INTEGRATED MYCOTOXIN MANAGEMENT STRATEGIES IN MAIZE GRAIN

By

Katlin Marie Fusilier

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences- Master of Science

2019

ABSTRACT

INTEGRATED MYCOTOXIN MANAGEMENT STRATEGIES IN MAIZE GRAIN

By

Katlin Marie Fusilier

In recent years, high levels of mycotoxins such as deoxynivalenol (DON) in Michigan maize (*Zea mays* L) grain have been an issue for growers. This is caused by the interaction between hybrid susceptibility to ear rot, ideal weather conditions, and western bean cutworm (WBC) damage. In 2017 and 2018, field experiments were conducted at nine locations to determine the best management strategies for mycotoxins in Michigan and the Great Lakes region. Treatments included three host plant resistance levels, fungicide application, and three insect trait packages. A subset of four site-years with DON levels greater than 2 $\mu g\ g^{-1}$ was used for in-depth analysis. Host plant resistance and fungicide application did not lower DON levels across any of the site-years. Data from this research provided further evidence that the Cry1F Bt trait is ineffective against WBC in the Great Lakes region. Hybrids with the Vip3a Bt protein lowered WBC incidence although DON control was limited to one out of three site-years. Western bean cutworm incidence showed a significant correlation with ear rot incidence at two of four site-years where the environment was not conducive to fungal infection. To evaluate the occurrence of multiple mycotoxins across Michigan environments, samples from one hybrid with no fungicide application were selected from each of the nine locations across two years and analyzed for 26 different mycotoxins. Contamination from multiple mycotoxins was found in every sample with all samples having at least four mycotoxins present. Overall, mycotoxin contamination continues to be a problem in the Great Lakes Region and limited options are available to growers for their management.

ACKNOWLEDGMENTS

I would first like to thank my Master's thesis advisor, Dr. Maninder Singh, without his support and guidance throughout researching and writing my thesis I would not be where I am today. I would also like to show appreciation for Dr. Martin Chilvers and Dr. Chris DiFonzo for serving on my committee, being collaborators on the project, and always being willing to talk about ideas.

I would like to thank Dr. Singh's technicians Bill Widdicombe and Lori Williams along with Dr. Martin Chilver's technician, Adam Byrne, for their help with technical aspects of the project including planting, spraying, and harvesting plots. I am grateful for my fellow graduate students in Dr. Singh's lab; Thomas Siler, Kalvin Canfield, and Harkirat Kaur for their idea sharing and for their friendship throughout my time here. I would also like to thank the undergraduate students who worked on my project; Steve Anderson, Emily Wildt, Austin Cornett, and Jake Carden. I appreciate all of these people and their countless hours spent helping in the field and with processing samples.

I would like to thank the following sponsors of this research: The Corn Marketing Program of Michigan for funding, MSU AgBioResearch, Monsanto, Bayer, and Syngenta for their donation of seed and chemicals, and to our farmer cooperators for allowing us to use their land for the study.

Finally, I am extremely grateful to my friends and family and my husband, Chad, for providing me with support and encouragement throughout my time in graduate school and while writing my thesis. This would have never been possible without their support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AB1	Aflatoxin
AME	Alternariolmethylether
AOH	Alternariol
BEA	Beuvericin
Bt	*Bacillus thuringiensis*
CTN	Citrinin
DIAS	Diacetoxyscirpenol
DON	Deoxynivalenol
D3G	Deoxynivelenol 3-β-D –glucoside
3- ADON	3-acetyl-deoxynivalenol
15-ADON	15-acetyl-deoxynivalenol
ENNA	Enniatin A
ENNA1	Enniatin A1
ENNB	Enniatin B
ENNB1	Enniatin B1
ERI	Ear Rot Incidence
ERS	Ear Rot Severity
FDA	U.S. Food and Drug Administration
FB1	Fumonisin B1
GDD	Growing Degree Day
RB2	Fumonisin B2
FB3	Fumonisin B3

FUSX	Fusarenon-X
HT-2	HT-2 Toxin
MON	Moniliformin
NEO	Neoxolaniol
NIV	Nivelenol
OTA	Ochratoxin A
OTB	Ochratoxin B
PENA	Penitrim A
ROC	Roquefortine C
STER	Sterigmatocystin
T-2	T-2 Toxin
WBC	Western Bean Cutworm
WBCI	Western Bean Cutworm Incidence
ZON	Zearalenone

CHAPTER 1

LITERATURE REVIEW

Mycotoxins

Mycotoxins have been a human health issue possibly since the time of the ancient Greeks and Romans. Ergotism, a disease caused by mycotoxins, was referenced in the Middle Ages and proved to be caused by ergoty rye in 1630 (Pitt and Miller, 2017). Between 2012 and 2015, an estimated 4.5 billion bushels of maize (*Zea mays* L) in the United States and Ontario were contaminated with mycotoxins, with annual totals ranging between 117.3 million and 2.6 billion contaminated bushels (Mueller, et al., 2016). Using a computer model, the U.S. Food and Drug Administration (FDA) estimated the economic cost of aflatoxins, fumonisins, and deoxynivelenol to be $932 million annually (CAST, 2003).

Deoxynivalenol (DON) is a specific mycotoxin, sometimes found in Michigan maize at high levels. DON belongs to the B group trichothecenes (Ueno, 1977) produced by two fungal species: *Fusarium graminearum* and *Fusarium colmorum*. The B group of trichothecenes are characterized by a carbonyl group at the C-8 position. The chemical is stable at high temperatures and is soluble in water and some polar solvents (EFSA, 2004). The stability of DON allows it to persist in food and feed, where it affects both human and animal health. Effects of DON vary depending on the type of digestive system in the animal. Swine, a monogastric animal, show the highest sensitivity to DON followed by chicken and turkey; while ruminant animals show the lowest sensitivity (Prelusky, et al., 1994). When DON is consumed by animals at rates often present in naturally contaminated feed, effects are associated with poor animal performance factors such as reduced weight gain. Swine fed DON contaminated feed consumed less and grained less weight (Rotter, et al., 1995). At higher concentrations, DON exposure in swine can cause vomiting 15 minutes after initial consumption (Young, et al., 1983), hence it is also referred to as "vomitoxin".

Besides animals, DON can also affect humans who consume infected grain. Humans have a monogastric digestive system similar to swine, therefore are also highly sensitive to the effects of DON (Prelusky, et al., 1994). However, less is known about the effects of DON on human health compared to animals. A study of urine from 300 adults in the United Kingdom detected DON in 98.7% of individuals. Based on the urinary levels the researchers estimate that some individuals may exceed the European Union recommended maximum daily intake of 1000 ng DON kg^{-1} body weight (Turner, et al., 2008).

Because of DON's potential effects on human and animal health, the FDA set advisory levels for the mycotoxin in wheat (*Triticum aestivum* L.) in 1982. In 1993 following an outbreak of DON contamination in hard red spring wheat in the Midwestern U.S., the FDA applied new advisory levels to all grain and grain products in the U.S. In July of 2010, the FDA again revised their advisory levels to reflect current studies stating that certain species of animals could tolerate higher levels of DON while still keeping a safe food supply (US Food and Drug Administration, 2010). Current advisory levels for products intended for human consumption are set at a maximum DON limit of 1 $\mu g\ g^{-1}$. Grain intended for swine feed has a maximum limit level of 5 $\mu g\ g^{-1}$, while chickens and beef cattle feed has a maximum limit of 10 $\mu g\ g^{-1}$ (US Food and Drug Administration, 2010). When mycotoxins are over set limits, grain loads are docked in price or rejected. This causes growers to look for strategies to reduce DON contamination in their grain.

Fusarium graminearum

In Michigan, the DON producing fungal species is *Fusarium graminearum* (teleomorph *Gibberella zeae),* which produces the disease known as Gibberella ear rot of maize. *Fusarium graminearum* is an important pathogen of maize that characteristically appears as a pink to red fungal mat on the ear (Wise, et al., 2016). *Fusarium graminearum* has multiple host plants and is the causal agent of head blight on wheat, barley, rice, and oats, as well as stalk and ear rots of maize (Goswami and Kistler, 2004).

Fusarium graminearum overwinters on maize and wheat residue left in the field, providing production of inoculum during subsequent growing seasons (Khonga and Sutton, 1988). Though maize and wheat residue both can serve as a source of inoculum, studies of head blight in wheat show that inoculum from maize residue generally produces greater rates of infection than wheat residue (Dill-Macky and Jones, 2000). *Fusarium graminearum* forms perithecia on residue, which then forcibly discharge ascospores into the air (Munkvold, 2003). Above 28.5° C, a decrease in production occurs (Tschanz, et al., 1976). Optimal temperatures for the discharge of ascospores from perithecia is 16.6° C which is lower than that for perithecial development (Tschanz, et al., 1976). Release of ascospores by the perithecia follows a daily pattern with most spore release occurring at night. The release of ascospores from the perithecia coincides with an increase of relative humidity at night (Paulitz, 1996). A heavy rainfall will stop spore release but then peak ascospore production occurs two to four days later (Paulitz, 1996). Besides ascospores, macroconidia can also infect the host. Macroconidia are produced in sporodochia, which form on crop residue left in the field (Munkvold, 2003). However, experiments on head blight in wheat showed that ascospores likely play the biggest role in infection (Munkvold, 2003).

Forcible discharge of the ascospores by the perithecia allow the spores to travel through the air to their infection site. *Fusarium graminearum* ascospores from 6-day-old perithecia had an average discharge distance of 4.6 mm (Schmale, et al., 2005) but spores are also able to travel for long distances in the air at altitudes of 3200 m (Stakman, et al., 1923). Thus, spores can be transported long distances through the atmosphere. Once spores are airborne they must settle out through a process known as deposition before landing on and infecting plant tissues. Ascospores of *F. graminearum* settle out of the air in less than three seconds if they do not become caught in air currents (Schmale, et al., 2005).

Silk channels appear to be the most common route for *F. graminearum* infection of maize ears (Miller, et al., 2007). Infections occur when macroconidia or ascospores land on exposed silks, germinate, and grown down the silk into the developing ear (Miller, et al., 2007). Because silk channel infections begin in the silks, symptoms begin at the tip and move downward toward the butt end of the ear (Hesseltine and Bothast, 1977, Koehler, 1942). Infectious macrodonidia or ascospores of *F. graminearum* can also enter the ear through punctures in the husk created by birds or insects (Christensen and Schneider, 1950, Sutton, et al., 1980). Under favorable conditions, *F. graminearum* can also be transferred from infected seeds to seedlings (Kabeere, et al., 1997).

Fusarium graminearum Management Strategies

Managing *F. graminearum* in maize is important for growers. Studies show that disease severity of *F. graminearum* is highly correlated with DON content in final grain (Reid, et al., 1999, Reid, et al., 1996). Because of this, growers look to reduce infection of *F. graminearum* to lower the risk of DON in the final grain product.

Changes in cultural practices such as planting date, tillage practices, and fertilization can have an effect on the amount of disease present in the field. Khonga and Sutton (1988) observed that *F. graminearum* on maize and wheat residue produced inoculum primarily in the first and second years. They also noted that more inoculum was produced by residue left on the surface compared to residue that was buried. They recommended burying infected residues or limiting planting wheat or corn to every three years. Dill-Macky and Jones (2000) demonstrated lower infection of wheat with *F. graminearum* with conventional tillage compared to reduced tillage or no-till systems. The difference between conventional, reduced, or no-till systems was small with disease incidences of 64%, 72%, and 71%, respectively. Corn grain DON concentrations in conventional, reduced, and no-till systems was 8.1, 10.6, and 11.1 μg g^{-1}, respectively. Miller et al. (1998) found inconclusive results when looking at similar tillage systems in wheat but data suggested that zero tillage resulted in increased infection compared to conventional tillage.

Changing planting date to avoid infestation periods of *F. graminearum* has also been suggested as a management strategy. In wheat, panting date may influence disease levels because seasonal patterns of temperature and moisture correlate with infection. Subedi et al. (2007) found that later planting dates increased the incidence of *F. graminearum* infection in spring wheat. The authors recommended planting spring wheat as early as possible to avoid high humidity and temperatures during flowering.

Planting population can also affect *F. graminearum* severity levels and DON concentration. Maize planted at 82,000 plants ha^{-1} had significantly higher DON concentrations than maize planted at 65,000 plants ha^{-1} (Blandino, et al., 2008). The increased plant population likely influenced the microclimate with in the maize crop early in the growing season. In the same study, plant density did not influence the type of fungal growth occurring on the ear, only the severity of growth; climactic conditions such as temperature, humidity, and rainfall determined which type of mycotoxin was present (Blandino, et al., 2008).

Having a balanced fertilizer program may also be important in controlling *F. graminearum* in maize. Amending soil with added N can reduce ear rot problems but too much N could increase problems (Reid, et al., 2001). In wheat, inconsistent results of N fertilizer on Fusarium head blight have been observed (Subedi, et al., 2007) with one study showing increased N can increase Fusarium head blight and DON (Lemmens, et al., 2004), while another showed the opposite (Teich and Nelson, 1984). Low levels of N could cause stress to the plants making them susceptible to infection (Teich and Nelson, 1984). Nitrogen treatments had significant effects on the incidence and severity but the differences were small and inconsistent over sites and years suggesting that N does not have a great effect on Fusarium head blight levels. Besides N, other nutrients could also play a role in *F. graminearum* infection. Studies showed that Fusarium head blight in wheat was increased on fields with medium or low P levels (Teich and Nelson, 1984).

Another option to reduce *F. graminearum* is the use of maize hybrids with resistance to fungal infection. Reid et al. (1992) demonstrated genotypic variations in maize hybrids for resistance to *F. graminearum*. Resistance mechanisms to infection by *F. graminearum* can be found in both silks and kernels (Chungu, et al., 1996, Reid, et al., 1992). Silk resistance to *F. graminearum* slows the growth of the fungus down the silk channel and into the ear (Miller, et al., 2007). Kernel resistance mechanisms inhibit the spread of fungus from kernel to kernel (Chungu, et al., 1996). In 1997, Schaafsma reported that most commercial hybrids were susceptible to infection by *F. graminearum* (Schaafsma, et al., 1997). Since then, breeding programs have developed inbred lines with higher resistance to infection (Reid, et al., 2003, Reid, et al., 2001a, Reid, et al., 2001b).

