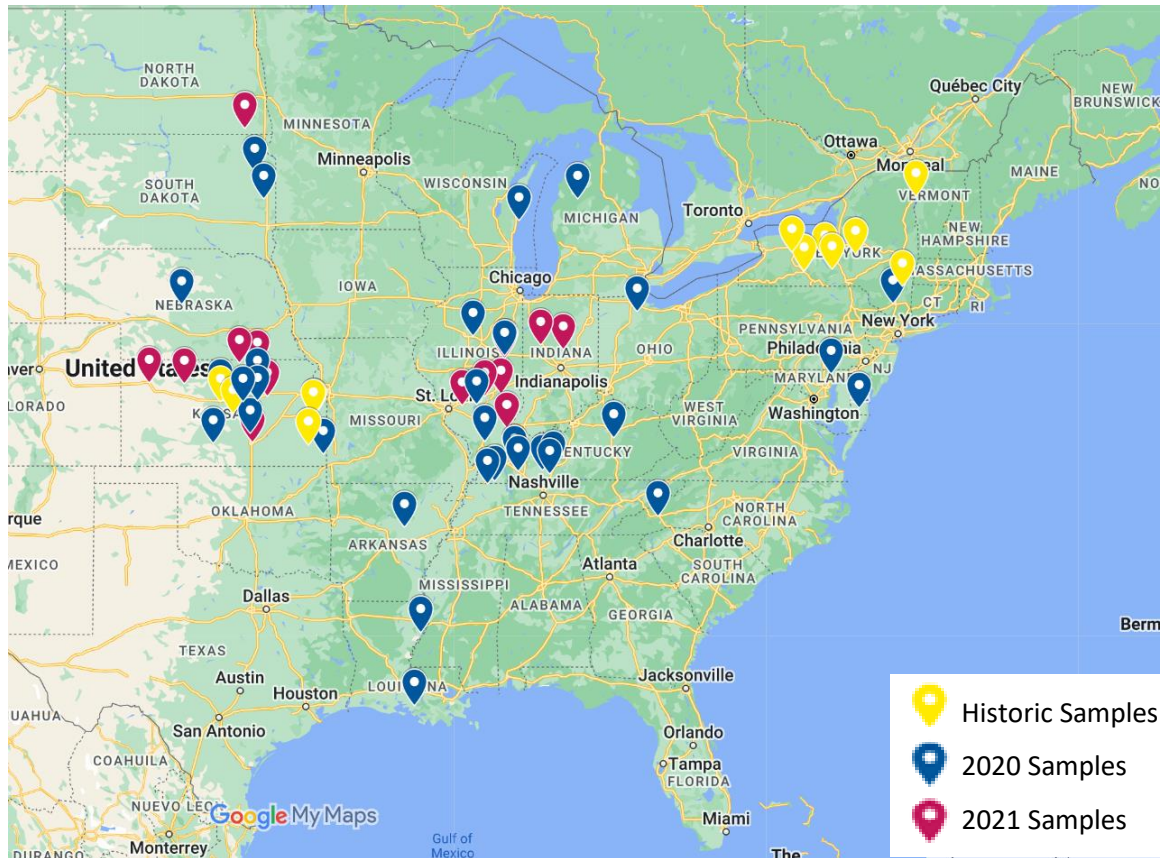


Figure 2.1: *Fusarium* isolate collection locations from 1991-2014 (yellow), 2020 (blue), and 2021 (red) used to determine baseline fungicide sensitivity to active ingredient pydiflumetofen.

Historic Isolate Mean EC50

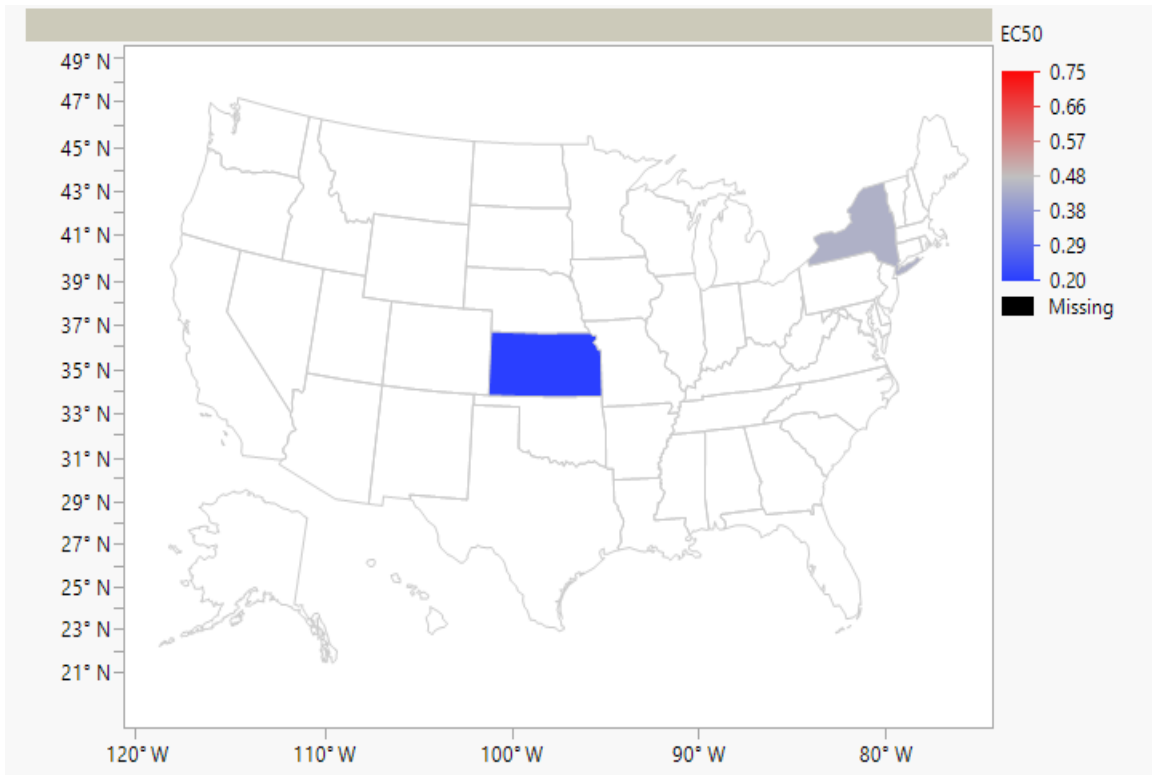


Figure 2.2: Pydiflumetofen baseline sensitivities of Historic *Fusarium graminearum* isolates collected between 1991-2014 in the states of New York and Kansas.

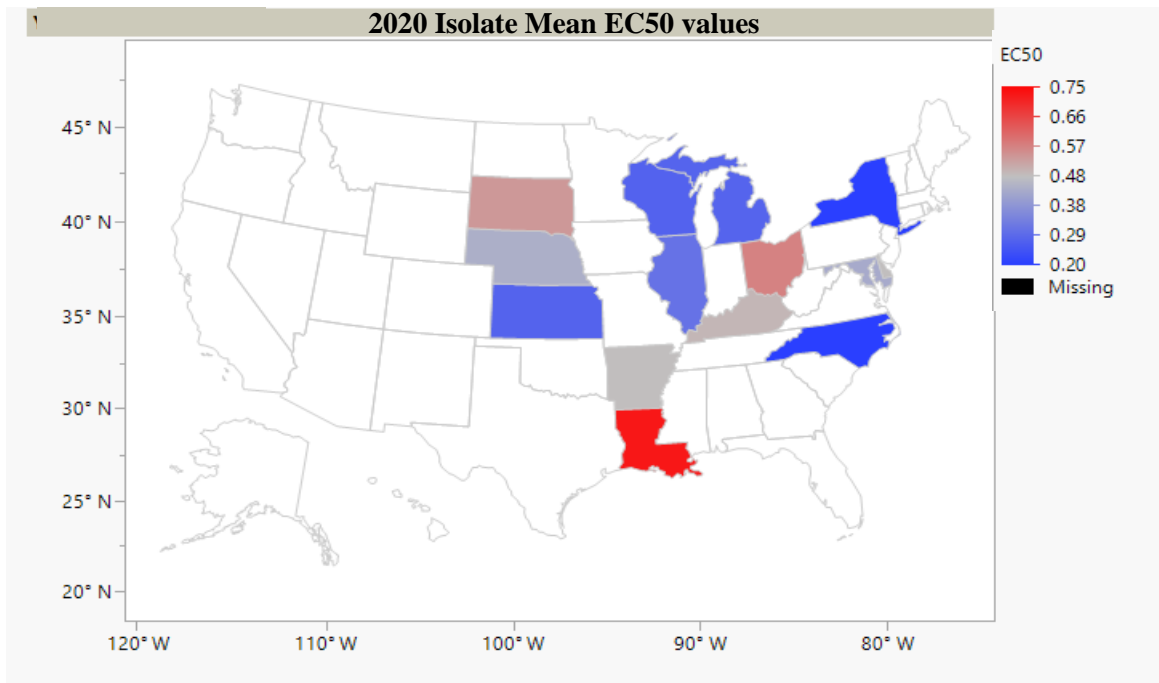


Figure 2.3: Pydiflumetofen baseline sensitivities of 2020 *Fusarium graminearum* isolates collected in states Arkansas, Delaware, Illinois, Kansas, Kentucky, Louisiana, Maryland, Michigan, Nebraska, New York, North Carolina, Ohio, South Dakota, and Wisconsin.