Resistance to *F. graminearum* decreased ear rot disease and DON under inoculated conditions. Vigier et al. (2001) demonstrated this for a hybrid with moderate resistance to *F. graminearum* through both the kernel and silk mechanisms compared to two moderately susceptible inbreds. In wheat, using cultivar resistance to manage *F. graminearum* infection is well-documented (Bai, et al., 2001, Miller, et al., 1985) and is regarded as the most practical and effective means of *F. graminearum* control (Rudd, et al., 2001).

The interaction between fungicide use and host plant resistance to control *F. graminearum* growth in maize is also an area of interest. Studies in wheat, demonstrated a benefit of using both cultivar resistance and fungicides to control Fusarium head blight. Wegulo et al. (2011) demonstrated an additive interaction between prothioconazole use and cultivar resistance on reducing Fusarium head blight and DON in wheat. The use of a fungicide on a moderately resistant cultivar compared to a susceptible cultivar resulted in lower levels of disease. Other studies found a similar additive effect between cultivar resistance and the use of a prothioconazole + tebuconazole mixture when controlling *F. graminearum* and DON in wheat (Paul, et al., 2019, Willyerd, et al., 2012).

Prothioconazole is a broad-spectrum fungicide that belongs in the triazolinthione chemical class. Prothioconazole works as a demethylation inhibitor, inhibiting the demethylation process at either position 14 of lanosterol or 24-methylene dihydrosterol, both of which are precursors to sterols in fungi. When sterol formation is blocked, fungal infection of the plant is effectively stopped. Without sterol production, appresoria and haustoria cannot form and mycelial growth and spore formation are stopped, and the plant is protected from infection

(Dutzmann and Suty-Heinze, 2004). Prothioconazole is a leaf-systemic fungicide (Dutzmann and Suty-Heinze, 2004). However, experiments show limited movement through the plant with only traces of the compound found far from the application site (Lehoczki-Krsjak, et al., 2013). Because of this limited movement, full coverage spray is important.

The efficacy of prothioconazole to control ear rots is inconsistent. Luna and Wise (2015) used both an *in vitro* and field experiment to test prothioconazole efficacy on *Stenocarpella maydis*, the causal organism for Diplodia ear rot. *In vitro,* the fungicide reduced fungal growth, but under field conditions no consistent reduction in Diplodia ear rot was found. Anderson et al. (2017) reported that fungicides reduced *F. graminearum* severity in two out of three site-years in maize but DON was not lowered in any site-year. Smith et al. (2017) found that an R1 (silking) application of prothioconazole in maize did not result in lower ear rot severities than a non-treated control. Researchers attributed the lack of efficacy of fungicides to *F. graminearum* to the barrier of the maize husk and the limited systemic movement of the fungicide in the plant.

Although studies have been inconsistent in maize, prothioconazole fungicide is very effective in lowering *F. graminearum* and DON levels in wheat. Reductions in Fusarium head blight severity were between 39 and 93% while reductions in DON were between 40 and 91% (Haidukowski, et al., 2012). Meta-analysis of fungicide trials on soft winter wheat also showed that prothioconazole reduced Fusarium head blight and DON levels in wheat (Paul, et al., 2008). Anderson et al. (2017) stated that the lack of a husk acting as a protective barrier in wheat might explain why fungicides were more effective in wheat than in maize.

Western Bean Cutworm

Western bean cutworm (WBC, *Striacosta albicosta),* is a moth native to the United States, first described in 1887 from a specimen collected in Arizona (Smith, 1887). Western bean cutworm has been on an eastward expansion for a while and was first found in Michigan in 2006. The first reported damage from WBC in Michigan occurred to fields in northwest Michigan in 2007 (DiFonzo and Hammond, 2008). Traditionally WBC was a secondary pest of maize, but is now a primary pest in the Great Lakes region due to lack of management options, overwintering populations and uncontrolled injury from the pest within the region (Smith, et al., 2018). Western bean cutworm can cause multiple issues including a loss in maize yield. Yield loss from WBC was estimated to be between 232.6 to 936.2 kg/ha with infestation of one larvae per maize ear (Appel, et al., 1993, Paula-Moraes, et al., 2013).

Adult moths are approximately 2 cm long and can be identified by a cream stripe along the outer edge of the forewing and a circular spot halfway along the length of the forewing. Moth flight begins in mid-June, peaks in mid to late July, and ends in September depending on environmental conditions. Females lay eggs in July or August in maize fields in the late whorl stage, moths favor pretassel to just tasseling maize (Smith, et al., 2019). Late whorl maize plants provide shelter and a favorable microclimate for WBC larvae (Paula-Moraes, et al., 2012). Eggs are often laid on newly unfurled whorl leaves, usually on the top side of the leaf. Eggs are white when they are first laid, but become darker as they approach hatching in about five to seven days. After eclosion, larvae eat their eggshells and most move upwards into the tassel (Smith, et al., 2019). Larvae that feed on tassel tissue are heavier and survive better than larvae that feed on leaf tissue alone (Paula-Moraes, et al., 2012). Once pollen shed ends, small larvae move downwards into the silks and ear tip. They feed on silks than on kernels at the tip or the side of the ear. Early fall larvae drop from the maize plant and burrow into the soil, approximately 12-25 cm down, to pupate. In the soil they make earthen chambers using secretions from their salivary glands. The following summer, moths emerge from the soil and begin the cycle again (Smith, et al., 2019).

Monitoring for WBC moths gives an indication for when to scout fields as part of a management strategy. Either blacklight or pheromone traps have been used to monitor moth flight, though pheromone traps are generally recommended (Mahrt, et al., 1987). Pheromone traps should be placed at least 1.2 m above the ground (Dorhout and Rice, 2008, Mahrt, et al., 1987) and monitored weekly to determine when peak moth flight occurs (Smith, et al., 2019). Pheromone traps should not be used in place of scouting, as they are poor predictors of potential damage in maize fields (Mahrt, et al., 1987). Scouting should focus on fields in the pre-tassel stage, which are attractive to female moths. At a minimum, 20 consecutive plants in five areas of the field should be scouted for egg masses and larvae on the upper surfaces of the new and not-yet unfolded leaves. Scouting is recommended every three to five days throughout oviposition (Smith, et al., 2019).

When considering management strategies for WBC, few cultural or biological practices are recommended, as they are unreliable. Soil disturbance through tillage may increase mortality of WBC overwintering in the soil but has not been studied directly (Smith, et al., 2019). A return to conventional tillage is unlikely due to benefits from reduced tillage practices. In addition, conventional tillage would need to be implemented across a wide area due to the movement of the WBC moths (Smith, et al., 2019). Nabids, ladybird beetle adults, and spiders can feed on eggs and larvae up the third instar. After the third instar, predation of WBC by birds can cause high levels of mortality (Seymour, et al., 2010). Overall options for cultural and biological control of WBC are limited and likely does not play a large factor in WBC control.

Due to challenges associated with WBC control, growers may consider options such as an insecticide to reduce insect populations. Treatment thresholds for insecticide sprays have changed over time and varied across regions. Using a yield loss of 232.6 kg/ha, the economic threshold for WBC was estimated at 33 eggs per plant when calculated as the control costs divided by the product of the commodity value and the yield loss per larvae (Appel, et al., 1993). Counting the number of egg masses in the field is impractical (Smith, er al., 2019). Thresholds have been estimated at 4-8% of plants with an egg mass or larvae (Paula-Moraes, et al., 2013, Seymour, et al., 2010). Economic thresholds have been estimated for maize in Nebraska based on price per bushel, larval survival rates, and management costs per acre. Based on these conditions recommended thresholds could range from 1-58% of plants infested (Paula-Moraes, et al., 2013). In the Great Lakes region, entomologists recommend using a cumulative threshold of 5% of plants with egg masses. Because this threshold is cumulative, egg masses are documented over several weeks until the action threshold has been reached (Smith, et al., 2019). Insecticide applications should be done before WBC colonize the ear. Before they colonize the ear, larvae are moving on the maize plant and would potentially be exposed to insecticides (Paula-Moraes, et al., 2012). After they enter the ear, larvae are protected from the insecticide spray. In the Great Lakes region, an insecticide-fungicide tank mix is recommended at the R1 growth stage to optimize fungicide protection and still provide control for WBC (Smith, et al., 2019).

Transgenic maize can also be used to control insect damage. Most maize acres are now planted to transgenic maize hybrids to control European corn borer. Some proteins for protection against European corn borer (Cry1A, Cry1Ab, and Cry2Ab) have no effect on WBC (Smith, et al., 2019). However, several early field studies showed that Cry1F had efficacy against WBC. Catangui and Berg (2006) found no WBC damage on Cry1F hybrids in a study in Iowa from

2003 to 2004. Experiments in Ontario showed significantly lower WBC damage on hybrids containing Cry1F in 2011 but from 2012 to 2015 no difference was found between hybrids containing Cry1F versus hybrids without Cry1F (Smith, et al., 2017). Because of issues with resistance of WBC to Cry1F, growers are looking for other transgenic control options. Vip3A is a newer insecticidal protein isolated from *B. thuringensis* to control WBC (Estruch, et al., 1996). Field studies have shown reduced damage from WBC on hybrids containing the Vip3A protein when compared to hybrids containing Cry1F (Bowers, et al., 2013). Initial susceptibility studies of WBC to the Vip3A protein show lower variation in susceptibility when compared to Cry1F (Farhan, et al., 2018). Growers must use proper integrated pest management strategies along with monitoring for resistance of WBC to Vip3A to continue to use Vip3A as a management strategy in the Great Lakes region.

Managing for WBC is important not only because of potential yield losses from WBC feeding but also because of the increased risk of ear rot infections and mycotoxin damage with WBC feeding. Damage from the larvae creates wounds in the husk, allowing for the entry of pathogens into the ear (Smith, et al., 2019). Western bean cutworm has been shown to have an influence on ear rot levels and mycotoxin levels in maize. It has been demonstrated that physically injured kernels of maize have a greater incidence of ear rot symptoms and consequently higher mycotoxin levels than uninjured kernels (Munkvold, 2003). This is because kernels that are injured by WBC serve as an entry point for the pathogen into the ear. A positive correlation has been found between the presence of WBC larvae and *F. graminearum* severity in maize (Parker, et al., 2017). Smith et al. (2017) found that higher incidences of WBC damage increased DON concentrations. Because of the link between WBC and the DON producing fungus, *F. graminearum*, managing WBC is an important consideration for growers when creating a DON management strategy.

Complex associations between *F. graminearum*, WBC, and the environment require that management strategies include strategic approaches working to address all of the variables in the system.

LITERATURE CITED

LITERATURE CITED

Ali, M.L., J.H. Taylor, L. Jie, G. Sun, M. William, K.J. Kasha, et al. 2005. Molecular mapping of QTLs for resistance to Gibberella ear rot, in corn, caused by Fusarium graminearum. Genome 48: 521-533. doi:10.1139/g05-014.

Anderson, N.R., M.P.R. Luna, J.D. Ravellette and K.A. Wise. 2017. Impact of foliar fungicides on Gibberella ear rot and deoxynivalenol levels in Indiana corn. Plant Health Progress: PHP-01-17-0010-RS. doi:10.1094/php-01-17-0010-rs.

Appel, L.L., R.J. Wright and J.B. Campbell. 1993. Economic injury levels for western bean cutworm, *Loxagroits albicosta* (Smith) (Lepidoptera, Noctuidae), eggs and larvae in field corn. Journal of the Kansas Entomological Society 66: 434-438.

Bai, G.H., R. Plattner, A. Desjardins and F. Kolb. 2001. Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breeding 120: 1-6. doi:10.1046/j.1439-0523.2001.00562.x.

Blandino, M., A. Reyneri and F. Vanara. 2008. Effect of plant density on toxigenic fungal infection and mycotoxin contamination of maize kernels. Field Crops Research 106: 234-241. doi:10.1016/j.fcr.2007.12.004.

Bowers, E., R. Hellmich and G. Munkvold. 2013. Vip3Aa and Cry1Ab proteins in maize reduce Fusarium ear rot and fumonisins by deterring kernel injury from multiple Lepidopteran pests. World Mycotoxin Journal 6: 127-135. doi:10.3920/wmj2012.1510.

CAST. 2003. Mycotoxins: Risks in plant, animal, and human systems. Council for Agricultural Science and Technology.

Catangui, M.A. and R.K. Berg. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera : Noctuidae), as a potential pest of transgenic Cry1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environmental Entomology 35: 1439-1452. doi:10.1093/ee/35.5.1439.

Christensen, J.J. and C.L. Schneider. 1950. European corn borer (*Pyrausta nubilalis* HBN) in relation to shank, stalk, and ear rots of corn. Phytopathology 40: 284-291.

Chungu, C., D.E. Mather, L.M. Reid and R.I. Hamilton. 1996. Inheritance of kernel resistance to *Fusarium graminearum* in maize. Journal of Heredity 87: 382-385. doi:10.1093/oxfordjournals.jhered.a023019.

Cote, L.M., A.M. Dahlem, T. Yoshizawa, S.P. Swanson and W.B. Buck. 1986. Excretion of deoxynivalenol and its metabolie in milk, urine, and feces of lactating dairy cows. Journal of Dairy Science 69: 2416-2423. doi:10.3168/jds.S0022-0302(86)80681-6.

Dai, S.W., M.D. Shulski, K.G. Hubbard and E.S. Takle. 2016. A spatiotemporal analysis of Midwest US temperature and precipitation trends during the growing season from 1980 to 2013. International Journal of Climatology 36: 517-525. doi:10.1002/joc.4354.

DiFonzo, C.D. and R. Hammond. 2008. Range expansion of western bean cutworm, *Striacosta albicosta* (Noctuidae), into Michigan and Ohio. Crop Management 7.

Dill-Macky, R. and R.K. Jones. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease 84: 71-76. doi:10.1094/pdis.2000.84.1.71.

Dorhout, D.L. and M.E. Rice. 2008. An evaluation of western bean cutworm pheromone trapping techniques (Lepidoptera : Noctuidae) in a corn and soybean agroecosystem. Journal of Economic Entomology 101: 404-408. doi: 10.1093/jee/101.2.404

Dutzmann, S. and A. Suty-Heinze. 2004. Prothioconazole: a broad spectrum demethylation-inhibitor (DMI) for arable crops. Pflanzenschutz-Nachrichten Bayer 57: 249-264.

European Food Safety Authority (EFSA). 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed. EFSA J. 73: 1-42.

Estruch, J.J., G.W. Warren, M.A. Mullins, G.J. Nye, J.A. Craig and M.G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proceedings of the National Academy of Sciences of the United States of America 93: 5389-5394. doi:10.1073/pnas.93.11.5389.

Farhan, Y., J.L. Smith and A.W. Schaafsma. 2018. Baseline susceptibility of *Striacosta albicosta* (Lepidoptera: Noctuidae) in Ontario, Canada to Vip3a *Bacillus thuringiensis* protein. Journal of Economic Entomology 111: 65-71. doi:10.1093/jee/tox296.

Folcher, L., M. Delos, E. Marengue, M. Jarry, A. Weissenberger, N. Eychenne, et al. 2010. Lower mycotoxin levels in Bt maize grain. Agronomy for Sustainable Development 30: 711-719. doi:10.1051/agro/2010005.

Goswami, R.S. and H.C. Kistler. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5: 515-525. doi:10.1111/j.1364-3703.2004.00252.x.

Groth, J.V., E.A. Ozmon and R.H. Busch. 1999. Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. Plant Disease 83: 1033-1038. doi:10.1094/pdis.1999.83.11.1033.

Haidukowski, M., A. Visconti, G. Perrone, S. Vanadia, D. Pancaldi, L. Covarelli, et al. 2012. Effect of prothioconazole-based fungicides on Fusarium head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions. Phytopathologia Mediterranea 51: 236-246.

Hammond, B.G., K.W. Campbell, C.D. Pilcher, T.A. Degooyer, A.E. Robinson, B.L. McMillen, et al. 2004. Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the

United States in 2000-2002. Journal of Agricultural and Food Chemistry 52: 1390-1397. doi:10.1021/jf030441c.

Hesseltine, C.W. and R.J. Bothast. 1977. Mold development in ears of corn from tasseling to harvest. Mycologia 69: 328-340. doi:10.2307/3758658.

Kabeere, F., J.G. Hampton and M.J. Hill. 1997. Transmission of *Fusarium graminearum* (Schwabe) from maize seeds to seedlings. Seed Science and Technology 25: 245-252.

Khonga, E.B. and J.C. Sutton. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 10: 232-239. doi:10.1080/07060668809501730.

Koehler, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. Journal of Agricultural Research 64: 0421-0442.

Lehoczki-Krsjak, S., M. Varga, A. Szabo-Hever and A. Mesterhazy. 2013. Translocation and degradation of tebuconazole and prothioconazole in wheat following fungicide treatment at flowering. Pest Management Science 69: 1216-1224. doi:10.1002/ps.3486.

Lemmens, M., K. Haim, H. Lew and P. Ruckenbauer. 2004. The effect of nitrogen fertilization on Fusarium head blight development and deoxynivalenol contamination in wheat. Journal of Phytopathology 152: 1-8. doi:10.1046/j.1439-0434.2003.00791.x.

Luna, M.P.R. and K.A. Wise. 2015. Timing and efficacy of fungicide applications for Diplodia ear rot management in corn. Plant health progress 16: 123-131.

Mahrt, G.G., R.L. Stoltz, C.C. Blickenstaff and T.O. Holtzer. 1987. Comparisons between blacklight and pheromone traps for monitoring the western bean cutworm (Lepidoptera, Noctuidae) in south central Idaho. Journal of Economic Entomology 80: 242-247. doi:10.1093/jee/80.1.242.

Miller, J.D., J. Culley, K. Fraser, S. Hubbard, F. Meloche, T. Ouellet, et al. 1998. Effect of tillage practice on Fusarium head blight of wheat. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 20: 95-103.

Miller, J.D., J.C. Young and D.R. Sampson. 1985. Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathologische Zeitschrift-Journal of Phytopathology 113: 359-367.

Miller, S.S., L.M. Reid and L.J. Harris. 2007. Colonization of maize silks by *Fusarium graminearum*, the causative organism of Gibberella ear rot. Canadian Journal of Botany-Revue Canadienne De Botanique 85: 369-376. doi:10.1139/b07-027.

Mueller, D.S., K.A. Wise, A.J. Sisson, T.W. Allen, G.C. Bergstrom, D.B. Bosley, et al. 2016. Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. Plant Health Progress 17: 211-222.

Munkvold, G.P. 2003. Epidemiology of Fusarium diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705-713. doi:10.1023/a:1026078324268.

Munkvold, G.P., R.L. Hellmich and L.G. Rice. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Disease 83: 130-138. doi:10.1094/pdis.1999.83.2.130.

Parker, N.S., N.R. Anderson, D.S. Richmond, E.Y. Long, K.A. Wise and C.H. Krupke. 2017. Larval western bean cutworm feeding damage encourages the development of Gibberella ear rot on field corn. Pest Management Science 73: 546-553. doi:10.1002/ps.4313.

Paul, P.A., P.E. Lipps, D.E. Hershman, M.P. McMullen, M.A. Draper and L.V. Madden. 2008. Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. Phytopathology 98: 999-1011. doi:10.1094/phyto-98-9-0999.

Paul, P.A., J.D. Salgado, G. Bergstrom, C.A. Bradley, E. Byamukama, A.M. Byrne, et al. 2019. Integrated effects of genetic resistance and prothioconazole plus tebuconazole application timing on Fusarium head blight in wheat. Plant Disease 103: 223-237. doi:10.1094/pdis-04-18-0565-re.

Paula-Moraes, S., T.E. Hunt, R.J. Wright, G.L. Hein and E.E. Blankenship. 2013. Western bean cutworm survival and the development of economic injury levels and economic thresholds in field corn. Journal of Economic Entomology 106: 1274-1285. doi:10.1603/ec12436.

Paula-Moraes, S.V., T.E. Hunt, R.J. Wright, G.L. Hein and E.E. Blankenship. 2012. On-plant movement and feeding of western bean cutworm (Lepidoptera: Noctuidae) early instars on corn. Environmental Entomology 41: 1494-1500. doi:10.1603/en12149.

Paulitz, T.C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Disease 80: 674-678. doi:10.1094/pd-80-0674.

Pitt, J.I. and J.D. Miller. 2017. A concise history of mycotoxin research. Journal of Agricultural and Food Chemistry 65: 7021-7033. doi:10.1021/acs.jafc.6b04494.

Prelusky, D.B., B.A. Rotter and R.G. Rotter. 1994. “Toxicology of mycotoxins”. Pp. 359-403 in Mycotoxins in grain: Compounds other than aflatoxin, edited by J.D. Miller and H.L. Trenholm. Minnestota: Eagan Press.

Reid, L., A. Bolton, R. Hamilton, T. Woldemariam and D. Mather. 1992. Effect of silk age on resistance of maize to *Fusarium graminearum*. Canadian Journal of Plant Pathology 14: 293-298.

Reid, L.M., D.E. Mather, R.I. Hamilton and A.T. Bolton. 1992. Genotypic differences in the resistance of maize silks to *Fusarium graminearum*. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 14: 211-214. doi:10.1080/07060669209500877.

Reid, L.M., G. McDiarmid, A.J. Parker and T. Woldemariam. 2003. CO441 corn inbred line. Canadian Journal of Plant Science 83: 79-80. doi:10.4141/p02-058.

Reid, L.M., G. McDiarmid, A.J. Parker, T. Woldemariam and R.I. Hamilton. 2001a. CO388 and CO389 corn inbred lines. Canadian Journal of Plant Science 81: 457-459. doi:10.4141/p00-053.

Reid, L.M., G. McDiarmid, A.J. Parker, T. Woldemariam and R.I. Hamilton. 2001b. CO430, CO431 and CO432 corn inbred lines. Canadian Journal of Plant Science 81: 283-284. doi:10.4141/p00-118.

Reid, L.M., R.W. Nicol, T. Ouellet, M. Savard, J.D. Miller, J.C. Young, et al. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: Disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89: 1028-1037. doi:10.1094/phyto.1999.89.11.1028.

Reid, L.M., D.W. Stewart and R.I. Hamilton. 1996. A 4-year study of the association between Gibberella ear rot severity and deoxynivalenol concentration. Journal of Phytopathology-Phytopathologische Zeitschrift 144: 431-436. doi:10.1111/j.1439-0434.1996.tb00319.x.

Reid, L.M., X. Zhu and B.L. Ma. 2001. Crop rotation and nitrogen effects on maize susceptibility to Gibberella (*Fusarium graminearum*) ear rot. Plant and Soil 237: 1-14. doi:10.1023/a:1013311703454.

Rotter, B.A., B.K. Thompson and M. Lessard. 1995. Effects of deoxynivalenol contamined diet on performance and blood parameters in growing swine. Canadian Journal of Animal Science 75: 297-302. doi:10.4141/cjas95-046.

Rudd, J.C., R.D. Horsley, A.L. McKendry and E.M. Elias. 2001. Host plant resistance genes for Fusarium head blight: Sources, mechanisms, and utility in conventional breeding systems. Crop Science 41: 620-627. doi:10.2135/cropsci2001.413620x.

Schaafsma, A.W., R.W. Nicol and L.M. Reid. 1997. Evaluating commercial maize hybrids for resistance to Gibberella ear rot. European Journal of Plant Pathology 103: 737-746. doi:10.1023/a:1008629629069.

Schmale, D.G., Q.A. Arntsen and G.C. Bergstrom. 2005. The forcible discharge distance of ascospores of *Gibberelia zeae*. Canadian Journal of Plant Pathology 27: 376-382.

Seymour, R., G. Hein and R. Wright. 2010. Western Bean Cutworm in Corn and Dry Beans. NebGuide G2013. Nebraska Cooperative Extension, Lincoln, NE.

Smith, J.B.. 1887. New Genera and Species of North American Noctuidae. Proceedings of the United States National Museum. The Smithsonian Institution, Washington: Government Printing Office p. 450-479.

Smith, J.L., T.S. Baute, M.M. Sebright, A.W. Schaafsma and C.D. DiFonzo. 2018. Establishment of *Striacosta albicosta* (Lepidoptera: Noctuidae) as a Primary Pest of Corn in the Great Lakes Region. Journal of Economic Entomology 111: 1732-1744. doi:10.1093/jee/toy138.

Smith, J.L., DiFonzo, C.D. Baute, T.S., Michel, A.P., & Krupke, C.H. 2019. Ecology and Management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans- revision with focus on the great lakes region. Journal of Integrated Pest Management, 10(1). Doi:10.1093/jipm/pmz025.

Smith, J.L., M.D. Lepping, D.M. Rule, Y. Farhan and A.W. Schaafsma. 2017. Evidence for Field-Evolved Resistance of *Striacosta albicosta* (Lepidoptera: Noctuidae) to Cry1F *Bacillus thuringiensis* Protein and Transgenic Corn Hybrids in Ontario, Canada. Journal of Economic Entomology 110: 2217-2228. doi:10.1093/jee/tox228.

Smith, J.L., V. Limay-Rios, D.C. Hooker and A.W. Schaafsma. 2017. Management of injury by *Striacosta albicosta* (Lepidoptera: Noctuidae) and deoxynivalenol content in maize. Canadian Journal of Plant Pathology 39: 580-581.

Smith, J.L., V. Limay-Rios, D.C. Hooker and A.W. Schaafsma. 2018. *Fusarium graminearum* Mycotoxins in Maize Associated With *Striacosta albicosta* (Lepidoptera: Noctuidae) Injury. Journal of Economic Entomology 111: 1227-1242. doi:10.1093/jee/toy005.

Stakman, E.C., A.W. Henry, G.C. Curran and W.N. Christopher. 1923. Spores in the upper air. Journal of Agricultural Research 24: 0599-0606.

Subedi, K.D., B.L. Ma and A.G. Xue. 2007. Planting date and nitrogen effects on Fusarium head blight and leaf spotting diseases in spring wheat. Agronomy Journal 99: 113-121. doi:10.2134/agronj2006.0171.

Sutton, J.C., W. Baliko and H.J. Liu. 1980. Fungal colonization and zearalenone accumulation in maize ears injured by birds. Canadian Journal of Plant Science 60: 453-461.

Teich, A.H. and K. Nelson. 1984. Survey of Fusarium head blight and possible effects of cultural practices in wheat fields in Lambton county in 1983. Canadian Plant Disease Survey 64: 11-13.

Tschanz, A., R. Horst and P.E. Nelson. 1976. The effect of environment on sexual reproduction of *Gibberella zeae*. Mycologia 68: 327-340.

Turner, P.C., J.A. Rothwell, K.L.M. White, Y. Gong, J.E. Cade and C.P. Wild. 2008. Urinary deoxynivalenol is correlated with cereal intake in individuals from the United Kingdom. Environmental Health Perspectives 116: 21-25. doi:10.1289/ehp.10663.

Ueno, Y. 1977. Mode of action of trichothecenes. Pure and Applied Chemistry 49: 1737-1745. doi:10.1351/pac197749111737.

US Food and Drug Administration. 2010. Guidance for Industry and FDA: Advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. US FDA. Silver Spring, MD.

Vigier, B., L.M. Reid, L.M. Dwyer, D.W. Stewart, R.C. Sinha, J.T. Arnason, et al. 2001. Maize resistance to Gibberella ear rot: symptoms, deoxynivalenol, and yield. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 23: 99-105. doi:10.1080/07060660109506915.

Wegulo, S.N., W.W. Bockus, J.H. Nopsa, E.D. De Wolf, K.M. Eskridge, K.H.S. Peiris, et al. 2011. Effects of Integrating Cultivar Resistance and Fungicide Application on Fusarium Head Blight and Deoxynivalenol in Winter Wheat. Plant Disease 95: 554-560. doi:10.1094/pdis-07-10-0495.

Willyerd, K.T., C. Li, L.V. Madden, C.A. Bradley, G.C. Bergstrom, L.E. Sweets, et al. 2012. Efficacy and Stability of Integrating Fungicide and Cultivar Resistance to Manage Fusarium Head Blight and Deoxynivalenol in Wheat. Plant Disease 96: 957-967. doi:10.1094/pdis-09-11-0763.

Wise, K., D. Mueller, A. Sisson, D. Smith, C. Bradley and A. Robertson. 2016. A farmers guide to corn diseases. APS Press, The American Pytopathological Society, St. Paul, MN.

Young, L.G., L. McGirr, V.E. Valli, J.H. Lumsden and A. Lun. 1983. Vomitoxin in corn fed to young-pigs. Journal of Animal Science 57: 655-664.