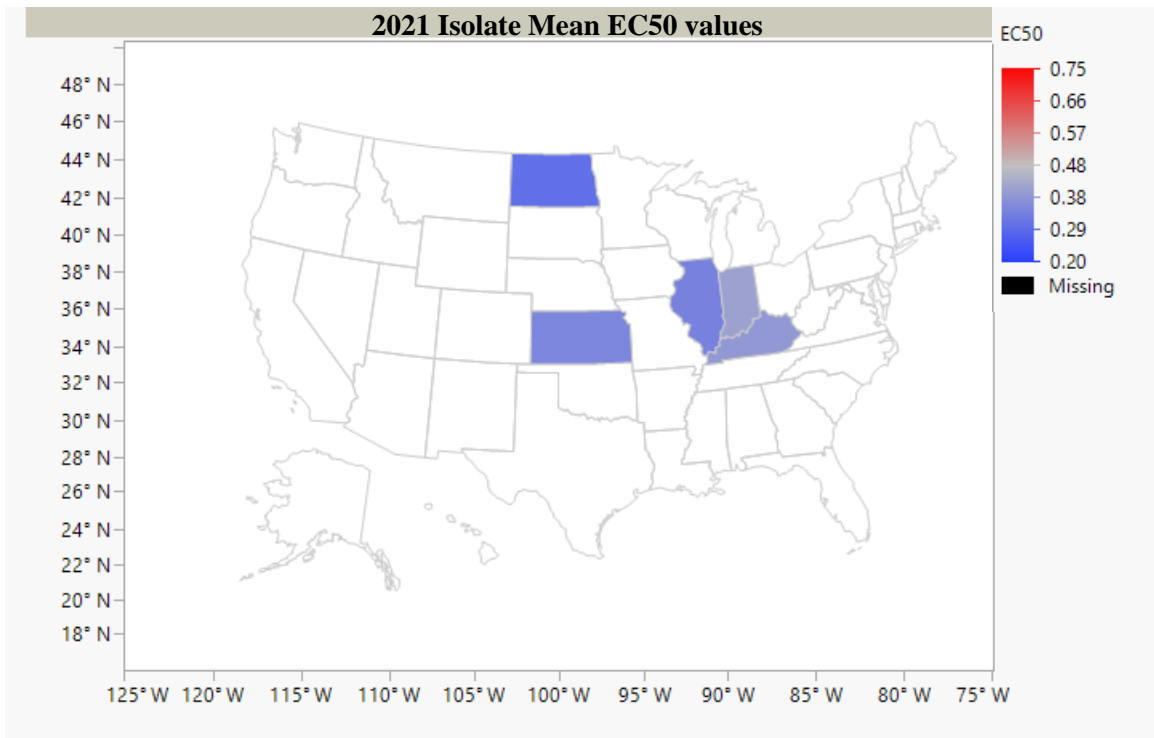


Figure 2.4: Pydiflumetofen baseline sensitivities of 2021 *Fusarium graminearum* isolates collected in states Illinois, Indiana, Kansas, Kentucky, and North Dakota

Year	State	Concentration (µg/mL)						EC50 (µg/mL)
		0 ug/mL	0.01 ug/mL	0.05 ug/mL	0.25 ug/mL	1.0 ug/mL	5.0 ug/mL	
Historic	Kansas							0.044
	New York							1.645
2020	Wisconsin							0.006
	Nebraska							1.789
2021	Indiana							0.01
	Indiana							1.453

Figure 2.5: Minimum and maximum EC50 baseline sensitivity values of individual *Fusarium graminearum* isolates collected from each year period; Historic (1991-2014), 2020, and 2021. From left to right Potato Dextrose Agar plates amended with pydiflumetofen to final concentrations of 0 µg/mL (Control), 0.01 µg/mL, 0.05 µg/mL, 0.25 µg/mL, 1.0 µg/mL, 5.0 µg/mL pydiflumetofen.

Chapter 3

BASELINE FUNGICIDE SENSITIVITY LEVELS OF *FUSARIUM* ISOLATES TO PYDIFLUMETOFEN IN EAST COAST MALTING BARLEY

Abstract

Fusarium Head Blight (FHB) is the most devastating plant disease affecting barley (*Hordeum vulgare*) production in the East Coast. While most frequently associated with *Fusarium graminearum*, recent studies in NY have shown multiple species associated with FHB in malting barley. Since 2008, demethylation inhibitors (DMI's, FRAC code 3) have been the major class of fungicide active ingredients used to control FHB but concerns of fungicide resistance developing have prompted the registration of new fungicides with additional modes of action. In 2019, pydiflumetofen, a succinate dehydrogenase inhibitor (SDHI, FRAC code 7), was labeled for control of FHB in small grains. In effort to document *Fusarium* diversity and baseline fungicide sensitivities in malting barley, isolates were collected in Delaware, Maryland, and New York during the 2021-22 growing seasons. Isolates were identified to species by amplification of Translation Elongation Factor 1 α (*TEF1*). In the preliminary set of 28 isolates, 79% were identified as *F. graminearum*, 7% *F. verticillioides*, 7% *F. acuminatum*, 3.5% *F. poae*, and 3.5% *F. asiaticum*. New York isolates were the most diverse containing four of the species recovered. An *in vitro* poison plate mycelial assay was conducted using yeast bacto agar (YBA) amended with pydiflumetofen to final concentrations of 0, 0.01, 0.05,

0.25, 1.0, and 5.0 µg/mL. A subset of *F. graminearum* isolates were also grown on PDA amended to the same concentrations for comparison of media types on mycelial growth. The effective concentration to reduce mycelial growth by 50% (EC50) was determined for each isolate and averaged by species. Average EC50 values were 1.09 µg/mL for *F. graminearum* (n=22), 1.07 µg/mL for *F. verticillioides* (n=2), 1.51 µg/mL for *F. acuminatum* (n=2), 1.09 µg/mL for *F. poae* (n=1), and 1.09 µg/mL for *F. asiaticum* (n=1). Averages of *F. graminearum* on PDA were 1.04 µg/mL (n=8). EC50 values were not impacted, but growth on YBA was more symmetric and this media is recommended for continued studies. The baseline fungicide sensitivity levels documented in this preliminary work will be expanded and used to monitor the response of *Fusarium* spp. to SDHI fungicides as use expands in malting barley.

Introduction

Fusarium Head Blight, primarily caused by *Fusarium graminearum*, is one of the most important small grain crop diseases in the world and can cause severe yield and quality losses in malting barley (McMullen et al. 1997). FHB may also be caused by several related *Fusarium* species, including *F. avenaceum*, *F. acuminatum*, *F. poae*, and *F. sporotrichioides* (Fulcher et al. 2021). FHB is most threatening to malting barley crops in the time leading up to and following anthesis (Osborne and Stein 2007) especially when anthesis coincides with warm, wet conditions conducive to infection (Goswami and Kistler, 2004). *F. graminearum* has a short generation cycle beginning in the spring, with spores traveling by wind and rain and infecting kernels on grain heads (McMullen et al.

1997). FHB can cause economic losses in malting barley production in two ways. First, FHB infection can significantly reduce the overall yield at harvest if not properly managed (Willyerd et al. 2012). Second, *F. graminearum* and its relatives can produce several mycotoxins during infection and colonization of grains (Cowger et al. 2020) which can greatly reduce grain quality, increasingly so for grain intended for brewing purposes (Bai and Shaner 2004).

Mycotoxins produced during the FHB infection cycle have been shown to induce deleterious health defects in humans and animals. Deoxynivalenol (DON), otherwise known as vomitoxin, administered at chronic low doses in animal studies induces growth abnormalities and immune suppression and administered at high doses causes vomiting, hemorrhaging, toxic shock, and death (Pestka 2010). For this reason, DON levels are closely monitored following guidelines set by the Food and Drug Administration (FDA). While DON contamination limits are set at <10 ppm DON in grain intended for cattle and chicken feed, <5 ppm DON in grain intended for swine, dairy cattle, and all other animals; and <1 ppm DON in finished grain products intended for human consumption (Food and Drug Administration, 2010), malting barley intended for brewing has an even stricter 0.5 ppm DON contamination limit (Bai and Shaner 2004). Grain contaminated with DON maybe subject to dockage and purchased at a reduced price or be rejected outright.

Small grains are most susceptible to FHB infection during anthesis when sensitive floral tissues emerge from the head. Barley cultivars grown in North America circumvent this critical infection timing by flowering when head tissues are still encapsulated in a

protective leaf sheath called the boot. Barley heads flower and self-pollinate while protected but become susceptible to disease initiation shortly thereafter once they emerge (Fernando et al. 2021). At emergence, floral tissues begin to senesce and can be colonized by fungal spores spread by wind, rain splash, or insect vectors. Infected barley florets can become discolored with dark necrotic lesions, orange-pink mycelial growth, black spots, or may bleach to tan (Tekauz et al. 2000). Infections in barley often have patchy distributions, with diseased and healthy kernels interspersed throughout the head (Tekauz et al. 2000). Infections are usually restricted to the first infected kernel, unlike in wheat with infections spreading through the head (Hoheneder 2022), and *Fusarium* damaged kernels (FDK) in barley are not as small or shriveled compared to FDK in wheat (Tekauz et al. 2000).

Barley is often designated 2-row or 6-row based on kernel arrangement on the head. Barley can be used as animal feed, in human food products, and is a major component of the brewing and distilling industries (Drakoupoulos et al. 2021). Malting barley can include both 2-row and 6-row barley, but 2-row is generally preferred by brewers due to lower protein and enzyme contents. Barley that is 6-row is most commonly used for feed. The brewing industry on the East Coast is experiencing a renaissance, creating new markets for malting barley production in the Mid-Atlantic. *Fusarium* species causing FHB in malting barley can be diverse in the Mid-Atlantic region. In New York, several ‘ancient grain’ species were cultivated to investigate FHB activity on less common *Triticum* varieties, including emmer (*T. dicoccum*), spelt (*T. spelta*), einkorn (*T. monococcum*) and a variety of *Fusarium* species were recovered. The

dominant species recovered included *F. graminearum* and *F. avenaceum* as well as *F. acuminatum*, *F. poae*, *F. sporotrichioides*, *F. armeniacum*, and members of the *F. incarnatum-equiseti* species complex (Fulcher et al. 2021). In Pennsylvania, wheat, barley, spelt, and rye (*Secale cereale*) samples were collected to investigate *Fusarium* species diversity. Although *F. graminearum* was the dominant species, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and a member of the *F. fujikuroi* species complex were also recovered (Duffeck et al. 2022). *Fusarium* diversity in malting barley has not been investigated as intensively as compared to wheat, but is merited as production becomes more widespread in the United States, especially in the context of mycotoxin regulations as *Fusarium* species can produce an array of toxins other than deoxynivalenol, the major mycotoxin produced by *F. graminearum* (Beccari et al. 2019; Cowger et al. 2020).