CHAPTER 2

ROLE OF HOST PLANT RESISTANCE, INSECT TRAIT PACKAGES, AND FUNGICIDE APPLICATION IN MAIZE EAR ROT AND DEOXYNIVALENOL MANAGEMENT

Introduction

Ear infection by fungal pathogens such as *Stenocarpella maydis, Aspergillus flavus, Trichoderma viride, Penicillium* spp., *Fusarium graminearum,* and *Fusarium verticillioides* is an important problem for Maize (*Zea mays* L) growers in North America. *Fusarium graminearum* is an especially important ear rot pathogen in Michigan and the Great Lakes region. This pathogen produces a pink fungal mat on the ear, known as Gibberella ear rot (Wise, et al., 2016). Infection occurs when spores enter through silk channels or wounds created by damage to the husk. Between 2012 and 2015, Gibberella ear rot accounted for an estimated loss between 350,000 and 2.1 million metric tons of maize grain yield per year in the United States and Ontario, Canada (Mueller, et al., 2016). In addition to reducing yield, an infection can also reduce grain quality. Under the optimal environmental conditions, Gibberella ear rot may produce multiple mycotoxins, including deoxynivalenol (DON) which are toxic to both humans and animals. High DON levels in grain result in feed refusal and vomiting, particularly in swine (Prelusky, et al., 1994). The United States Food and Drug Administration (FDA) has set advisory limits regulating the amount of DON in maize grain (US Food and Drug Administration, 2010). In grain and grain by-products intended for swine production, DON concentration cannot exceed 5 $\mu g\ g^{-1}$; for chickens, ruminating beef cattle, or feedlot cattle, the limit is 10 $\mu g\ g^{-1}$ (US Food and Drug Administration, 2010). Growers are not only concerned with DON levels in the grain that is fed directly to animals, but also with the quality of the grain taken to the local elevator. Price docking can occur if DON levels exceed a set limit. In addition, mycotoxin contamination in maize grain sold for ethanol production is of concern as mycotoxin concentration increases by three times in dried distiller's grain, a product of ethanol production. Because of this, ethanol production plants seek to limit the amount of mycotoxins in maize grain.

Multiple factors play into the development of Gibberella ear rot in maize grain and subsequent mycotoxin production. First, the environment must be favorable for spore production by the fungus. Gibberella ear rot generally infects under cool wet conditions. The optimal temperature for ascospore release by *F. graminearum* is 16.6°C (Tschanz, et al, 1976). Once released, ascospores can infect the ear. Gibberella ear rot grows best under moderate temperatures (24 to 28°C) and high humidity (greater than 80%, Mansfield et al., 2005). The timing of favorable conditions is also key. For example, an increase in precipitation in July increased Gibberella ear rot in maize grain (Vigier, et al. 1997). Cool wet conditions around silking time result in more infection.

The presence of ear feeding insects also contributes to ear rots. Feeding by Lepidoptera species, in particular, creates wounds in the husk, which are points of entry for fungal spores into the maize ear. Species that damage maize ears include European corn borer (*Ostrinia nubilalis*), western bean cutworm (WBC, *Striacosta albicosta*), fall armyworm (*Spodoptera frugiperda*), and corn earworm (*Helicoverpa zea*). Feeding by these insects at the ear tip and into the husk opens the maize ear, leaving it vulnerable to fungal infection. Physically injured kernels of maize result in a greater incidence of ear rot and higher mycotoxin levels than uninjured kernels (Munkvold, 2003). Strong correlations have been found between insect-injured kernels and Fusarium ear rot infection, as well as correlations between insect injury and mycotoxin levels (Bowers, Hellmich, & Munkvold, 2013).

The management of Gibberella ear rot requires an integrated strategy. One component is host plant resistance. When inoculated with *F. graminearum*, maize hybrids rated as resistant had less ear rot and lower levels of DON than susceptible hybrids (Vigier, et al., 2001). However, Gibberella ear rot resistance is limited in current hybrids on the market. Because hybrid resistance to Gibberella ear rot is limited, growers may choose to use a fungicide application to reduce fungal infection. Multiple products are marketed to reduce ear rot in maize grain. For example, prothioconazole products such as Proline® (Bayer Crop Science, Research Triangle Park, NC) are labeled for the suppression of Fusarium, Gibberella, and Aspergillus ear rots and the reduction of both disease symptoms and levels of mycotoxin in the grain (Bayer Crop Science, 2016). Prothioconazole is a broad-spectrum fungicide that inhibits the demethylation process in fungi (Dutzmann and Suty-Heinze, 2004). Studies have found the optimum spraying time for prothioconazole fungicides is up to eight days after silks emerge (Limay-Rios and Schaafsma, 2018). At this timing, prothioconazole decreased DON levels by 59% compared to non-treated control (Limay-Rios and Schaafsma, 2018). However, the ability of prothioconazole fungicides to manage Gibberella ear rot and DON production in maize has been inconsistent (Anderson, et al., 2017, Smith, et al., 2018).

Because of the link between insect feeding and ear rot in maize, controlling Lepidoptera species is another component to managing Gibberella ear rot. Because of their ease of use, many growers plant maize hybrids with *Bacillus thuringiensis* (Bt) proteins as an insect protection strategy. The use of Bt hybrids to control Lepidoptera species can reduce mycotoxin levels. For example, mycotoxins in maize grain are reduced through the control of European corn borer with Bt hybrids (Bowers, et al., 2013, Folcher, et al., 2010, Hammond, et al., 2004, Munkvold, et al., 1999). Using an insecticide is another method to control insects in the ear zone (Blandino, et al., 2008).

In the last decade, there has been increased concern about Gibberella ear rot infection in Michigan and the Great Lakes region. This concern has developed because of several factors. First, changes in climatic conditions in the region affect fungal ear rot development. Between 1980 and 2013, the growing season average temperature has increased by 0.15° C decade^{-1} in the Midwestern U.S. (Dai, et al., 2016). During the same period, growing season precipitation has increased by 12.20 ± 21.27 mm decade^{-1} in the Midwest (Dai, et al., 2016). Within the growing season, early-season precipitation (April to June) has increased while late-season precipitation (July to October) has decreased (Dai, et al., 2016). Furthermore, during the last three decades, the number of extreme rainfall events has been well above average and is expected to increase (Wuebbles, Fahey, & Hibbard, 2017). This means that there will be more dry and low precipitation days with an increase in heavy precipitation days (Wuebbles, et al., 2017). Changing climate conditions affect diseases as certain conditions promote growth for each species. Another change in the last decade is an increase in ear feeding insect injury in the region. Beginning in 2000, WBC, a pest of maize in the western plains, expanded its range eastward (Rice, 2000, Smith, et al., 2019), reaching Michigan and Ohio by 2006 (DiFonzo & Hammond, 2008). By 2010, it was a frequent pest in maize ears in the Great Lakes region (Smith, et al., 2019). Most of the Bt proteins targeted for European corn borer do not control WBC (Smith, et al., 2019). The Herculex Bt trait (Cry1F) initially provided control (Catangui and Berg, 2006), but within a few years, WBC evolved resistance to Cry1F (Smith, et al., 2017; Unglesbee, 2016). Resistance became widespread across the Corn Belt, and in 2017, WBC was removed as a target from the Herculex label (Unglesbee, 2017). Only the Vip3a Bt protein retains efficacy against WBC, although hybrids with this protein are not widely available in the Great Lakes region. As a result of the movement of WBC into the region, and the lack of Bt options to control it, ear injury has increased dramatically in frequency and level in the Great Lakes region, and grain quality issued have returned. This insect injury, combined with the changing and favorable environment, has renewed concern about ear rots and mycotoxin contamination among farmers and agribusinesses in the region.

To address grower concerns, the goal of this research was to determine the best management strategies to minimize DON production in maize grain. Specific objectives of the study were to determine the effects of host plant resistance, Bt traits, and fungicide application and their interactions on WBC damage, ear rot infection, and DON levels under varying environmental conditions.

Methods

Design

Experiments were conducted in Michigan in 2017 and 2018 at nine locations in conjunction with the Michigan Corn Performance Trails (https://varietytrials.msu.edu/corn/) for a total of 18 site-years. A high number of locations were used to attempt to capture environmental conditions for fungal ear rot and WBC pressure. The locations were spread across three zones (Figure 2-1), established based on long-term accumulated growing degree day (GDD) data. The 30 year (1981-2010) normal accumulated GDD from May 1 to October 31 were 2557, 2478, and 2342 for zones 1, 2, and 3 respectively. All field sites were on commercial farms with naturally occurring insect and disease pressure. Plots were planted with a four-row Almaco packet planter (Almaco, Nevada, IA) with a row spacing of 0.76 m (Table 2-1). Plots measured 6.71 m by 3.05 m with 0.91 m alleyways between the ends of plots. The center two rows of each plot were used for data collection including stand counts, insect scouting, grain samples, and yield, while the outer two rows acted as a buffer. Plots were managed according to grower standards for the area. Treatments (ear rot tolerance, insect trait package, and fungicide) were replicated five times at each location, in a randomized complete block design.

Maize hybrids were selected to represent three levels of host plant resistance. These levels were represented by three different ear rot tolerance rating levels: below average, average, and above average. Hybrid ear rot tolerance ratings were determined by the seed company based on inoculated field trials. The hybrids were similar in growing degree day requirements, ranging from 2550 to 2600 GDD to black layer. Within each ear rot tolerance rating, two near isoline Bt hybrids were selected. These hybrids differed in protection against WBC. SmartStax hybrids with the Lepidoptera proteins Cry1A.105, Cry2AB2, Cry1F was assumed to protect against WBC, VT Double Pro hybrids with only Cry1A.105 and Cry2AB2 would not (Difonzo and Porter, 2019). The SmartStax and VT Double PRO differed in the presence and absence of the Cry1F Bt protein, targeted to study the effect of WBC damage. These treatments resulted in six hybrids in 2017. In 2018, a seventh hybrid with a GDD requirement of 2560 to black layer expressing the Agrisure Viptera 3220 E-Z Refuge (Viptera) trait package was added to the study. The Vip3a protein found in the hybrid trait package is effective against WBC with reduced levels of insect damage compared to hybrids without the Vip3a trait (Bowers, et al., 2013). This hybrid was added based on published reports of WBC resistance to Cry1F (Smith et al, 2017, Unglesbee, 2016) and findings during the first growing season for the experiment.

A third treatment, fungicide, was applied at six of the nine locations in 2017 and seven of the nine locations in 2018 (Figure 2-1). A subset of locations was chosen to receive the fungicide treatment based on where disease pressure was expected to be high. A prothioconazole fungicide, Proline® 480 SC (Bayer, Research Triangle Park, NC), was applied at a rate of 416.32 ml ha^{-1} in a randomized complete block design. The trial also included control plots with no fungicide application at each location. Applications were made using a pressurized CO_2 high clearance backpack sprayer with a 3.05 m wide boom, fitted with TeeJet 8001VS nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 50.8 cm apart. Boom height was approximately 30 cm above the tassel. Fungicide applications were made at full silk emergence (approximately 4-8 days after silking) before the browning of the silks (Table 2-1). The combination of ear rot resistance, insect control, and fungicide application resulted in 12 treatments in 2017 and 14 treatments in 2018, for a total sample size (n) of 450 in 2017 and 580 in 2018.

Harvest Assessments

In both years, ten consecutive ears from the middle rows in each plot (total= 20 ears) were hand-harvested after plants reached physiological maturity (Abendroth, et al., 2011) to assess WBC and ear rot injury. Ears were rated as damaged or not damaged from WBC and the number of damaged kernels was counted. Western bean cutworm incidence (WBCI) was calculated as the number of damaged ears per plot. Damage from other insects (e.g. sap beetle) was minimal. Ears were also rated as infested or not infested by fungal mycelium and the number of infested kernels was counted. Ear rot incidence (ERI) was calculated as the number of infected ears per plot. Ear rot severity (ERS) was calculated as the number of infected kernels on each infected ear divided by the total number of kernels per ear (Groth, et al., 1999). Various ear rots were not separated and a single score was recorded.

Deoxynivalenol Quantification

In both years, the 20 ears from each plot for harvest assessments were shelled using a Haban Husker-Sheller (Haban Manufacturing Co, Racine, WI). The shelled grain from the 20 ears was mixed thoroughly into one bulk sample per plot before a 500 g subsample was taken. From this subsample, 50 g was subsampled and ground using a cyclone sample mill (UDY Corporation, Fort Collins, CO) with a 1 mm screen. Ground samples were sent to the University of Minnesota DON Testing Lab (St. Paul, MN) for gas chromatography-mass spectrometry testing using the methods described in Fuentes, et al., 2005.

Data Analyses

Four site years in the study were chosen for in-depth statistical analysis, each with DON values greater than 2 $\mu g\ g^{-1}$ (Figure 2-2). These locations also generally had high WBCI, ERI, or ERS (Figure 2-3 to 2-5). Very low DON levels are generally acceptable in animal feed, and discount schedules for grain elevators in the Great Lakes region generally begin at 3 to 4 $\mu g\ g^{-1}$ (Greig, 2019). Management strategies for Gibberella ear rot and DON when DON levels are high is of the most importance to growers, thus locations with low DON levels were not analyzed in this experiment. The locations chosen for in-depth analysis were Washtenaw 2017, Huron 2018, Montcalm 2018, and Saginaw 2018. All other locations in the study had average DON values less than 2 $\mu g\ g^{-1}$.

Statistical analysis was performed using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc.) at an alpha of 0.05. For locations with a fungicide treatment (Huron 2018 and Montcalm 2018), the model consisted of ear rot tolerance rating, insect trait package, and fungicide as well as their interactions as fixed effects with replication as a random effect. For locations without a fungicide treatment (Washtenaw 2017 and Saginaw 2018), the statistical model consisted of ear rot tolerance rating and insect trait package and their interaction as fixed effects with replication as a random effect. With the failure of the Cy1F protein in the SmartStax hybrid against WBC, a Viptera hybrid was included for the 2018 season to study the effect of

WBC control on ear rots and DON. Because SmartStax and VT Double PRO hybrids showed no difference in WBC control in the main models these hybrids were pooled into a non-Viptera category to compare to Viptera hybrids. The model was run with insect trait as the fixed effect and replication as a random effect. Where significant ($P<0.05$) fixed effects were observed, pairwise comparisons were made using the lsmeans statement with Tukey's adjustment.

Results

In total, data on 18 site years was collected throughout the two years of study. Out of the 18 site years, the four locations whose DON values were greater than 2 μg g^{-1} were chosen for statistical analysis based on a selected threshold. The threshold of 2 μg g^{-1} was chosen because growers do not generally get discounted for high DON levels until 3-4 μg g^{-1} in the Great Lakes region (Greig, 2019). The locations chosen for analysis were Washtenaw 2017, Huron 2018, Montcalm 2018, and Saginaw 2018 (Figure 2-2). All other locations in the study had an average DON value below 2 μg g^{-1}. Locations with an average DON level below 2 μg g^{-1} had DON incidences between 43%-100%.

Washtenaw 2017

Silking occurred between July 22 and July 25 in Washtenaw 2017. Weather conditions around silking were relatively dry, with no rainfall during the 10 days after silking and relative humidity around 70% (Figure 2-6). The average temperature during this same period was 21.8° C. At the end of August and early September, temperatures were cool with an increase in temperature at the end of September (Figure 2-6).

Western bean cutworm incidence differed significantly by ear rot tolerance rating levels (Table 2-2). Hybrids with above average ear rot tolerance rating had significantly more ears with WBCI (97%) than hybrids with an average (89%) or below average (78%) ear rot tolerance rating. There was no difference in WBC feeding between hybrids with a SmartStax trait package or a VT Double PRO trait package. Western bean cutworm incidence was moderately correlated with ERI in Washtenaw 2017 (Figure 2-10). WBCI was weakly correlated with DON values (R^2=0.19, p=0.0195).

Ear rot incidence differed significantly by the interaction between ear rot tolerance rating and insect trait package (Table 2-3). Ear rot severity also differed significantly by the interaction between ear rot tolerance rating and insect trait package (Table 2-4).

At Washtenaw 2017, DON was present in 96.7% of samples with an average DON concentration of 4.18 $\mu g\ g^{-1}$. DON levels differed significantly by both the ear rot tolerance rating and trait package (Table 2-2). Hybrids with an average ear rot tolerance had higher DON levels (6.04 $\mu g\ g^{-1}$) than below average ear rot tolerance hybrids (2.37 $\mu g\ g^{-1}$). Hybrids with above average ear rot tolerance rating (4.16 $\mu g\ g^{-1}$) were not significantly different from either the below average or above average ear rot tolerance rating hybrids. Hybrids with a VT Double PRO trait package had significantly lower DON levels (2.79 $\mu g\ g^{-1}$) than hybrids with a SmartStax trait package (5.59 $\mu g\ g^{-1}$).

Huron 2018

Silking in Huron during the 2018 growing season occurred between July 29 and August 1. During the 10 days following the silking period, 54.3 mm of precipitation fell over seven separate days. During this same period relative humidity averaged 83.7 % while average temperatures were 21.8° C (Figure 2-7).

Western bean cutworm incidence was only impacted by the presence of the Viptera trait package. Neither of the insect trait packages (SmartStax or VT Double PRO) affected WBCI. Non-Viptera hybrids had higher WBCI than the hybrid with the Viptera trait package (Table 2-

7). Western bean cutworm incidence was very weakly correlated with ERI in Huron 2018 (Figure 2-6), while WBCI and DON were not correlated (p=0.84).

Ear rot incidence was not significantly impacted by ear rot tolerance rating, trait package (SmartStax, VT Double PRO), or fungicide applications. The hybrid with the Viptera trait package had lower ERI levels (51%) compared to non-Viptera hybrids (78%).

The interaction between ear rot tolerance rating and insect trait package significantly affected ERS in Huron 2018 (Table 2-5). Ear rot severity was reduced in fungicide treated plots (14.7%) compared to non-treated plots (18.5%) (p=0.038). There was no interaction between fungicide application and ear rot tolerance rating.

At Huron 2018, DON was present in 100% of samples with an average DON concentration of 7.87 μg $g^{-}1$. DON levels in Huron 2018 were significantly impacted by the interaction between ear rot tolerance rating and insect trait package (Table 2-6). Hybrids with the Viptera trait package were found to have lower DON levels than non-Viptera hybrids (Table 2-7). Fungicide applications did not affect DON levels (Table 2-2).

Montcalm 2018

Silking occurred approximately between July 24 and July 30 in Montcalm 2018. In the 10 days following silking 5.1 mm of rain fell over four separate days. Relative humidity averaged 79.6% and temperatures averaged 21.8°C (Figure 2-8).

Hybrids with the Viptera trait package had significantly lower WBCI levels than non-Viptera hybrids (Table 2-7). Western bean cutworm incidence was weakly correlated with ERI in Montcalm 2018 (Figure 2-10). Western bean cutworm and DON were not correlated (p=0.35). No experimental treatments significantly affected ERI or ERS.

At Montcalm 2018, DON was present in 100% of samples with an average concentration of 4.87 μg g^{-1}. DON levels differed by ear rot tolerance rating. Hybrids with average ear rot tolerance (6.70 μg g^{-1}) had significantly higher DON levels than the above average hybrids (3.33 μg g^{-1}), below average hybrids (4.64 μg g^{-1}) were not significantly different either from the average or above average hybrids. Insect trait and fungicide did not effect DON levels in Montcalm 2018 (Table 2-2). Fungicide was not found to interact with ear rot tolerance rating.

Saginaw 2018

Silking occurred approximately between August 2 and August 5 in Saginaw 2018. During the 10 days following silking, precipitation totaled 47 mm over three separate days. Humidity averaged 70.5% and temperatures averaged 23.3° C (Figure 2-9).

In Saginaw 2018, Hybrids with Viptera had lower WBCI levels than non-Viptera hybrids (Table 2-7). Western bean cutworm incidence was very weakly correlated with ERI in Saginaw 2018 (Figure 2-10) and was not correlated with DON (p=0.61).

Ear rot incidence and ERS were not impacted by any treatment in the study.

At Huron 2018, DON was present in 100% of samples with an average concentration of 9.87 μg g^{-1}. Ear rot tolerance rating and insect trait package had a significant impact on DON levels in Saginaw 2018 (Table 2-2Table 2-2). Hybrids with an above average ear rot tolerance rating level had significantly higher DON levels (3.80 μg g^{-1}) than either the average (2.74 μg g^{-1}) or below average (2.48 μg g^{-1}) hybrids. SmartStax hybrids had significantly higher DON levels (3.51 μg g^{-1}) than VT Double PRO hybrids (2.51 μg g^{-1}).

Discussion

At high DON levels (>2 μg g^{-1}), host plant resistance and fungicide applications did not show a benefit for the management of ear rots and DON in maize grain at the four study locations. In addition, weather conditions around silking may have played a role in determining whether factors such as WBCI played a part in fungal ear rot development at the study locations. Furthermore, a reduction in WBCI using a Viptera hybrid only resulted in a reduction of DON at one out of three study locations in 2018.

Environmental factors at silking that promote infection of the maize silks by Gibberella ear rot include cool and wet conditions. Wet conditions can arise from rainfall, a high dew point, or high humidity levels. The environment around silking varied for each of the locations studied due to weather pattern variability across the state and differences in silking dates. This variability lead to certain locations with more favorable conditions for fungal development than others. Huron 2018 and Saginaw 2018 each had rainfall amounts greater than 45mm during the 10 days following silking. Washtenaw 2017 and Montcalm 2018 each had much lower rainfall during this period, totaling less than 6mm, less than ideal conditions for fungal infection of the silks.

DON levels differed significantly by host plant resistance level at each of the four locations. Though host plant resistance did affect DON levels, the pattern of DON reduction from host plant resistance was not consistent across locations. In addition, the reduction of DON with host plant resistance did not follow the expected pattern. This lack of consistency may be due to environmental factors at each location along with how ear rot tolerance ratings are determined. Host plant resistance to Gibberella ear rot is determined by the seed company by inoculating ears and later rating for ear rot development. Hybrids are likely tested at a limited number of testing facilities, which may not translate to all environmental conditions. In addition, ear rot tolerance ratings may not be a good indicator of DON accumulation. Because of this lack of consistency, using host plant resistance to manage DON production by Gibberella ear rot was not beneficial in this study. Although an advantage from host plant resistance was not observed in this study, the lack of efficacy was based on a small number of hybrids. Other studies have shown promise when using host plant resistance to lower DON levels. Vigier et al. (2001) demonstrated that a hybrid with moderate resistance in both the kernels and the silks had lower ear rot and DON levels than two moderately susceptible inbred lines. Future studies should look at a wider range of hybrids with varied genetics. Reid et al. (1992) demonstrated genotypic variations in maize hybrids for resistance to Gibberella ear rot. Resistance mechanisms can be found both in the silks and kernels and each works to stop infection by Gibberella ear rot in different ways (Chungu, et al., 1996, Miller, et al., 2007, Reid, et al., 1992). Because of known resistance mechanisms in hybrids, along with differences in DON between hybrids in the current study, proper selection of hybrids in the future could help reduce DON accumulation.

If optimal environmental conditions do not occur around silking, then other factors may play a role in ear rot development. During the study, both Huron 2018 and Saginaw 2018 had rainfall amounts greater than 45 mm during the 10 days following silking. These locations each had very weak correlations between WBCI and ERI and WBCI and DON. Both Washtenaw 2017 and Montcalm 2018 had much lower rainfall during the ten days following silking, totaling less than 6 mm causing less than ideal conditions for fungal infection of the silks. These two site years showed much stronger correlations between WBCI and ERI. This demonstrates that WBCI may have a larger role in Gibberella ear rot fungal infection and possibly DON production when environmental conditions are not perfect for fungal infection during or after silking. Although the locations had correlations between WBCI and ERI, only Washtenaw 2017 had a correlation between WBCI and DON. Washtenaw had the highest WBCI level. This indicates that although WBCI may have played a role in ERI, it may not play a role in DON production unless WBCI levels are extremely high.

Because of demonstrated correlations between insect damage and fungal infection (Bowers, et al., 2013), growers have looked to control Lepidoptera insects using insect protection trait packages or thorough scouting and spraying, thus hoping to manage disease levels in their fields. Recently WBC has become a major issue for Michigan farmers as the pest moved into the region. At the beginning of this study, growers in the Midwest U.S. relied on hybrids with the Cry1F Bt protein to control WBC. Since the initiation of the study, Cry1F has been removed from seed labels for control of WBC due to field observations of resistance. This study provided further evidence from the state of Michigan in the northern maize belt, that hybrids containing Cry1F (SmartStax) and hybrids without the Cry1F protein (VT Double PRO) did not differ in WBC damage. Other studies also demonstrated the resistance of WBC to the Cry1F protein (Ostrem, et al., 2016, Smith, et al., 2017). Though hybrids with Cry1F did not provide WBC control, hybrids with the Vip3a protein did provide control. The Viptera hybrid reduced WBCI at the three 2018 study locations. Though Viptera reduced WBCI at all three locations, a reduction in DON was only found at one location, Huron 2018. This reduction in DON levels in Huron 2018 was not expected. Environmental conditions were expected to be the driver for fungal infection in Huron 2018 rather than WBC, due to the low correlation between WBCI and ERI. The reduction in DON levels by the Viptera hybrid may possibly be attributed to the fact that Huron 2018 had relatively higher WBI. Smith et. al (2017) stated that a high level of WBC injury is greater than 30% incidence across non-protected plots. Only Huron 2018 had WBCI greater than 30% in non-Viptera plots. To consider the effects of insect damage on ear rot and DON levels, more study environments with high WBC levels are needed.

At the two locations with a fungicide treatment, fungicide was not shown to affect DON or ERI. Fungicide did reduce ERS at Huron 2018. Because fungicide did not reduce DON or

ERI, it would not be considered a beneficial strategy when managing ear rot and DON levels at these locations. Other studies showed varied results when using a prothioconazole fungicide to manage Gibberella ear rot and DON in maize. Anderson et al. (2017) reported that fungicides reduced Gibberella ear rot severity in two out of three site-years in maize but did not reduce DON. Smith et al. (2018) found that an R1 prothioconazole application in maize did not result in lower ear rot severities when compared to the non-treated control. Anderson et al. (2017) states that the lack of fungicide efficacy on Gibberella ear rot could be due to the thick husk barrier preventing the fungicide from entering the ear along with the limited systemic movement of the fungicide. In wheat (*Triticum spp*.), prothioconazole fungicides are very effective at lowering Fusarium head blight, caused by *F. graminearum*, and DON levels (Haidukowski, et al., 2012). To better understand the effect of fungicide, more locations with sufficient disease and high DON levels would be needed.

Overall, an integrated management strategy is needed to manage fungal ear rot infection and mycotoxin contamination in maize grain. Differences in management strategies used by growers could depend on environmental conditions. Western bean cutworm damage may have more of an effect on ear rots when conditions around silking are not ideal for fungal development. Adequate control of insects through scouting and spraying or Bt traits may be a strategy useful to growers with high insect levels. Across high DON level locations in this study, host plant resistance and fungicide were not effective in managing DON levels. Based on previous research growers may still consider these strategies in some scenarios. Future research should use a larger set of hybrids with varying ear rot tolerance ratings and genetic backgrounds across a large range of environments to obtain sufficient DON levels. Information from this research can also be used to create a predictive model for DON levels in maize grain in Michigan dependent on environmental conditions and management strategies.