Triazole/demethylation inhibitor (DMI) fungicides (FRAC Group 3) fungicides function by inhibiting the proper biosynthesis of fungal membranes and have been used since 1998 to control FHB. By 2008, full federal registration was obtained for four DMI active ingredients on barley, including tebuconazole, prothioconazole, metconazole, and propiconazole, which have been the major source of chemical management of FHB since. In effort to reduce the risk of fungicide resistance, there was interest in adding new fungicides with additional modes of action to management options (Anderson et al. 2020). In 2014, the first tebuconazole-resistant *F. graminearum* isolate was identified in New York (Spolti et al. 2014) Worldwide, *F. graminearum* isolates collected between 1987 and 2004 in Europe exhibited a yearly 0.22% reduction in epoxiconazole sensitivity (Klix et al. 2007), and two *F. asiaticum* isolates and one *F. graminearum* isolate collected

in China showed resistance to the DMI products tebuconazole and prochloraz (Yin et al. 2009). Pydiflumetofen (FRAC code 7), the first succinate dehydrogenase inhibitor (SDHI) active ingredient labeled for use in small grains, was registered in 2019 as Miravis Ace (Syngenta Crop Protection Inc., Switzerland). This product is in combination with propiconazole (DMI) and is the first SDHI/DMI dual mode of action product registered for FHB management in barley. Succinate Dehydrogenase Inhibitors function by inhibiting respiration and preventing energy production in fungal cells. The pydiflumetofen trademark name is Adepidyn™ with Miravis™ Ace labeled for wheat and malting barley and Miravis™ Neo labeled for *Fusarium* ear molds in corn.

Pydiflumetofen is classified as medium to high risk for resistance development (FRAC code list 2022, www.frac.info/publications) by the fungicide resistance action committee (FRAC). Resistance to pydiflumetofen has not been documented in *Fusarium* to date, but other pathosystems developing resistance to SDHI fungicides has been observed (FRAC 2020, Peng et al. 2021). Three of the four subunits of the SDH enzyme (subunits B, C, and D) are associated with fungicide binding and have been connected with development of resistance (Peng et al. 2021). Baseline pydiflumetofen sensitivity was first screened *in vitro* in *F. asiaticum* (Hou et al. 2017), *Cercospora zea-maydis* (Neves and Bradley 2019), *Venturia inaequalis* (Ayer et al. 2019), *F. oxysporum* f. sp. *niveum* (Miller et al. 2020), *Cercospora soja* and *Corynespora cassiicola* (Neves and Bradley 2021). The first sensitivity data of *F. graminearum* to pydiflumetofen was conducted on a subset of 94 isolates from wheat, corn, dry bean, and soybean surveys originating from 2011-2017 in Michigan (Breunig and Chilvers 2021). Prior to

widespread use of the chemistry, there is need to expand observations on baseline sensitivities to encompass grain production regions across the US, particularly in malting barley. The objectives of this study were i) to investigate diversity of *Fusarium* isolated from east coast malting barley and ii) identify baseline fungicide sensitivities of *Fusarium* species isolated from malting barley to pydiflumetofen.

Materials and Methods

Isolate Collection

Isolates used in this assay were collected from malting barley fields in the east coast Mid-Atlantic region and submitted as isolate samples or as bulk grain where single spore isolations were made upon sample arrival. In 2021 samples were collected in New York and Delaware, and in 2022 samples were collected from Maryland and Delaware. All isolates were collected after the commercial release of pydiflumetofen and may have had exposure to the compound. All isolates were maintained on full-strength potato dextrose agar (PDA; Difco) amended with 0.05 mg Penicillin G (100 ug ml⁻¹) and Streptomycin sulfate (100 ug ml⁻¹) prior to assays. For long term storage, a colonized hyphal plug was transferred to a 9 cm diam Petri dish containing PDA, and four pieces of sterile 2.5 cm diameter filter paper were placed around the plug (Whatman, Global Life Sciences Solutions, USA). Fully colonized discs were removed and placed into coin envelopes for storage at -80 C.

Molecular Confirmation of Isolates

Fungal isolates used in this assay were identified to species using polymerase chain reaction (PCR) amplification of Transcription Elongation Factor 1-alpha (TEF1) (O'Donnell et al. 1998). Two 6.75 mm plugs from each isolate were transferred into 60 x 15 mm Petri dishes containing 8 ml of potato dextrose broth (PDB) (Difco) and stored at 23°C until fully colonized. Isolates showing no contamination were vacuum-aspirated through autoclave-sterilized filter paper in a Buchner funnel. Dried tissue was separated into 1.5 ml microcentrifuge tubes and stored at -20°C for DNA extraction. Genomic DNA from dried, frozen tissue was extracted by macerating tissue with 400 µl of cell lysis solution (Qiagen, Hilden, Germany) using autoclave-sterilized plastic pestles. Following the addition of 3 µl Puregene proteinase K (Qiagen) to each sample, tubes were vortexed for 20 s, and incubated at 55°C for 1 h. The supernatant of microcentrifuged samples was treated with RNase (3 µl; Qiagen) and incubated at 37°C for 30 min followed by the addition of 200 µl of protein precipitation solution (Qiagen) and 1 h incubation on ice. Tubes were then centrifuged for 5 min and supernatant was pipetted into microcentrifuge tubes containing 400 µl isopropanol to precipitate DNA. Tubes were centrifuged for 3 min, the supernatant was poured off, and the DNA pellet was washed with 200 µl ethanol. After centrifuging, the supernatant was poured off and samples were allowed to dry on a heating block before being rehydrated with 50 µl of sterile, nuclease-free water. DNA concentration and purity were measured by spectrophotometry and standardized to a concentration of 20 ng ml⁻¹ before storage at -20°C.

For each isolate, the elongation factor 1 α gene region was amplified using 50 μ l PCR reactions that consisted of 10 X DreamTaq Master Mix, 10 μ M of forward and reverse primer: EF1 (5'-ATG GGT AAG GAG GAC AAG AC-3') and EF2 (5'-GGA AGT ACC AGT GAT CAT G-3') and (Cerón-Bustamante et al. 2018) and 20 ng of genomic DNA. Thermal cycling parameters were set as: 95°C for 3 min followed by 35 cycles of DNA denaturation for 40 s at 95°C; primer annealing for 30 s at 55°C; and primer extension for 1 min at 72°C. A final extension step of 72°C for 10 min completed the cycle. Amplification was verified using gel electrophoresis, at 90 V for 40 min. Aliquots of amplified products were cleaned with ExoSAP-IT (USB products Affymetrix, Inc. ThermoFisher Scientific, Waltham Massachusetts). Samples were sequenced at Eurofins Genomics (Louisville, KY). Analysis of sequence data was completed with CLC Main Workbench 20.0.4 (CLC Bio: a Qiagen Company, Germany) and copied into the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) for comparison against published, identified specimens.

Mycelial Growth Assay

A poison plate mycelial assay was performed to determine EC50 values, the effective concentration to inhibit mycelial growth by 50 percent, for each isolate. Isolates were grown on amended (Streptomycin, Penicillin) PDA for seven days prior to assay plating. A 7 mm diameter plug was transferred from the leading edge of PDA-grown colonies and transferred to the center of an amended 60 x 15 mm Petri dish

(Fischerbrand, Fischer Scientific USA). Fungicide stock solutions were prepared using pydiflumetofen (Miravis, Syngenta Crop Protection Inc., Greensboro, North Carolina) added to sterile deionized water and making serial dilutions of 5000, 1000, 250, 50, and 10 µg/mL pydiflumetofen. Stock solutions were added to yeast bacto agar (YBA) cooled to approximately 55 C in a 1:1000 mL ratio to produce YBA poison plates with final pydiflumetofen concentrations of 5.0, 1.0, 0.25, 0.05, 0.01, and 0 µg/mL (Breunig and Chilvers 2021; Hou et al. 2017). The non-treated control was amended with 1 mL sterile deionized water. A subset of *Fusarium graminearum* samples were also grown on potato dextrose agar (PDA) amended to the same fungicide concentrations to compare growth responses and fungicide sensitivity on both media. Using a media pump (Digital Mini Pump Dispensing Systems, Argos Technologies, Radnor, Pennsylvania), 8 mL of amended media was allotted to each assay plate.

Once transferred, assay plates were incubated in the dark for 5 days at 24 C. Each isolate was replicated three times and the trial was performed twice on YBA and once on PDA. Mycelial growth was measured at the widest point and transverse to the widest point going through the original plug with a digital caliper (iGaging ABSOLUTE ORIGIN 0-6" Digital Electronic Caliper, iGaging, San Clemente, California). The two measurements were averaged, and the width of the original plug (7 mm) was subtracted to give the total growth. The relative growth of each isolate at each concentration was determined by dividing the total growth on a treatment plate by the total growth on the non-treated control.

$$\% \text{ Inhibition} = \left(1 - \frac{\text{diameter of control} - \text{diameter of treatment}}{\text{diameter of control}} \right) * 100.$$

Data Analysis

Mycelial EC50 values for each isolate were assessed using JMP Statistical Software. Percent inhibition was graphed against log treatment concentration and a line of best fit was generated. The line equation was set to $Y=50$ to determine the effective concentration to inhibit mycelial growth by 50%. Absolute EC50 values were estimated separately for each run and mean of the two runs presented. Data from the replicated experiments were combined and sorted by species.

Results

Molecular Confirmation of Isolates

Isolates included 6 from New York 2021, 2 from Maryland 2022, 19 from Delaware 2022, and 1 from Delaware 2021. All isolates collected from Delaware in 2022 were identified as *F. graminearum* (n=19). The single isolate from Delaware in 2021 was identified as *F. acuminatum* (n=1). New York 2021 isolates were identified to be *F. graminearum* (n=2), *F. verticillioides* (n=2), *F. acuminatum* (n=1), and *F. poae* (n=1). Maryland isolates were identified as *F. graminearum* (n=1) and *F. asiaticum* (n=1). In total, 28 isolates consisting of five *Fusarium* species were identified (Figure 3.1).