APPENDICES

APPENDIX A

TABLES AND FIGURES

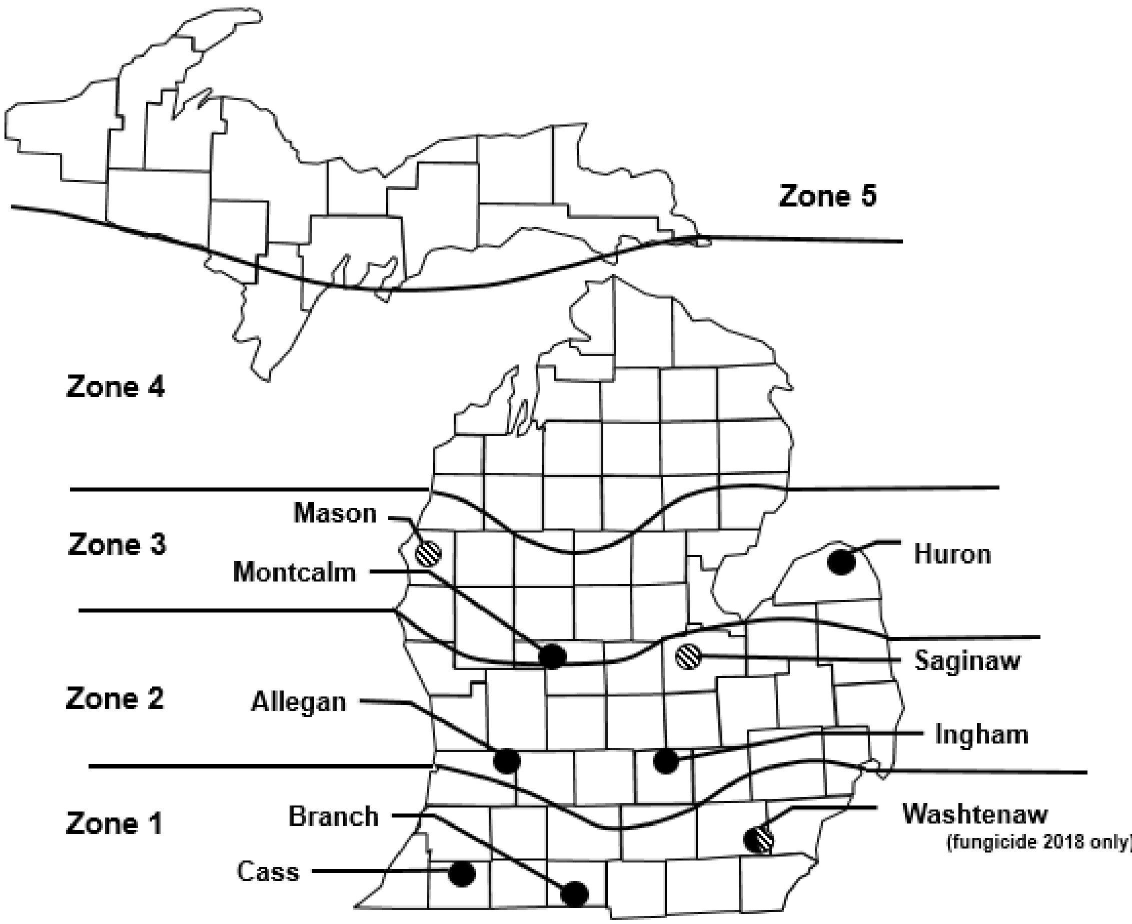

Figure 2-1. Field locations throughout Michigan in 2017 and 2018. Zones reflect 30 year (1981-2010) normal accumulated growing degree days from May 1 to October 31, 2557, 2478, and 2342 for zones 1, 2, and 3 respectively. Black circles indicate trials where fungicide applications were made while hatched circles were non-treated locations.

Table 2-1. Agronomic management details at each of nine locations in 2017 and 2018.

Location	Town	Previous Crop	Irrigation	Soil Classification	Soil Test Values pH	P	K	Planting Date	Average Population (plants hectare^{-1})[1]	Fertilizer N-P-K	Fungicide Application Date	Harvest Date
2017												
Allegan	Martin	Soybean	Dryland	Ockley loam	6.05	86	218	May 12	80,956	109-9-3 +manure	July 21	October 20
Branch	Coldwater	Soybean	Irrigated	Oshtemo sandy loam	6.4	115	117	May 31	85,586	221-9-3	August 1	October 17
Cass	Vandalia	Soybean	Irrigated	Kalamazoo loam	6.7	50	142.5	May 14	83,890	240-9-3	July 21	October 17
Huron	Bad Axe	Maize	Dryland	Kilmanagh loam	7.5	61	118	May 17	84,917	127-9-3 +manure	August 3	October 11
Ingham	East Lansing	Soybean	Dryland	Conover loam	6.85	52	197.5	May 23	83,646	190-9-3	July 26	October 13
Mason	Scottville	Carrots w/ rye cover	Irrigated	Fern-Marlette complex	6.1	139	153	May 10	81,575	109-9-3 +manure	-	October 27
Montcalm	Greenville	Soybean	Dryland	McBride and Isabella sandy loam	5.85	73	111.5	May 16	82,863	154-9-3	July 28	October 31
Saginaw	New Lothrop	Soybean	Dryland	Conover loam	6.8	44	118	May 29	85,325	154-9-3	-	October 15
Washtenaw	Milan	Soybean	Dryland	Pella silt loam	6.8	41.5	164.5	May 15	81,445	184-9-3	-	October 24
2018												
Allegan	Martin	Soybean	Dryland	Ockley loam	6.1	95	170	May 18	77,219	160-9-3 +manure	July 30	October 18
Branch	Coldwater	Maize	Irrigated	Oshtemo sandy loam	6.2	80	110	May 29	83,314	190-9-3	August 6	November 1
Cass	Vandalia	Maize	Irrigated	Kalamazoo loam	6.4	25	198	May 9	82,796	245-9-3	July 24	October 19
Huron	Bad Axe	Maize	Dryland	Kilmanagh loam	7.9	77.5	236	May 16	83,509	160-9-3 +manure	July 31	October 22
Ingham	East Lansing	Soybean	Dryland	Conover loam	6.0	49	196	May 8	78,351	160-9-3	July 23	October 17

Table 2-1 (cont'd)

Mason	Scottville	Soybean	Irrigated	Ithica-Arkona complex	6.6	47	121	May 23	82,671	160-9-3 +manure	-	October 23
Montcalm	Greenville	Maize	Dryland	McBride and Isabella sandy loams	5.95	96	198	May 18	82,153	160-9-3	July 30	October 25
Saginaw	New Lothrop	Soybean	Dryland	Conover loam 61% Brookston loam 39%	6.75	47	219	May 30	82,671	160-9-3	-	October 30
Washtenaw	Milan	Sweet Corn	Dryland	Boyer loamy sand 64% Wasepi sandy loam 36%	5.95	107	325.5	June 13	81,580	160-9-3	August 15	November 20

[1] In the first year of the experiment (2017), plots were thinned to a maximum population of 87,053 plants hectare $^{-1}$. In the second year (2018) plots were not thinned due to the use of a precision planter

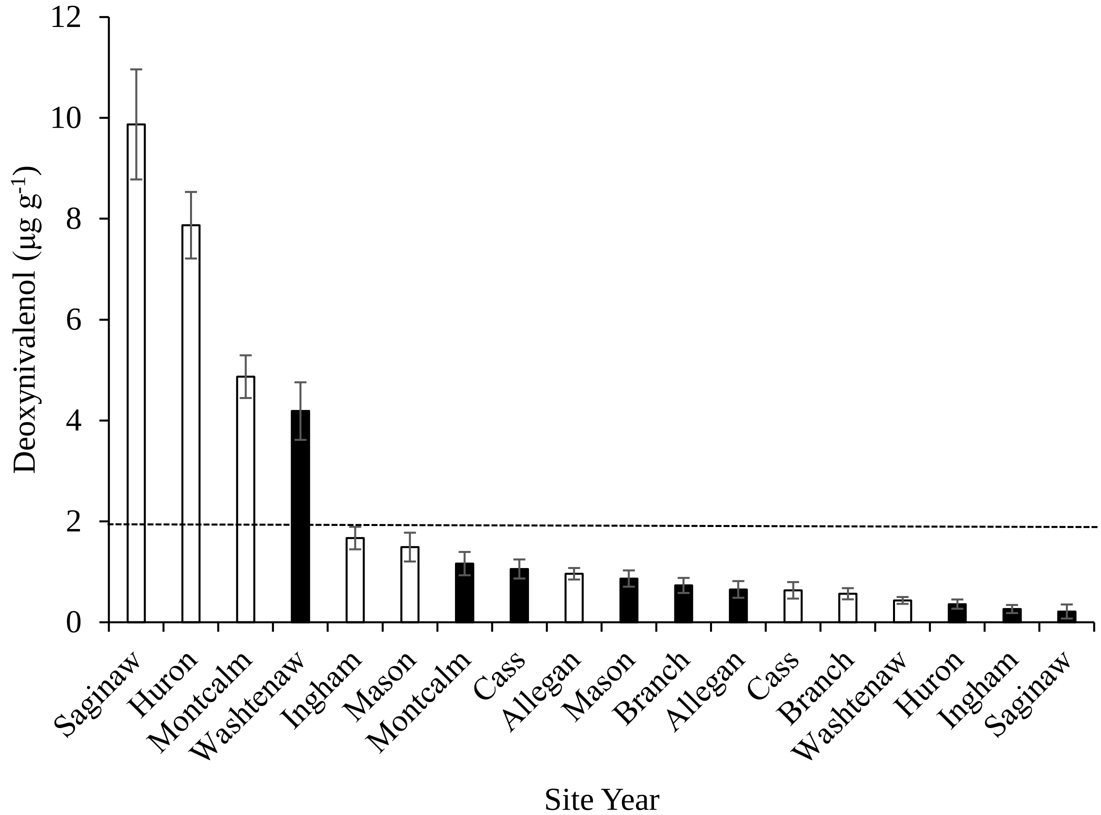

Figure 2-2. Average deoxynivalenol level (µg g^{-1}) by location in 2017 (black bars) and 2018 (white bars) across all treatments. Dotted line indicates the minimum DON threshold used to select locations for detailed statistical analysis. Error bars represent plus or minus one standard error.

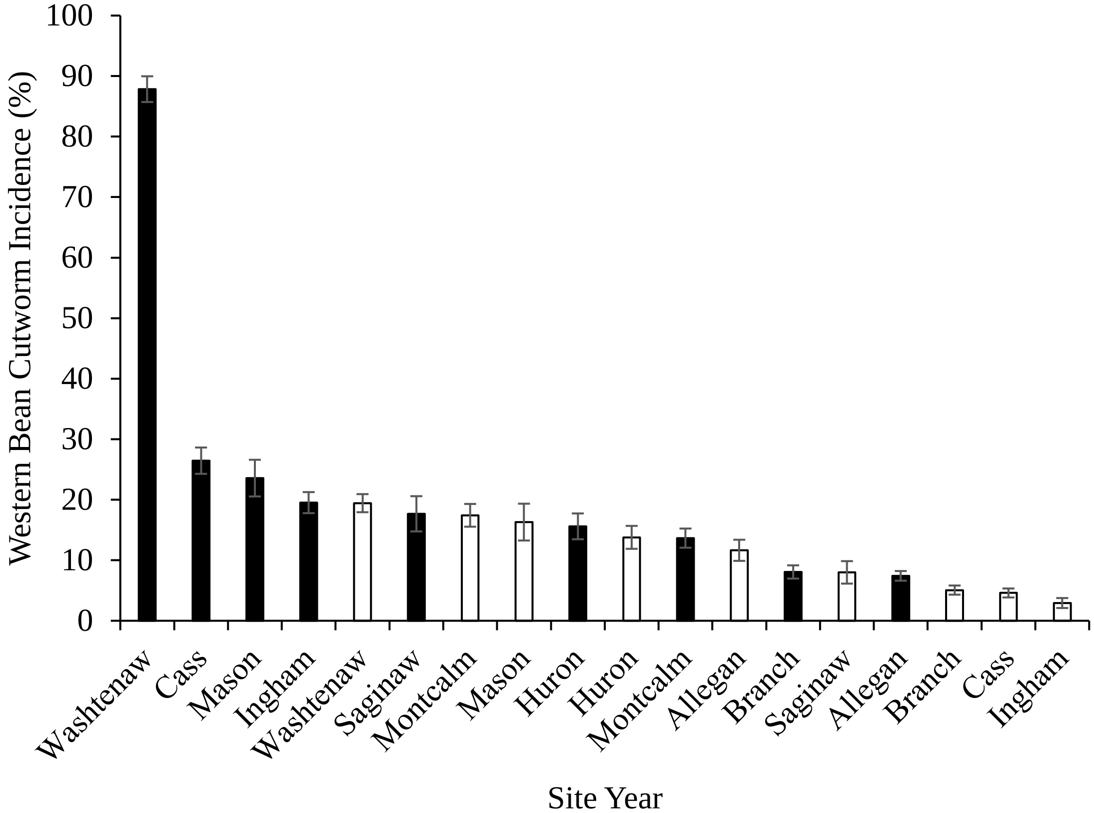

Figure 2-3. Average western bean cutworm incidence by location in 2017 (black bars) and 2018 (white bars) across all treatments. Error bars represent plus or minus one standard error.

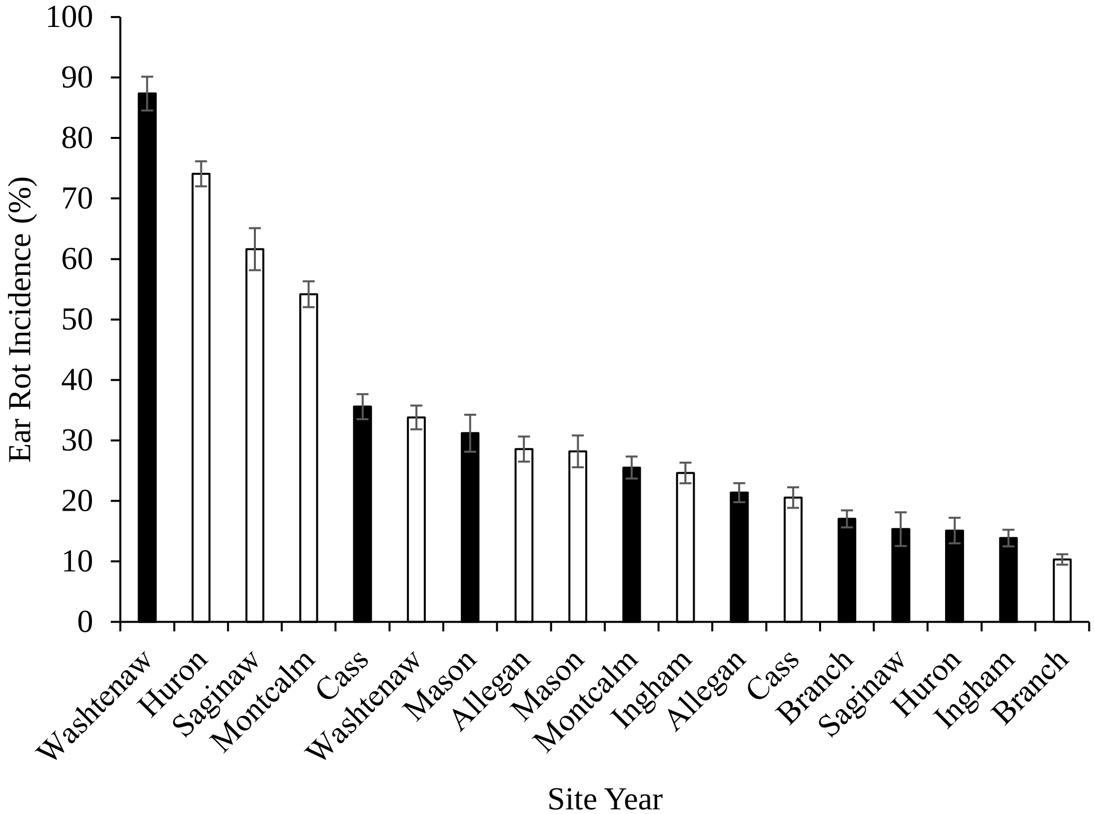

Figure 2-4. Average ear rot incidence by location in 2017 (black bars) and 2018 (white bars) across all treatments. Error bars represent plus or minus one standard error.