Sensitivity of Mycelial Growth to Pydiflumetofen

Yeast Bacto Agar (YBA) was amended with pydiflumetofen to final concentrations of 5.0, 1.0, 0.25, 0.05, 0.01, and 0 $\mu\text{g/mL}$ and 28 *Fusarium* isolates were cultured to determine EC50 values by species (Figure 3.2). For *F. graminearum* (n=22),

mean EC50 values were 1.09 µg/mL and ranged from 1.05 µg/mL to 1.25 µg/mL. The *F. verticillioides* (n=2) mean EC50 value was 1.07 µg/mL and ranged from 1.067 to 1.073 µg/mL. *F. acuminatum* (n=2) isolate EC50's averaged 1.51 µg/mL and ranged from 1.42 µg/mL to 1.61 µg/mL. The EC50 values of *F. poae* (n=1) and *F. asiaticum* (n=1) were both 1.09 µg/mL.

Comparison of Media Types

A subset of eight *F. graminearum* isolates from Delaware were grown on Potato Dextrose Agar (PDA) at the same pydiflumetofen concentrations to investigate the effect of media on growth morphology and if percent inhibition varied by media. The average EC50 value on PDA plates was 1.04 µg/mL. The average EC50 value of the *F. graminearum* subset on YBA was 1.08 µg/mL and the average EC50 value of all *F. graminearum* isolates tested on YBA was 1.09 µg/mL. While the mean EC50 values of YBA- and PDA- were similar, isolates grown on YBA were whiter in color and had more symmetric growth than on PDA where isolates had a classic pink mycelial mat and grew asymmetrically (Figure 3.3).

Discussion

In this preliminary study, baseline sensitivities of several *Fusarium* species to pydiflumetofen were determined. *Fusarium* isolates tested were diverse, representing five FHB-causing species active on the East Coast of the United States. New York isolates were the most diverse, containing four of the five species identified in this study. Of the

28 isolates assayed, 79% were identified as *F. graminearum*, 7% *F. verticillioides*, 7% *F. acuminatum*, 3.5% *F. poae*, and 3.5% *F. asiaticum*. *F. acuminatum* isolates were collected in 2021 from New York and Delaware and had the highest EC50 values of 1.42 and 1.61 $\mu\text{g/mL}$, respectively. Yearly differences in environmental conditions can select for different FHB-causing *Fusarium* species as well as influence disease pressures experienced in fields. For instance, during the Delaware 2021 growing season, weather conditions were very hot and dry during barley flowering and kernel development resulting in suppressed- almost non-existent- disease pressures with few *Fusarium* samples recovered that year and a single *F. acuminatum* isolate identified. The 2022 growing season, in comparison, had weather conditions more conducive to disease development resulting in higher disease pressures and an abundance of *Fusarium* samples recovered with numerous *F. graminearum* isolates identified. *F. graminearum*, *F. asiaticum*, and *F. poae* mean EC50 values were all 1.09 $\mu\text{g/mL}$. The highest *F. graminearum* isolate EC50 value was 1.25 $\mu\text{g/mL}$ from New York. *F. asiaticum* and *F. poae* were each represented by a single isolate, collected from Maryland in 2022 and New York in 2021, respectively. *F. verticillioides* had the lowest mean EC50 value of all the species represented, 1.07 $\mu\text{g/mL}$. All isolates of all species screened were sensitive to pydiflumetofen, with individual EC50 values ranging from 1.05 $\mu\text{g/mL}$ to 1.61 $\mu\text{g/mL}$. Studies performed on *F. graminearum* isolated from wheat, corn, dry bean, and soybean in Michigan had mean EC50 values of 0.06 $\mu\text{g/mL}$ (Breunig and Chilvers 2021). Closely related *F. asiaticum* isolates collected from wheat in China were also found to be sensitive to pydiflumetofen with mycelial with average EC50 values of 0.0745 $\mu\text{g/mL}$ or

below (Hou et al. 2017). EC50 values generated in this study were higher than previously reported pydiflumetofen sensitivities; 1.09 µg/mL compared to 0.06 µg/mL for *F. graminearum* and 0.0745 µg/mL for *F. asiaticum*, which may warrant further investigation.

Fusarium species identified in this study included members of the *Fusarium graminearum* species complex (FGSC) *F. graminearum* and *F. asiaticum* which can be grouped into the larger *Fusarium sambucinum* species complex (FSAMSC) along with *F. poae*. *F. acuminatum* is a member of the *Fusarium triticum* species complex (FTSC) and *F. verticillioides* is a member of the *Fusarium fujikuroi* species complex (FFSC). Members of the FGSC can produce type B trichothecenes deoxynivalenol (DON) and its derivative 15A-DON and 3A-DON, nivalenol (NIV), type A trichothecene 3 α -acetoxy-7 α ,15-dihydroxy-12,13-epoxytrichothec-9-ene (NX-2), and zearalenone (ZEA) and fumonisins. (Langevin et al. 2004; Varga et al. 2015; Valverde-Bogantes et al. 2019; Cowger et al. 2020). *F. poae* can produce NIV and type A trichothecenes T-2 and HT-2 as well as beauvericin (BEA) and enniatins (ENNs) (Beccari et al. 2019). *F. verticillioides* can produce BEA, moniliformin (MON), ENNs, and fumonisins (Duffeck et al. 2022) and *F. acuminatum* can produce ENNs and MON (Beccari et al. 2019). Although a range of mycotoxin contaminants can be produced within United States malting barley production regions, DON is the only one regularly tested at grain intake facilities (Cowger et al. 2020).

A subset *F. graminearum* isolates were grown on both YBA and PDA to investigate differences in growth morphology and effect of media on pydiflumetofen

sensitivity. Isolates grown on YBA were more symmetric allowing for improved consistency in lateral mycelial growth values than asymmetric isolates grown on PDA. The improved measurement of YBA plates coupled with the lack of media effect on EC50 values make YBA a more desirable media for use in future studies. YBA has been used previously in fungicide sensitivity assays to assess baseline sensitivity of *Botrytis cinerea* to SDHI products boscalid, penthiopyran, and fluopyram to (Amiri et al. 2014).

DMI products have been the major source of FHB chemical control since they were first registered in 2006. In the past, fungicide resistance to DMI products posed significant risk to food safety and security (Morton and Staub 2008) as they were once the only chemical control mechanism available. The SDHI pydiflumetofen was first registered for control of FHB in 2019, and use has rapidly expanded. In 2018, this active ingredient was also registered for use in several field and vegetable crop products. In combining multiple modes of action, the SDHI/DMI Miravis Ace reduces the risk of pathogens developing resistance because multiple forms of resistance would need to arise before field efficacy is lost. Resistance development can also be slowed by rotating application of fungicides of the same or different chemical class. Alternating between SDHI products and DMI products can help decrease the risk of fungicide resistance developing. Even with fungicides of the same chemical class the actual target, or method of inhibition, can vary. Another recently released combination product is Prosaro Pro (Bayer Cropscience, Research Triangle Park, NC) that combines the SDHI fluopyram with DMI's prothioconazole and tebuconazole. There have been multiple reports in the past of pathogens developing resistance and cross-resistance to SDHI fungicides (FRAC

2020, Peng et al. 2021). SDHI-resistant pathogens that have emerged in small grain production systems include *Mycosphaerella graminicola* (Leaf Spot of Wheat), *Pyrenophora teres* (Net Blotch of barley), *Ramularia collo-cygni* (Leaf Spot of Barley), and *Ustilago nuda* (Loose Smut of Barley), and *U. maydis* (Corn Smut) (FRAC 2020). Future studies should investigate the baseline sensitivity of *Fusarium* species to pydiflumetofen and fluopyram to track baseline sensitivities and identify the development of resistance and cross-resistance.

Baseline sensitivity to pydiflumetofen for *F. graminearum*, *F. acuminatum*, *F. verticillioides*, *F. poae*, and *F. asiaticum* has been generated in this preliminary assay of *Fusarium* species derived from malting barley. Continued identification and assessment of *Fusarium* active in the Mid-Atlantic small grain production region can be used to characterize *Fusarium* diversity and record fungicide sensitivity in our region to inform researchers and growers on best management practices for *Fusarium* head blight, track fungicide efficacy, and identify any reductions in sensitivity as they emerge.

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REFERENCES

- Amiri, A., Zuniga, A. I., Mertely, J., & Peres, N. A. (2014). First report on resistance to Pyraclostrobin, thiophanate-methyl, fenhexamid and boscalid in *Botrytis cinerea* from eucalyptus seedlings in Florida greenhouses. *Plant Disease*, 98(6), 851–851. <https://doi.org/10.1094/pdis-10-13-1107-pdn>
- Anderson, N. R., Freije, A. N., Bergstrom, G. C., Bradley, C. A., Cowger, C., Faske, T., Hollier, C., Kleczewski, N., Padgett, G. B., Paul, P., Price, T., & Wise, K. A. (2020). Sensitivity of *Fusarium graminearum* to metconazole and tebuconazole fungicides before and after widespread use in wheat in the United States. *Plant Health Progress*, 21(2), 85–90. <https://doi.org/10.1094/php-11-19-0083-rs>
- Breunig, M., & Chilvers, M. I. (2021). Baseline sensitivity of *Fusarium graminearum* from wheat, corn, dry bean and soybean to Pydiflumetofen in Michigan, USA. *Crop Protection*, 140, 105419. <https://doi.org/10.1016/j.cropro.2020.105419>
- Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to fusarium head blight. *Annual Review of Phytopathology*, 42(1), 135–161. <https://doi.org/10.1146/annurev.phyto.42.040803.140340>
- Beccari, G., Arellano, C., Covarelli, L., Tini, F., Sulyok, M., & Cowger, C. (2019). Effect of wheat infection timing on fusarium head blight causal agents and secondary metabolites in grain. *International Journal of Food Microbiology*, 290, 214–225. <https://doi.org/10.1016/j.ijfoodmicro.2018.10.014>
- Cerón-Bustamante, M., Ward, T. J., Kelly, A., Vaughan, M. M., McCormick, S. P., Cowger, C., Leyva-Mir, S. G., Villaseñor-Mir, H. E., Ayala-Escobar, V., & Nava-Díaz, C. (2018). Regional differences in the composition of fusarium head blight pathogens and mycotoxins associated with wheat in Mexico. *International Journal of Food Microbiology*, 273, 11–19. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.003>
- Cowger, C., Ward, T. J., Nilsson, K., Arellano, C., McCormick, S. P., & Busman, M. (2020). Regional and field-specific differences in *Fusarium* species and mycotoxins associated with blighted North Carolina Wheat. *International Journal of Food Microbiology*, 323, 108594. <https://doi.org/10.1016/j.ijfoodmicro.2020.108594>
- Drakopoulos, D., Sulyok, M., Jenny, E., Kägi, A., Bänziger, I., Logrieco, A. F., Krska, R., & Vogelgsang, S. (2021). Fusarium head blight and associated mycotoxins in grains and straw of barley: Influence of agricultural practices. *Agronomy*, 11(4). <https://doi.org/10.3390/agronomy11040801>

- Duffeck, M. R., Bandara, A. Y., Weerasooriya, D. K., Collins, A. A., Jensen, P. J., Kuldau, G. A., Del Ponte, E. M., & Esker, P. D. (2022). Fusarium head blight of small grains in Pennsylvania: Unravelling species diversity, toxin types, growth, and triazole sensitivity. *Phytopathology*, 112(4), 794–802. <https://doi.org/10.1094/phyto-02-21-0070-r>
- FDA. (2010). Guidance on advisory levels for deoxynivalenol (Don) in finished wheat. U.S. Food and Drug Administration. Retrieved September 25, 2022, from <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-fda-advisory-levels-deoxynivalenol-don-finished-wheat-products-human>
- Fernando, W. G. D., Oghenekaro, A. O., Tucker, J. R., & Badea, A. (2021). Building on a foundation: Advances in epidemiology, resistance breeding, and forecasting research for reducing the impact of fusarium head blight in wheat and Barley. *Canadian Journal of Plant Pathology*, 43(4), 495–526. <https://doi.org/10.1080/07060661.2020.1861102>
- FRAC. 2020. List of first confirmed cases of plant pathogenic organisms resistant to disease control agents. https://www.frac.info/docs/default-source/publications/list-of-resistant-plant-pathogens/list-of-first-confirmed-cases-of-plant-pathogenic-organisms-resistant-to-disease-control-agents_05_2020.pdf?sfvrsn=7073499a_2
- Fulcher, M. R., Winans, J. B., Benscher, D., Sorrells, M. E., & Bergstrom, G. C. (2021). Triticum varieties grown as ‘ancient grains’ in New York differ in susceptibility to fusarium head blight and harbor diverse *Fusarium* flora. *European Journal of Plant Pathology*, 159(3), 693–699. <https://doi.org/10.1007/s10658-020-02183-7>
- Goswami RS, Kistler HC. Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, 2004 Nov 1;5(6):515-25. Doi: 10.1111/j.1364-3703.2004.00252.x. PMID: 20565626.
- Hoheneder, F., Biehl, E. M., Hofer, K., Petermeier, J., 84rowth, J., Herz, M., Rychlik, M., Heß, M., & Hückelhoven, R. (2022). Host genotype and weather effects on fusarium head blight severity and mycotoxin load in spring barley. *Toxins*, 14(2), 125. <https://doi.org/10.3390/toxins14020125>
- Hou, Y.-P., Mao, X.-W., Wang, J.-X., Zhan, S.-W., & Zhou, M.-G. (2017). Sensitivity of *Fusarium asiaticum* to a novel succinate dehydrogenase inhibitor fungicide pydiflumetofen. *Crop Protection*, 96, 237–244. <https://doi.org/10.1016/j.cropro.2017.02.011>

- Klix, M. B., Verreet, J.-A., & Beyer, M. (2007). Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development. *Crop Protection*, 26(4), 683–690. <https://doi.org/10.1016/j.cropro.2006.06.006>
- Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during fusarium head blight development in six cereal species. *European Journal of Plant Pathology*, 110(7), 735–746. <https://doi.org/10.1023/b:ejpp.0000041568.31778.ad>
- McMullen M, Jones R, Gallenberg D. Scab of Wheat and Barley: A Re-emerging Disease of Devastating Impact. *Plant Disease*, 1997 Dec;81(12):1340-1348. Doi: 10.1094/PDIS.1997.81.12.1340. PMID: 30861784.
- Morton, V. and Staub, T. 2008 A Short History of Fungicides. Online, APSnet Features. Doi: 10.1094/APSnetFeature-2008-0308.
- O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., Broders, K., Kim, H.-S., McCormick, S. P., Busman, M., Aoki, T., Torres-Cruz, T. J., & Geiser, D. M. (2022). DNA sequence-based identification of *Fusarium*: A work in progress. *Plant Disease*, 106(6), 1597–1609. <https://doi.org/10.1094/pdis-09-21-2035-sr>
- Osborne LE, Stein JM. Epidemiology of Fusarium head blight on small-grain cereals. *International Journal of Food Microbiology*, 2007 Oct 20;119(1-2):103-8. Doi: 10.1016/j.ijfoodmicro.2007.07.032. Epub 2007 Jul 31. PMID: 17716761.
- Peng, J., Sang, H., Proffer, T. J., Gleason, J., Outwater, C. A., Jung, G., & Sundin, G. W. (2021). A method for the examination of SDHI fungicide resistance mechanisms in phytopathogenic fungi using a heterologous expression system in *Sclerotinia sclerotiorum*. *Phytopathology*, 111(5), 819–830. <https://doi.org/10.1094/phyto-09-20-0421-r>
- Pestka JJ. (2010). Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Archives of Toxicol.* 2010 Sep;84(9):663-79. doi: 10.1007/s00204-010-0579-8. Epub 2010 Aug 27. PMID: 20798930.
- Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., & Bergstrom, G. C. (2014). Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York Wheat and competitiveness of a tebuconazole-resistant isolate. *Plant Disease*, 98(5), 607–613. <https://doi.org/10.1094/pdis-10-13-1051-re>

- Tekauz, A., McCallum, B., & Gilbert, J. (2000). Review: Fusarium head blight of Barley in western Canada. *Canadian Journal of Plant Pathology*, 22(1), 9–16. <https://doi.org/10.1080/07060660009501156>
- Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., & Hallen-Adams, H. E. (2019). Recent population changes of fusarium head blight pathogens: Drivers and implications. *Canadian Journal of Plant Pathology*, 42(3), 315–329. <https://doi.org/10.1080/07060661.2019.1680442>
- Varga, E., Wiesenberger, G., Hametner, C., Ward, T. J., Dong, Y., Schöfbeck, D., McCormick, S., Broz, K., Stückler, R., Schuhmacher, R., Krska, R., Kistler, H. C., Berthiller, F., & Adam, G. (2015). New tricks of an old enemy: Isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. *Environmental Microbiology*, 17(8), 2588–2600. <https://doi.org/10.1111/1462-2920.12718>
- Willyerd, K. T., Li, C., Madden, L. V., Bradley, C. A., Bergstrom, G. C., Sweets, L. E., McMullen, M., Ransom, J. K., Grybauskas, A., Osborne, L., Wegulo, S. N., Hershman, D. E., Wise, K., Bockus, W. W., Groth, D., Dill-Macky, R., Milus, E., Esker, P. D., Waxman, K. D., Paul, P. A. (2012). Efficacy and stability of integrating fungicide and cultivar resistance to manage fusarium head blight and deoxynivalenol in wheat. *Plant Disease*, 96(7), 957–967. <https://doi.org/10.1094/pdis-09-11-0763>
- Yin, Y., Liu, X., Li, B., & Ma, Z. (2009). Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. *Phytopathology*®, 99(5), 487–497. <https://doi.org/10.1094/phyto-99-5-0487>

Table 3.1: List of 28 *Fusarium* isolates collected in barley used in baseline

pydiflumetofen sensitivity assay, including assay ID, year and state of collection, species, and GenBank accession number.

Isolate ID	Year Collected	State	Species
1L- <i>F. graminearum</i>	2021	New York	<i>F. graminearum</i>
1I	2021	New York	<i>F. verticillioides</i>
9A- <i>F. graminearum</i>	2021	New York	<i>F. graminearum</i>
10H	2021	New York	<i>F. verticillioides</i>
13B	2021	New York	<i>F. acuminatum</i>
8H- <i>F. poae</i>	2021	New York	<i>F. poae</i>
MD.Bar1.7.1.2022	2022	Maryland	<i>F. graminearum</i>
MD.Bar1.8.1.2022	2022	Maryland	<i>F. asiaticum</i>
Con.Bar1.14.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar1.20.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar1.54.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar2.8.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar2.39.2.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar2.48.1.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar4.10.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar4.15.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar5.4.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar5.19.2.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar5.32.1.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar6.9.1.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar6.15.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar7.2.1.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar7.32.1.2022	2022	Delaware	<i>F. graminearum</i>
Con.Bar7.32.2.2022	2022	Delaware	<i>F. graminearum</i>
Lewes.Malt.5.2.2022	2022	Delaware	<i>F. graminearum</i>
Lewes.6R.5.3.2022	2022	Delaware	<i>F. graminearum</i>
Lewes.6R.6.3.2022	2022	Delaware	<i>F. graminearum</i>
TwinBar.39.2021	2021	Delaware	<i>F. acuminatum</i>