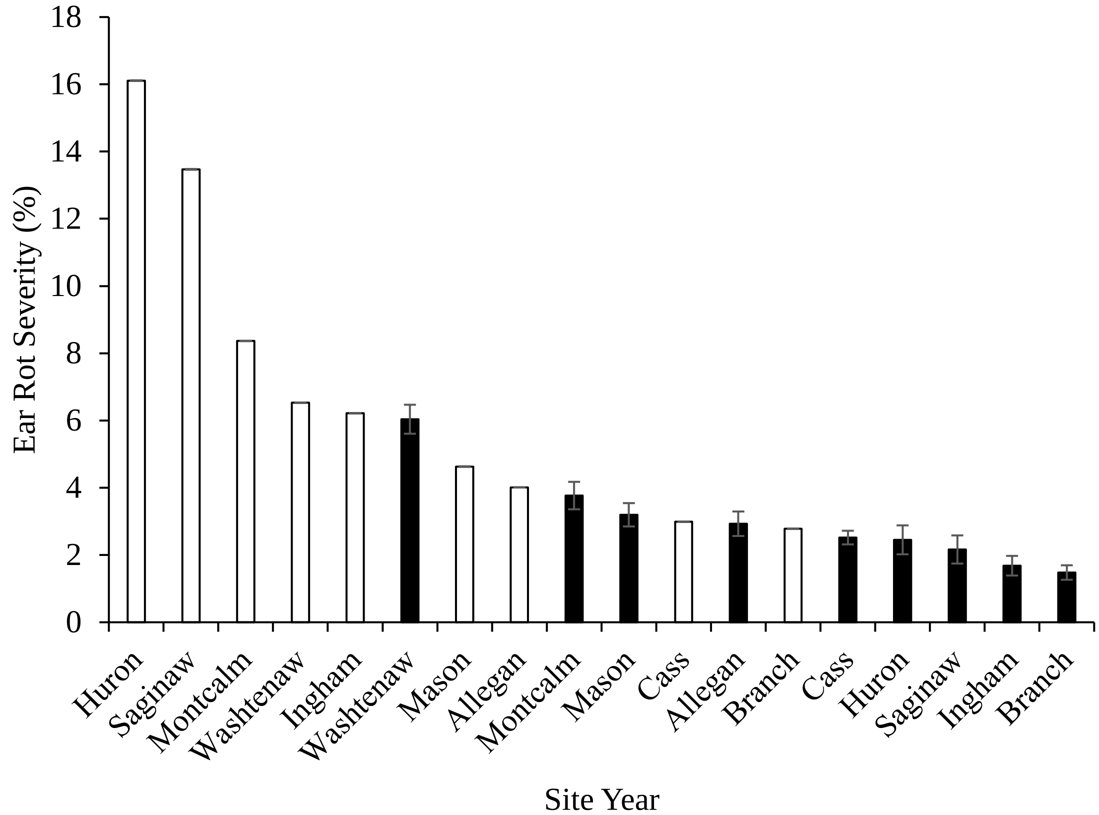

Figure 2-5. Average ear rot severity by location in 2017 (black bars) and 2018 (white bars) across all treatments. Error bars represent plus or minus one standard error.

Table 2-2. Analysis of variance results for deoxynivalenol (DON) concentration, western bean cutworm incidence (WBCI), ear rot incidence (ERI), and ear rot severity (ERS) of ear rot tolerance rating, insect trait, and fungicide from four locations in Michigan in 2017 and 2018. The four locations were chosen from a set of 18 site-years based on an average DON value greater than 2 μg g^{-1}.

Location	Num DF	WBCI		ERI		ERS		DON	
		F Value	P-value	F Value	P-value	F Value	P-value	F Value	P-value
Washtenaw 2017									
Ear Rot Tolerance Rating	2	14.42	0.0007	17.12	0.0005	4.65	0.0219	5.94	0.0080
Insect Trait	1	2.43	0.1418	3.99	0.0638	5.64	0.0277	10.37	0.0037
Ear Rot Tolerance Rating * Insect Trait	2	3.31	0.0692	4.88	0.0325	5.19	0.0152	2.73	0.0854
Huron 2018									
Ear Rot Tolerance Rating	2	1.87	0.1671	3.19	0.0511	1.29	0.2865	1.00	0.3744
Insect Trait	1	2.98	0.0916	2.15	0.1494	17.78	0.0002	12.62	0.0009
Ear Rot Tolerance Rating * InsectTrait	2	2.19	0.1239	0.46	0.6372	4.29	0.0210	3.95	0.0258
Fungicide	1	0.43	0.5163	0.28	0.5978	4.65	0.0375	1.32	0.2562
Ear Rot Tolerance Rating*Fungicide	2	0.54	0.5851	0.77	0.4697	0.33	0.7216	1.59	0.2153
Insect Trait*Fungicide	1	0.06	0.8024	0.1	0.7479	1.07	0.3077	0.05	0.8210
Ear Rot Tolerance Rating *Insect Trait* Fungicide	2	0.73	0.4863	0.38	0.6847	0.07	0.9289	0.94	0.3964
Montcalm 2018									
Ear Rot Tolerance Rating	2	0.6	0.5554	0.02	0.9799	1.63	0.2072	3.69	0.0367
Insect Trait	1	1.15	0.2887	0.29	0.5930	0.01	0.9242	1.48	0.2304
Ear Rot Tolerance Rating * InsectTrait	2	0.08	0.9212	1.28	0.2886	0.24	0.7843	0.71	0.5010
Fungicide	1	1.89	0.1760	0.54	0.4669	0.01	0.9296	0.33	0.5671
Ear Rot Tolerance Rating*Fungicide	2	0.81	0.4510	0.62	0.5447	1.47	0.2400	0.46	0.6353
Insect Trait*Fungicide	1	1.26	0.2678	0.98	0.3272	0.05	0.8180	0.29	0.5932
Ear Rot Tolerance Rating *Insect Trait* Fungicide	2	1.05	0.3585	1.42	0.2507	0.74	0.4821	1.11	0.3415
Saginaw 2018									
Ear Rot Tolerance Rating	2	0.27	0.7642	0.01	0.9863	0.06	0.9433	13.44	0.0002

Table 2-2 (cont'd)

Insect Trait	1	0.82	0.3754	3.52	0.0729	1.11	0.3054	20.45	0.0002
Ear Rot Tolerance Rating * Insect Trait	2	0.48	0.6271	1.17	0.3264	0.16	0.8561	2.20	0.1374

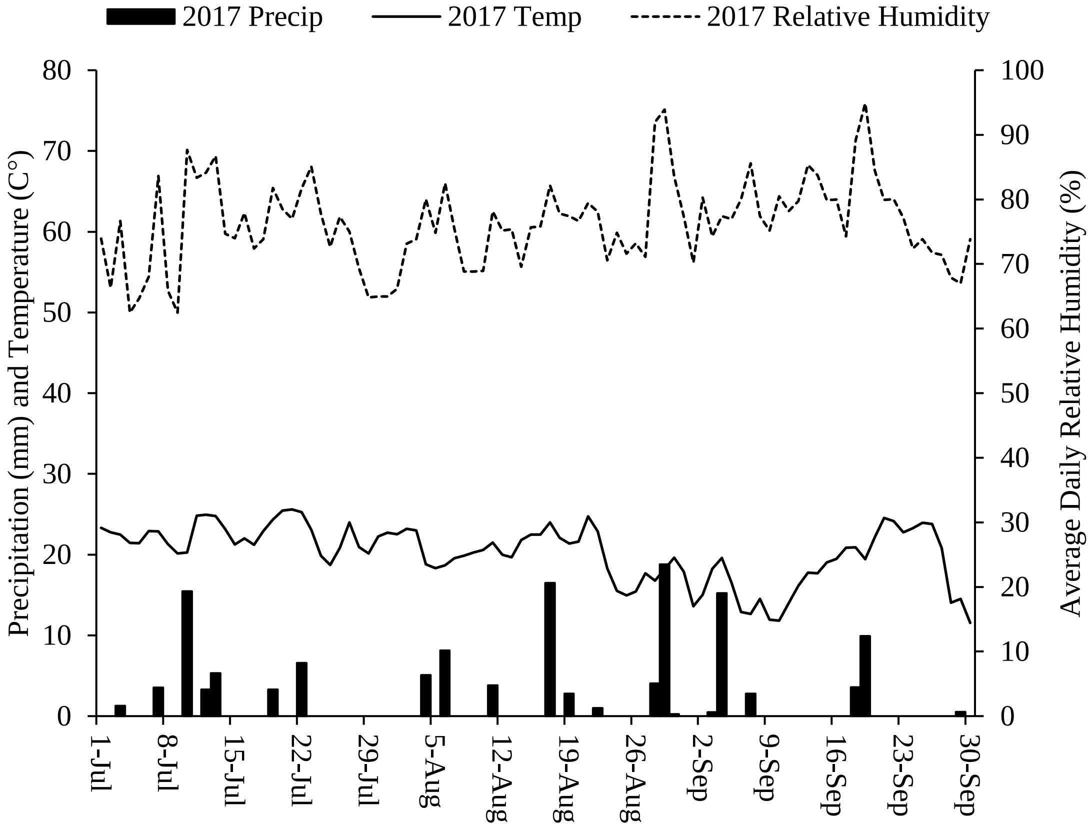

Figure 2-6. Average daily weather conditions in Washtenaw 2017. Bars indicate daily total precipitation values (mm). Solid line indicates daily average temperature and the dashed line indicates average daily relative humidity. Weather data obtained from the Michigan Automated Weather Network https://mawn.geo.msu.edu/. Silking occurred approximately between July 22 and July 25 2017.

Table 2-3. Interaction effect between ear rot tolerance rating level and insect trait package at Washtenaw 2017 on ear rot incidence levels (%).

Ear Rot Tolerance Rating	Insect Trait Package	Ear Rot Incidence (%)
Below Average	SmartStax	76.0 b
	VT Double PRO	78.0 ab
Average	SmartStax	98.0 ab
	VT Double PRO	73.0 b
Above Average	SmartStax	100.0 a
	VT Double PRO	99.1 a

† Numbers followed by a different letter are significantly different (P<0.05)

Table 2-4. Interaction effect between ear rot tolerance rating and insect trait package at Washtenaw 2017.

Ear Rot Tolerance Rating	Insect Trait Package	Ear Rot Severity (%)	
Below Average	SmartStax	4.2	b
	VT Double PRO	5.3	ab
Average	SmartStax	8.2	a
	VT Double PRO	4.1	b
Above Average	SmartStax	8.0	a
	VT Double PRO	6.4	ab

† Numbers followed by a different letter are significantly different (P<0.05)

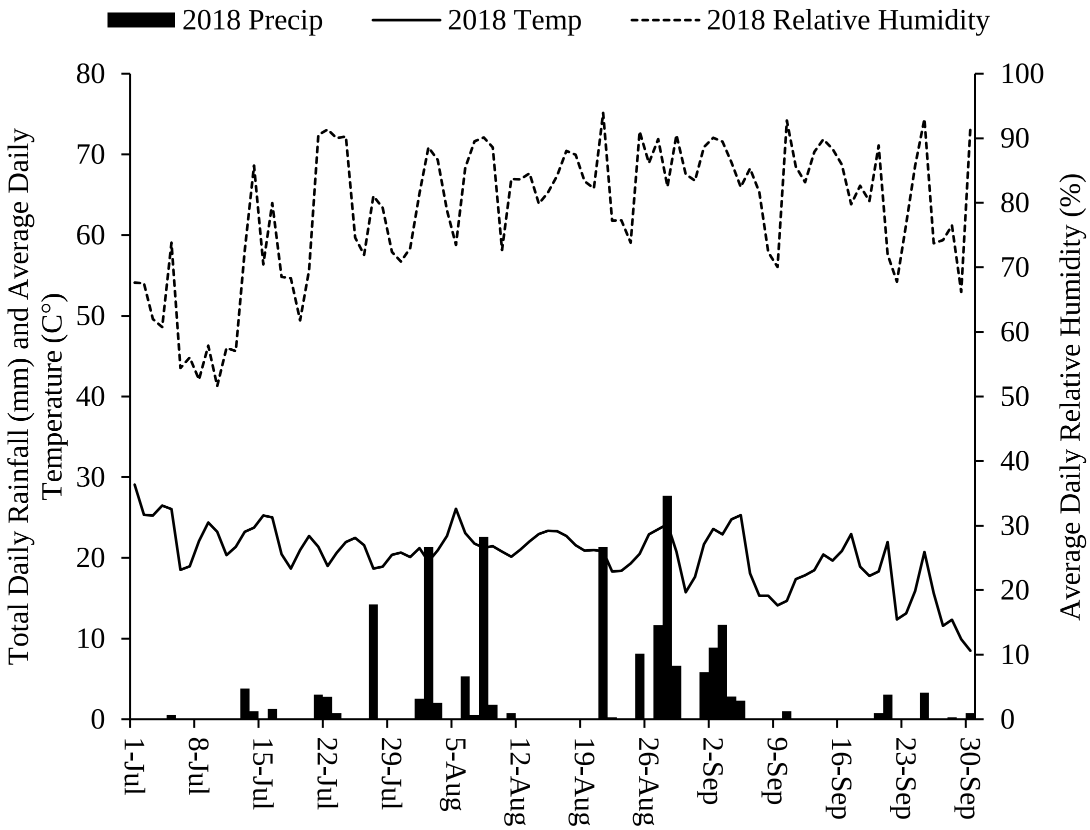

Figure 2-7. Average daily weather conditions for Huron 2018. Bars indicate daily total precipitation values (mm). Solid line indicates daily average temperature and the dashed line indicates average daily relative humidity. Weather data obtained from the Michigan Automated Weather Network https://mawn.geo.msu.edu/. Silking occurred approximately between July 29 and August 1 2018.