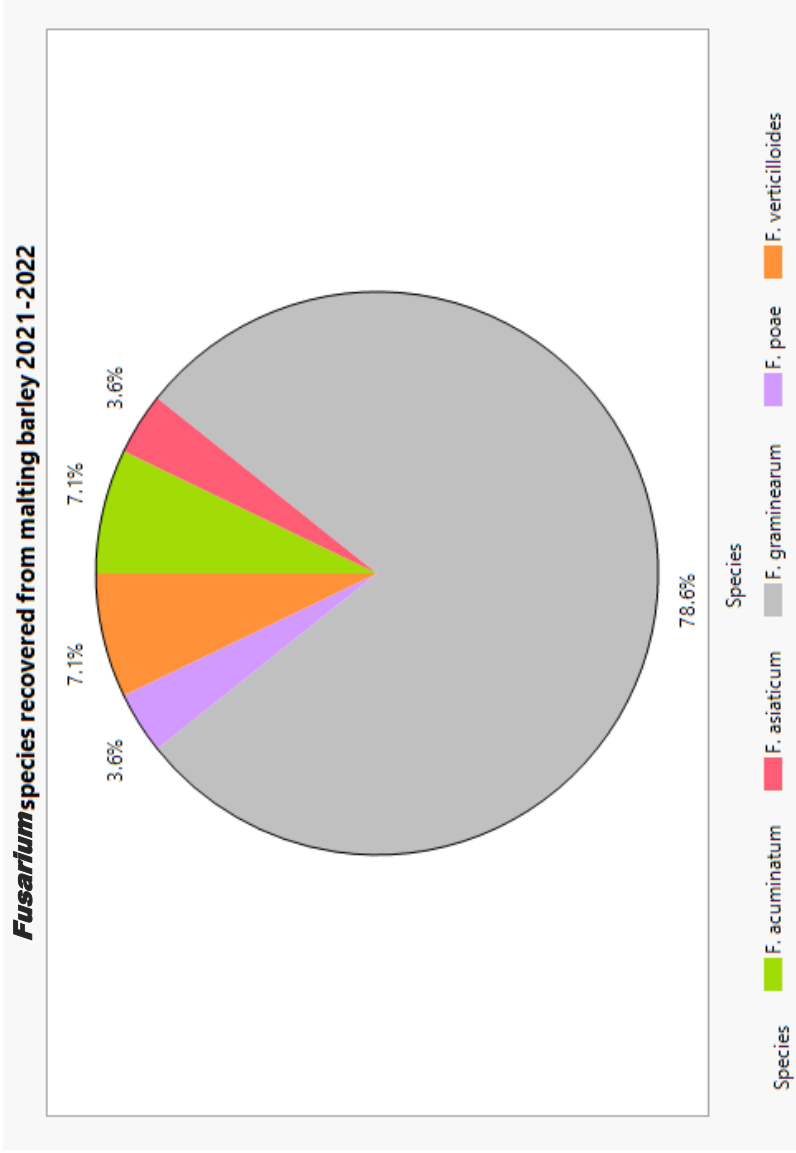


Figure 3.1: Composition of 28 *Fusarium* species tested in baseline pydiflumetofen sensitivity assay from isolates collected from malting barley in New York, Maryland, and Delaware.

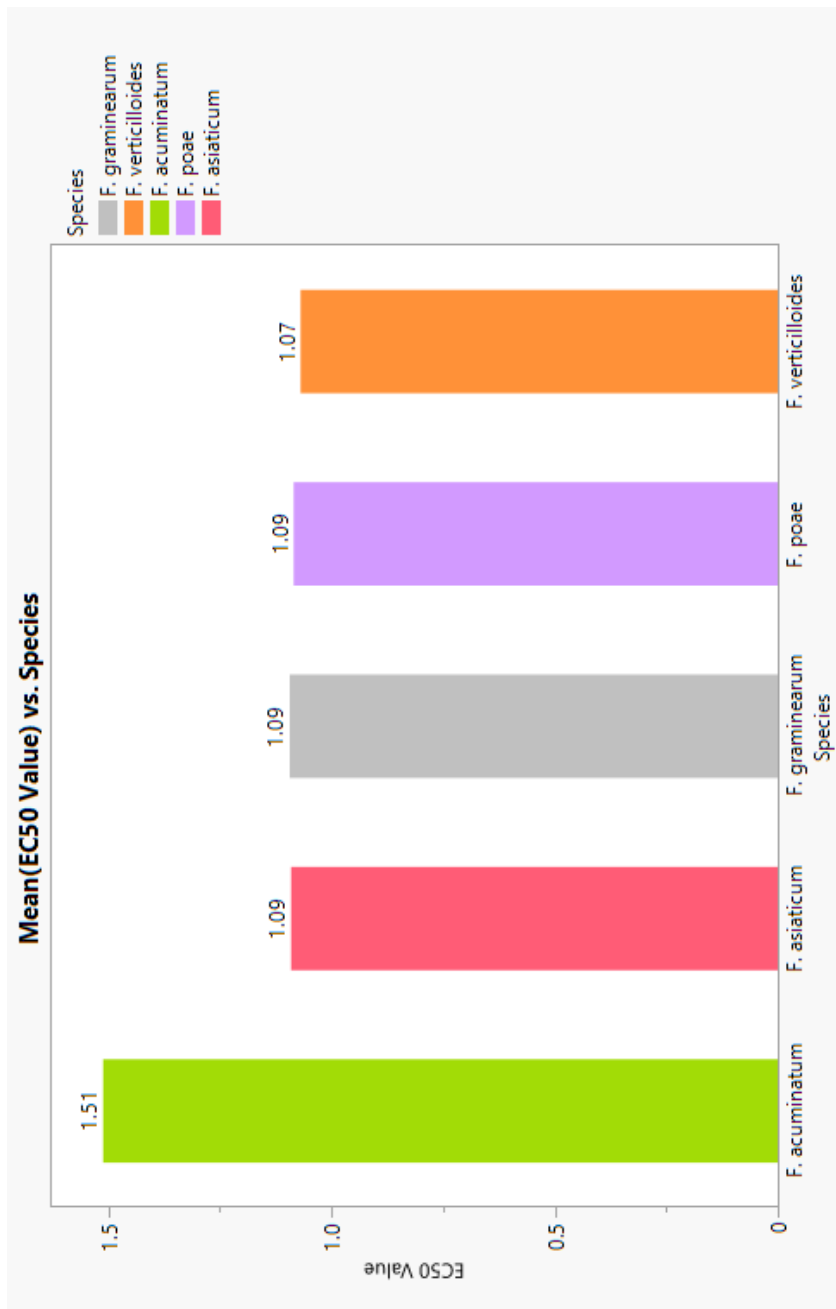


Figure 3.2: Mean EC50 baseline sensitivity value of *Fusarium* isolates from malting barley to pydiflumetofen on amended Yeast Bacto Agar.

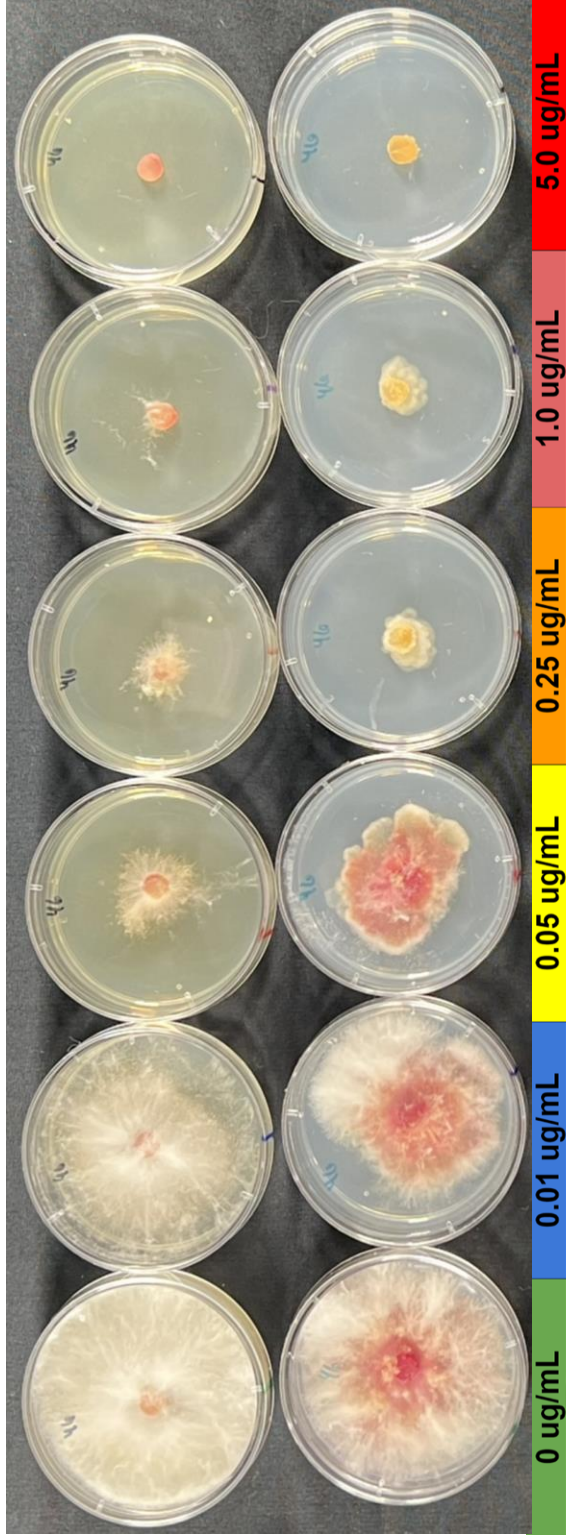


Figure 3.3: Comparison of a *Fusarium graminearum* isolate from malting barley growing on Yeast Bacto Agar (top) and Potato Dextrose Agar (bottom). From left to right; 0 $\mu\text{g/mL}$ (Control), 0.01 $\mu\text{g/mL}$, 0.05 $\mu\text{g/mL}$, 0.25 $\mu\text{g/mL}$, 1.0 $\mu\text{g/mL}$, 5.0 $\mu\text{g/mL}$ pycnidium.

Appendix A

**ASSESSMENT OF FUNGICIDES AND APPLICATION TIMINGS OF BARLEY
HEAD AND LEAF DISEASES IN GEORGETOWN, DE, 2020.**

Published in *Plant Disease Management Network*

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Barley (*Hordeum vulgare*; 'Violetta')
Fusarium head blight (*Fusarium graminearum*)
Septoria nodorum blotch (*Parastagonospora nodorum*)

Assessment of fungicides and application timings of barley head and leaf diseases in Georgetown, DE, 2020

Malting barley 'Violetta' was grown at the Carvel Research and Education Center in Georgetown, DE to evaluate the effectiveness of fungicides applied at various timings for disease control and impact on yield. Barley was planted on 4 Oct 2019 into corn residue in Rosedale loamy sand. The experimental design was a randomized block with five replicates. Plots were 22 feet long and 10 feet wide with alleys five feet wide. Production practices outlined by the University of Delaware Cooperative Extension Service were followed. Treatments included a non-treated control and five treatments of Miravis Ace 2.3 SE, Caramba 90 EC, and Prosaro 421 SC applied at different timings. Fungicide timings included application at half head visible (Feekes 10.3/Zadoks 55) on 14 Apr, at anthesis (Feekes 10.5.1/Zadoks 59) on 19 Apr, and four days after anthesis on 23 Apr. Applications were made using a CO₂ backpack sprayer equipped with Turbo TwinJet 11002 nozzles calibrated to deliver 20 GPA at 30 psi. Natural sources of inoculum were relied upon for disease development. Fusarium head blight (FHB) and Septoria nodorum blotch (SNB) were each rated for incidence (% of 20 barley heads showing symptoms per plot) and severity (amount of head covered with symptoms averaged across 20 barley heads per plot) on 26 May 2020. Barley flag leaves were also rated for SNB incidence (% of 20 flag leaves showing symptoms per plot) and severity (% of flag leaf covered in lesions averaged across 20 flag leaves per plot) on 18 May. Plots were harvested 10 Jun and yields were corrected to 13.5% moisture. Deoxynivalenol (DON) concentrations were obtained from grain subsampled from each plot. Disease and yield data were analyzed using ANOVA and means were separated using Fisher's least significant difference ($\alpha = .05$).

Environmental conditions were conducive to FHB and SNB disease development. All fungicide treatments and timings reduced FHB incidence and severity when compared to the control and did not significantly vary among treatments. All treatments and timings significantly reduced the incidence and severity of SNB lesions on the flag leaf. DON concentrations were less than 0.2 ppm in all treatments. SNB incidence in grain heads was reduced for all treatments. All treatments except Caramba applied at anthesis reduced SNB severity in grain heads compared to the control. All treatments and timings had higher yield than the control. Phytotoxicity was not observed in any treatment.

Treatment and Rate/A ^z	Growth Stage at Application (Feekes/Zadoks)	FHB Incidence ^y	FHB Severity	SNB Leaf Lesion Incidence	SNB Leaf Lesion Severity	SNB Incidence	SNB Severity	Yield (bu/A) ^x
Caramba 90 EC, 13.5 oz	Anthesis (10.5.1/59)	19.0 a	0.31 a	63.0 b	7.0 b	63.0 c	2.3 bc	96.9 b
Miravis Ace 2.3 SE, 13.7 oz	Half Head Visible (10.3/55)	12.0 a	0.15 a	18.0 a	2.5 ab	54.0 bc	1.1 a	102.1 b
Miravis Ace 2.3 SE, 13.7 oz	Anthesis (10.5.1/59)	16.0 a	0.23 a	19.0 a	0.9 ab	38.0 ab	0.8 a	105.6 ab
Miravis Ace 2.3 SE, 13.7 oz	4 Days After Anthesis	16.0 a	0.20 a	40.0 ab	4.0 ab	37.0 ab	0.6 a	102.9 b
Prosaro 421 SE, 6.5 oz	Anthesis (10.5.1/59)	23.0 a	0.36 a	21.0 a	1.3 ab	52.0 abc	1.4 ab	100.0 b
Control	--	53.0 b	1.17 b	100.0 c	40.2 c	86.0 d	3.1 c	86.7 c
<i>p</i> -value	--	0.0006	0.0015	0.0001	0.0001	0.0001	0.0004	0.0012
LSD ($\alpha=0.05$)	--	17.50	0.47	26.18	6.40	21.66	1.13	10.19

^z Non-ionic surfactant, Induce 90 SL, was added to all fungicide treatments at 0.125 % v/v, fb= followed by.

^y Percentage of 20 randomly selected barley heads per plot displaying Fusarium head blight symptoms on glumes. Treatments followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; $\alpha=0.05$).

^x Yields were obtained by harvesting from the center 6.6 ft of each plot and calculated by adjusting to 13.5% moisture.

Appendix B

ASSESSMENT OF FUNGICIDES AND APPLICATION TIMINGS FOR CONTROL OF WHEAT HEAD DISEASES IN GEORGETOWN, DE, 2020.

Published in *Plant Disease Management Network*

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Wheat (*Triticum aestivum*; ‘Shirley’)
Fusarium head blight; *Fusarium graminearum*
Septoria nodorum blotch; *Parastagonospora nodorum*

**Assessment of fungicides and application timings for control of wheat head diseases
in Georgetown, DE, 2020**

Soft red winter wheat, ‘Shirley’, was planted into corn stubble on 24 Oct 2019 at the Carvel Research and Education Center in Georgetown, DE to evaluate the effectiveness of fungicides applied at various timings for disease control and impact on yield. The field was a Pepperbox loamy sand soil type. The experimental design was a randomized complete block with five replicates. Plots were 24 ft long and 10 ft wide with 5-ft alleys between blocks. University of Delaware Cooperative Extension Service standard wheat production practices were followed. Treatments included a non-treated control and eight treatments of Miravis Ace, Prosaro, Caramba, and Tebuconazole applied at various application timings and combinations. Fungicide timings included applications at half of head visible (Feekes 10.3) on 28 Apr, at anthesis (Feekes 10.5.1) on 8 May, and five days after anthesis (5DAA) on 13 May. Fungicides were applied using a CO₂ pressurized backpack sprayer equipped with Turbo TwinJet 11002 nozzles calibrated to deliver 20 GPA at 30 psi. Corn grain was colonized with a local *Fusarium graminearum* isolate and applied on 7 Apr at a rate of 1.7 lb/plot to supplement the corn stubble as a natural inoculum source for Fusarium disease development. Plots relied on natural sources of inoculum for Septoria disease development and were not irrigated. Fusarium head blight (FHB) was evaluated on 2 Jun by visually rating disease incidence (% heads with symptoms) and severity (% of symptomatic glumes per head) of 40 randomly selected wheat heads per plot. Septoria nodorum blotch (SNB) was evaluated on 2 Jun by visually rating disease incidence and severity of 40 randomly selected heads per plot. Plots were harvested 23 Jun and yield was corrected to 13.5% moisture. Deoxynivalenol (DON) concentrations were determined from subsamples of grain collected from each plot. Disease and yield data were analyzed using ANOVA and means were separated using Fisher’s least significant difference ($\alpha = 0.05$).

Warm weather and frequent rainfall during flowering kept the region at high risk for FHB during anthesis, but SNB was the major disease observed. A late frost during flowering was detrimental to kernel formation, resulting in poor overall grain fill. All treatments and timings reduced FHB incidence and severity when compared to the non-treated control. DON concentrations did not vary and were below 0.2 ppm in all treatments. All Miravis Ace treatments and timings significantly reduced SNB incidence when compared to the non-treated control as well as to Prosaro and Caramba. All treatments and timings reduced SNB severity when compared to the non-treated control. No treatments significantly affected yield or test weight in comparison to the non-treated control. Phytotoxicity was not observed in any treatment.

Treatment and Rate/A ^z	Growth stage at application (Feekes)	FHB Incidence ^y	FHB Severity	SNB Incidence	SNB Severity	Yield (bu/A) ^x
Non-Treated Control	---	18.00 d	3.50 b	100.00 e	43.54 f	76.40 a
Prosaro 421 SC, 6.5 oz	10.5.1	4.00 c	0.46 a	95.00 de	27.79 e	75.00 a
Caramba 90 EC, 13.5 oz	10.5.1	3.00 bc	0.21 a	96.50 e	20.54 cd	75.50 a
Miravis Ace 2.3 SE, 13.7 oz	10.3	3.00 bc	0.21 a	48.50 bc	5.04 ab	77.90 a
Miravis Ace 2.3 SE, 13.7 oz	10.5.1	1.00 ab	0.07 a	22.50 a	2.07 a	75.20 a
Miravis Ace 2.3 SE, 13.7 oz	5 DAA 10.5.1	3.00 bc	0.21 a	41.00 b	4.97 ab	74.60 a
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1 fb		0.14 a			
Prosaro421 SC, 6.5 oz	5 DAA	2.00 abc		36.00 ab	3.54 ab	78.00 a
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1 fb					
Caramba 90 EC, 13.5 oz	5 DAA	0.00 a	0.00 a	36.50 ab	3.06 ab	76.50 a
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1 fb					
Tebuconazole 3.6F 4 oz	5 DAA	1.00 ab	0.07 a	34.50 ab	3.61 ab	78.00 a
<i>p</i> -value	---	0.0001	0.0001	0.0001	0.0001	NS
LSD ($\alpha=0.05$)	---	2.99	0.57	17.36	5.73	6.35

^z Non-ionic surfactant, Induce 90 SL, was added to all fungicide treatments at 0.125 % v/v, fb= followed by.

^y Percentage of 40 randomly selected wheat heads per plot displaying Fusarium head blight symptoms on glumes. Treatments followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; $\alpha=0.05$). NS= Not Significant.

^x Yields were calculated from the center 6.6 ft of each plot and adjusted to 13.5% moisture.

Appendix C
ASSESSMENT OF FUNGICIDES AND APPLICATION TIMINGS FOR
CONTROL OF BARLEY HEAD AND LEAF DISEASES IN GEORGETOWN, DE,
2021.

Published in *Plant Disease Management Network*

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Barley (*Hordeum vulgare*; ‘Thoroughbred’)
Fusarium head blight (*Fusarium graminearum*)
Septoria nodorum blotch (*Parastagonospora nodorum*)

Assessment of fungicides and application timings for control of barley head and leaf diseases in Georgetown, DE, 2021

Malting barley ‘Thoroughbred’ was grown at the Carvel Research and Education Center in Georgetown, DE to evaluate the effectiveness of fungicides applied at various timings for disease control and impact on yield. Barley was planted on 5 Nov 2020 into corn residue in Rosedale loamy sand. The experimental design was a randomized block with five replicates. Plots were 22 feet long and 10 feet wide with alleys five feet wide. Production practices outlined by the University of Delaware Cooperative Extension Service were followed. Treatments included a non-treated control and nine treatments of Miravis Ace, Sphaerex, Caramba, Prosaro, and Tebuconazole applied at different timings. Fungicide timings included application at half head visible (Feekes 10.3/Zadoks 55) on 26 Apr, at heading (Feekes 10.5/Zadoks 59) on 29 Apr, and four days after heading (4 dah) on 4 May. Applications were made using a CO₂ backpack sprayer equipped with Turbo TwinJet 11002 nozzles calibrated to deliver 20 GPA at 30 psi. Natural sources of inoculum were relied upon for disease development. Fusarium head blight (FHB) was evaluated on 24 May by visually rating disease incidence (% heads with symptoms) and severity (% of symptomatic glumes per head) of 20 randomly selected barley heads per plot. Septoria nodorum blotch (SNB) flag leaf lesion incidence and severity were evaluated on 17 May by visually rating lesion development and coverage of 10 randomly selected barley flag leaves per plot. Plots were harvested 24 Jun and yields were corrected to 13.5% moisture. Deoxynivalenol (DON) concentrations were obtained from grain subsampled from each plot. Disease and yield data were analyzed using ANOVA and means were separated using Fisher’s least significant difference ($\alpha=0.1$).

Environmental conditions were not conducive to FHB and SNB disease development. FHB incidence and severity were low and did not vary significantly among treatments. SNB head infections were not observed. Incidence of SNB did not vary among treatments. All treatments and timings significantly reduced the severity of SNB lesions on the flag leaf except Caramba. DON concentrations were less than 0.2 ppm in all treatments (data not shown). All treatments with Miravis Ace had significantly higher yields than the control. Phytotoxicity was not observed in any treatment.

Treatment and Rate/A ^z	Growth stage at application (Feekes)	Yield (Bu/A) ^y	SNB Flag Leaf Incidence ^x	SNB Flag Leaf Severity	FHB % Incidence ^w	FHB % Severity
Miravis Ace 2.3 SE, 13.7 oz	10.3	77.9 a	62.0	1.3 d	2.0	0.002
Caramba 90 EC, 13.5 oz	10.5.1	73.2 bcd	80.0	7.6 ab	1.0	0.001
Miravis Ace 2.3 SE, 13.7 oz	10.5.1	78.0 a	70.0	1.7 d	0.0	0.000
Prosaro 421 SC, 6.5 oz	10.5.1	72.7 cd	80.0	5.7 bc	1.0	0.001
Sphaerex 7.3 oz	10.5.1	72.8 cd	78.0	5.5 bc	0.0	0.000
Miravis Ace 2.3 SE, 13.7 oz	4 dah	78.4 a	78.0	4.2 cd	1.0	0.001
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1	77.7 ab	62.0	1.6 d	0.0	0.000
Prosaro 421 SC, 6.5 oz	4 dah					
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1	75.5 abc	76.0	2.1 d	0.0	0.000
Caramba 90 EC, 13.5 oz	4 dah					
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1	75.8 abc	64.0	1.7 d	2.0	0.001
Tebuconazole 3.6 F, 4 oz	4 dah					
Control	--	70.4 d	98.0	10.1 a	2.0	0.006
<i>p</i> -value	--	0.05	0.26	0.0002	0.86	0.67
LSD ($\alpha=0.1$)	--	4.62	NS	3.18	NS	NS

^z Non-ionic surfactant, Induce 90 SL, was added to all fungicide treatments at 0.125 % v/v, fb= followed by.

^y Yields were obtained by harvesting from the center 6.6 ft of each plot and calculated by adjusting to 13.5% moisture. Treatments followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; $\alpha=0.1$). NS = not significant.

^x Percentage of 10 randomly selected barley flag leaves displaying Septoria nodorum blotch lesions.

^w Percentage of 20 randomly selected barley heads per plot displaying Fusarium head blight symptoms on glumes.

Appendix D

**ASSESSMENT OF FUNGICIDES AND APPLICATION TIMINGS FOR
CONTROL OF WHEAT HEAD DISEASES IN GEORGETOWN, DELAWARE,
2021.**

Published in *Plant Disease Management Network*

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Wheat (*Triticum aestivum*; ‘Shirley’)

Fusarium head blight; *Fusarium graminearum*

Septoria nodorum blotch; *Parastagonospora nodorum*

Assessment of fungicides and application timings for control of wheat head diseases in Georgetown, Delaware, 2021

Soft red winter wheat, ‘Shirley,’ was planted into corn stubble on 9 Nov 2020 at the Carvel Research and Education Center in Georgetown, DE to evaluate the effectiveness of fungicides applied at various timings for disease control and impact on yield. The field was a Pepperbox loamy sand soil type. The experimental design was a randomized complete block with five replicates. Plots were 24 ft long and 10 ft wide with 5-ft alleys between blocks. University of Delaware Cooperative Extension Service standard wheat production practices were followed. Treatments included a non-treated control and thirteen treatments of Miravis Ace, Prosaro PRO, Sphaerex, Prosaro, Caramba, and Tebuconazole applied at various application timings and combinations. Fungicide timings included applications at half of head visible (Feekes 10.3) on 26 Apr, at anthesis (Feekes 10.5.1) on 12 May, and five days after anthesis (5 daa) on 17 May. Fungicides were applied using a CO₂ pressurized backpack sprayer equipped with Turbo TwinJet 11002 nozzles calibrated to deliver 20 GPA at 30 psi. Corn grain was colonized with a local *Fusarium graminearum* isolate and applied on 20 Apr at a rate of 1.7 lb/plot to supplement the corn stubble as a natural inoculum source for disease development. Plots relied on natural sources of inoculum for Septoria disease development and were mist-irrigated to promote disease development. Fusarium head blight (FHB) was evaluated on 10 Jun by visually rating disease incidence (% heads with symptoms) and severity (% of symptomatic glumes per head) of 20 randomly selected wheat heads per plot. Septoria nodorum blotch (SNB) was evaluated on 10 Jun by visually rating disease incidence and severity of 20 randomly selected heads per plot. SNB flag leaf lesion incidence and severity were evaluated on 10 Jun by visually rating lesion development and coverage on 10 randomly selected flag leaves per plot. Plots were harvested 24 Jun and yield was corrected to 13.5% moisture. Deoxynivalenol (DON) concentrations were determined from subsamples of grain collected from each plot. Disease and yield data were analyzed using ANOVA and means were separated using Fisher’s least significant difference ($\alpha = 0.05$).

Hot, dry weather and infrequent rainfall during flowering kept the region at low risk for FHB during anthesis, and SNB was the major disease observed. Only treatments that included Miravis Ace significantly reduced SNB flag leaf lesion incidence. All treatments significantly reduced SNB flag leaf lesion severity. FHB incidence did not vary among treatments. All treatments and timings except Miravis Ace at Feekes 10.3 and Prosaro and Sphaerex at anthesis reduced FHB severity when compared to the non-treated control. DON concentrations did not vary and were below 0.2 ppm in all treatments (data not shown). All Miravis Ace treatments and timings significantly reduced SNB head incidence. All treatments and timings reduced SNB head severity except Prosaro PRO at

Feekes 10.3 and Caramba and Sphaerex at anthesis when compared to the non-treated control. Only treatments of Miravis Ace applied at 10.3, 10.5.1, 5 daa, and when followed by Prosaro 5 daa significantly increased yields when compared to the control. Phytotoxicity was not observed in any treatment.

Treatment and Rate/A ^z	Growth stage at application (Feekes)	Yield (Bu/A) ^y	SNB % Flag Leaf Incidence ^x	SNB % Flag Leaf Severity	FHB % Incidence ^w	FHB % Severity	SNB % Incidence	SNB % Severity
Control	--	92.8 d	100.0 a	9.94 a	0.08	0.022 a	100.0 a	81.4 a
Miravis Ace 2.3 SE, 13.7 oz	10.3	99.9 abc	4.0 b	0.04 c	0.07	0.012 ab	27.0 b	2.9 e
Prosaro PRO 400 SC, 10.3 oz	10.3	96.6 cd	90.0 a	3.43 b	0.03	0.003 bc	100.0 a	71.9 ab
Caramba 90 EC, 13.5 oz	10.5.1	95.4 cd	96.0 a	1.43 bc	0.0	0.000 c	100.0 a	72.1 ab
Miravis Ace 2.3 SE, 13.7 oz	10.5.1	100.5 abc	8.0 b	0.08 c	0.05	0.006 bc	12.0 cd	1.2 e
Prosaro 421 SC, 6.5 oz	10.5.1	98.4 a-d	100.0 a	2.4 b	0.05	0.013 ab	100.0 a	66.1 b
Prosaro PRO 400 SC, 10.3 oz	10.5.1	97.1 cd	94.0 a	2.66 b	0.01	0.002 bc	95.0 a	39.2 d
Sphaerex 7.3 oz	10.5.1	96.0 cd	92.0 a	2.15 bc	0.06	0.012 abc	100.0 a	69.8 ab
Miravis Ace 2.3 SE, 13.7 oz	5 daa	102.9 ab	2.0 b	0.01 c	0.02	0.003 bc	20.0 bc	4.5 e
Prosaro PRO 400 SC, 10.3 oz	5 daa	96.8 cd	94.0 a	2.56 b	0.01	0.003 bc	99.0 a	44.6 cd
Sphaerex 7.3 oz	5 daa	97.6 bcd	92.0 a	1.37 bc	0.03	0.006 bc	100.0 a	52.2 c
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1	97.9 a-d	4.0 b	0.04 c	0.02	0.001 bc	18.0 bc	1.4 e
Caramba 90 EC, 13.5 oz	5 daa	97.8 bcd	0.0 b	0.0 c	0.04	0.004 bc	8.0 d	0.6 e
Miravis Ace 2.3 SE, 13.7 oz fb	10.5.1	104.0 a	2.0 b	0.02 c	0.0	0.000 c	6.0 d	0.6 e
Prosaro 421 SC, 6.5 oz	5 daa							
<i>p</i> -value	--	0.03	0.0001	0.0001	0.11	0.03	0.0001	0.0001
LSD ($\alpha=0.05$)	--	5.76	12.75	2.24	NS	0.01	9.57	12.51

^z Non-ionic surfactant, Induce 90 SL, was added to all fungicide treatments at 0.125 % v/v, fb= followed by.

^y Yields were calculated from the center 6.6 ft of each plot and adjusted to 13.5% moisture. Treatments followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; $\alpha=0.05$). NS= Not Significant.

^x Percentage of 10 randomly selected flag leaves per plot displaying *Septoria nodorum* blotch lesions.

^w Percentage of 40 randomly selected wheat heads per plot displaying *Fusarium head blight* symptoms on glumes. Treatments followed by the same letter are not significantly different based on Fisher's Least Significant Difference (LSD; $\alpha=0.05$). NS= Not Significant.