Table 2-5. Interaction effect between ear rot tolerance rating and Insect Trait Package at Huron 2018.

Ear Rot Tolerance Rating	Insect Trait Package	Ear Rot Severity (%)	
Below Average	SmartStax	14.5	bc
	VT Double PRO	15.1	bc
Average	SmartStax	12.4	bc
	VT Double PRO	21.0	ab
Above Average	SmartStax	11.7	c
	VT Double PRO	24.8	a

† Numbers followed by a different letter are significantly different (P<0.05)

Table 2-6. Interaction effect between ear rot tolerance rating and Insect Trait Package at Huron 2018

Ear Rot Tolerance Rating	Insect Trait Package	DON Level	
Below Average	SmartStax	6.0	b
	VT Double PRO	8.1	ab
Average	SmartStax	8.2	ab
	VT Double PRO	10.2	ab
Above Average	SmartStax	3.9	b
	VT Double PRO	13.7	a

† Numbers followed by a different letter are significantly different (P<0.05)

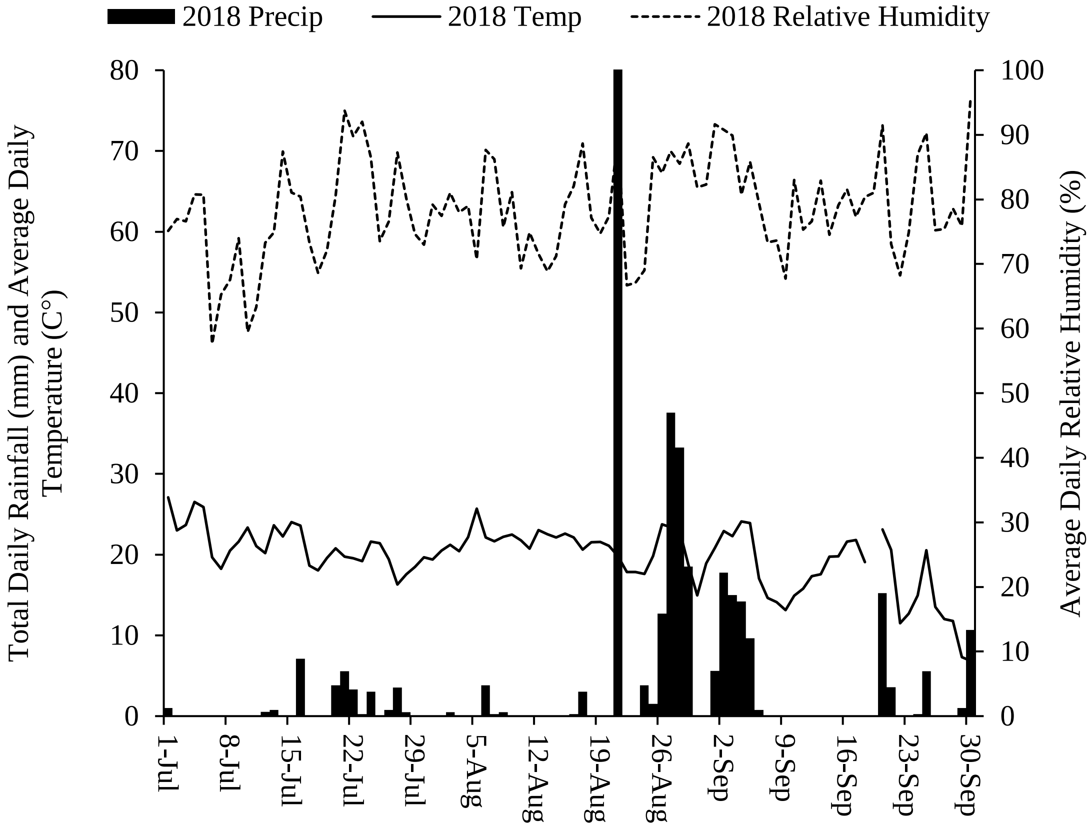

Figure 2-8. Average daily weather conditions for Montcalm 2018. Bars indicate daily total precipitation values (mm). Solid line indicates daily average temperature and the dashed line indicates average daily relative humidity. Precipitation, temperature, and relative humidity (July 1- August 12 and September 21-September 30) obtained from the Michigan Automated Weather Network https://mawn.geo.msu.edu/. Relative humidity data for August 12 through September 20 obtained from NASA POWER Agroclimatology. Silking occurred approximately between July 24 and July 30 2018.

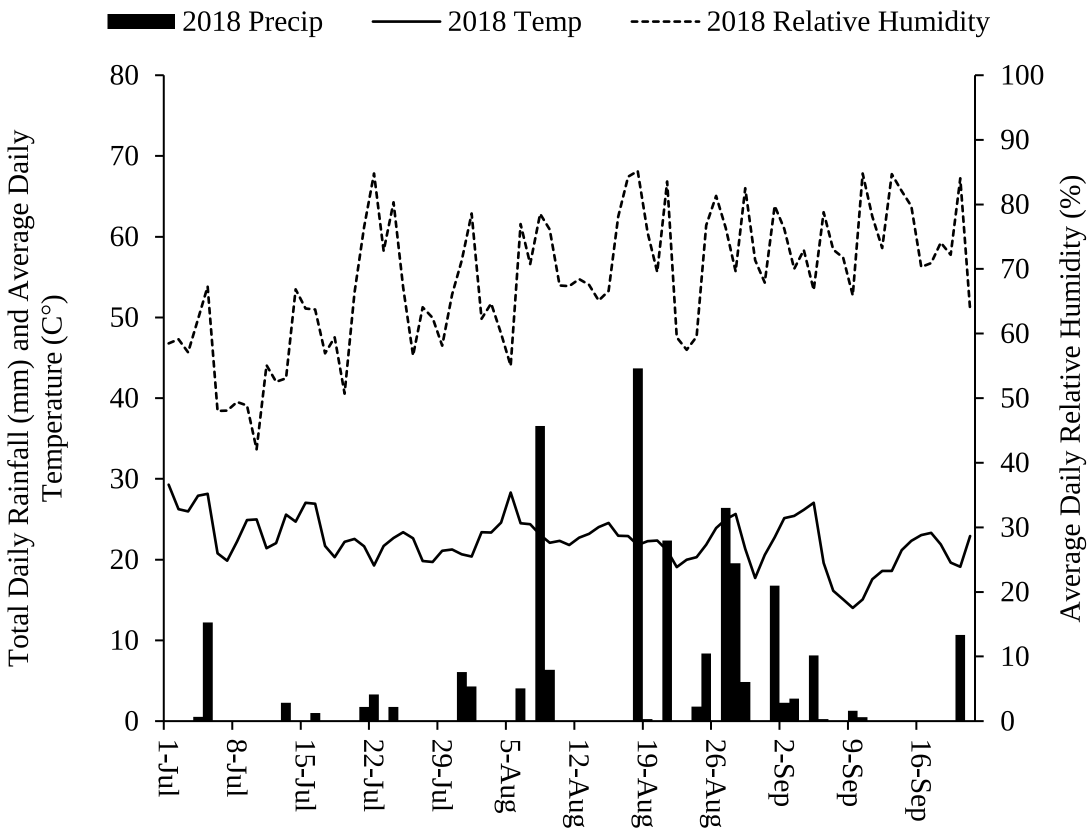

Figure 2-9. Average Daily weather conditions for Saginaw 2018. Bars indicate daily total precipitation values (mm). Solid line indicates daily average temperature and the dashed line indicates average daily relative humidity. Weather data obtained from the Michigan Automated Weather Network https://mawn.geo.msu.edu/. Silking occurred approximately between August 2 and August 5 2018.

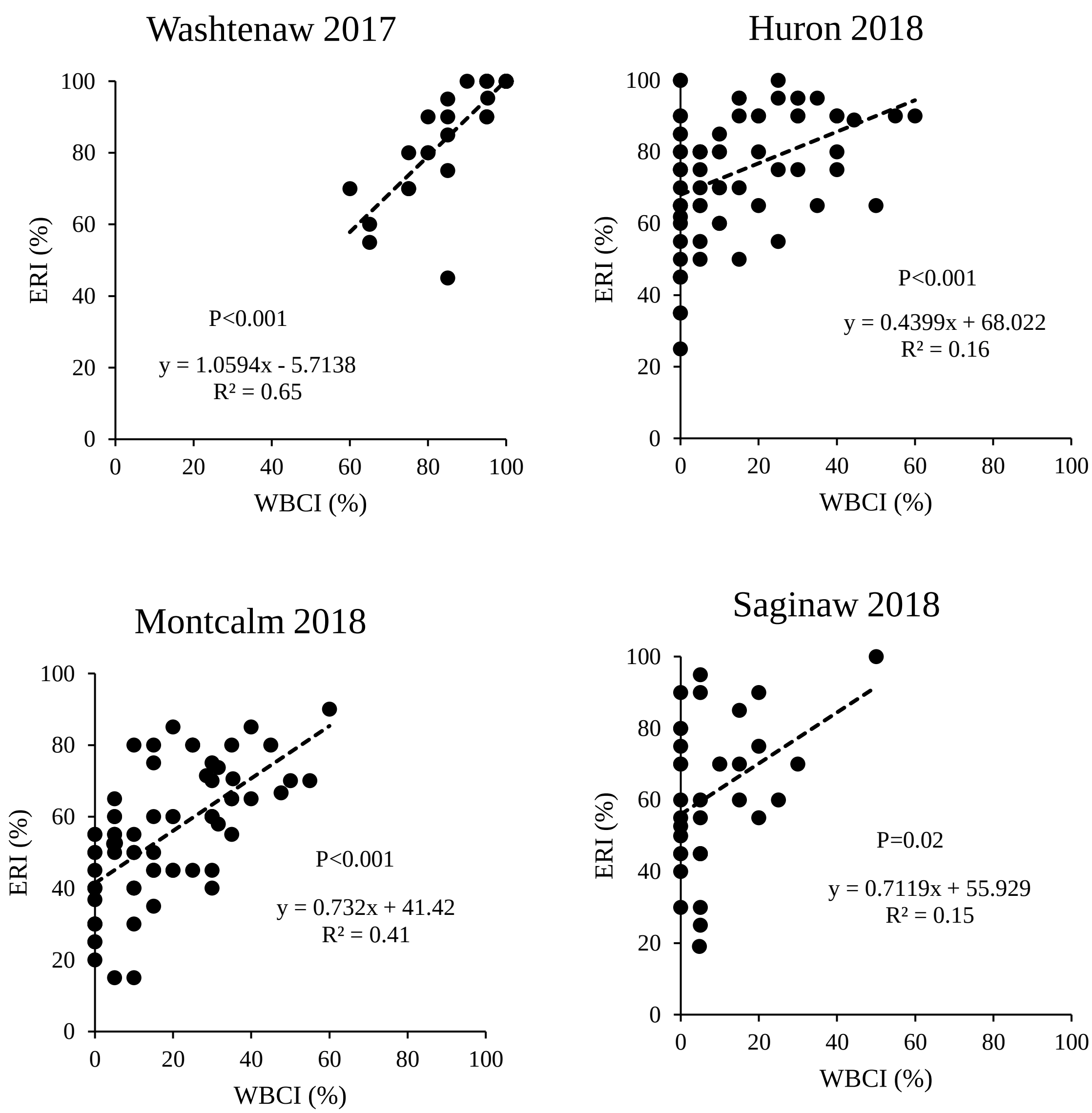

Figure 2-10. Correlation between western bean cutworm (WBCI) and ear rot incidence (ERI) at each of four site years in Michigan.

Table 2-7. Deoxynivalenol (DON), western bean cutworm incidence (WBCI), ear rot incidence (ERI), and ear rot severity (ERS) in Viptera and non-Viptera hybrids at the three site-years in 2018.

Location	Trait	WBCI (%)	ERI (%)	ERS (%)	DON (μg g^{-1})
Huron 2018	Viptera	2.23 b	50.7 b	13.3 a	5.0 b
	Non-Viptera	32.2 a	78.0 a	16.6 a	8.4 a
Montcalm 2018	Viptera	7.03 b	54.8 a	10.8 a	4.5 a
	Non-Viptera	19.2 a	62.8 a	13.9 a	4.2 a
Saginaw 2018	Viptera	1.95 b	48.6 a	8.37 a	8.7 a
	Non-Viptera	9.00 a	55.1 a	8.30 a	9.0 a

† Numbers followed by a different letter within a location are significantly different (P<0.05)

APPENDIX B

FUNGICIDE EFFECT ON YIELD

The prothioconazole fungicide Proline® 480 SC (Bayer, Research Triangle Park, NC) was applied at a labeled rate of 416.32 ml ha^{-1} in a randomized complete block design along with non-fungicide control plots. Applications were made using a pressurized CO^2 high clearance backpack sprayer with a 3.05 m wide boom, fitted with TeeJet 8001VS nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 50.8 cm apart. Boom height was approximately 30.48 cm above the tassel. Fungicide applications were made at full silk emergence (approximately 4-8 days after R1) before the browning of the silks (Table 2-1). One location (Cass 2018) received a blanket fungicide application of Delaro® fungicide (prothioconazole and trifloxystrobin) via overhead irrigaion at 365.56 ml ha^{-1} prior to the application of prothioconazole. Delaro is not labeled for the control of Gibberella ear rot but is used for the control of certain leaf diseases in maize.

Yield was determined for each plot in each of the 18 site-years by harvesting the middle two rows using a Kincaid 8-XP (Kincaid Equipment Manufacturing, Haven, KS) plot combine, except the Washtenaw location in 2018. In Washtenaw 2018, 6.096 m of row was hand harvested, threshed using a Haban Husker-Sheller (Haban Manufacturing Co, Racine, WI), and used to calculate plot yield. Yield from 20 hand harvested ears for mycotoxin analysis was added back into yield data from the plot combine to determine final overall yield for each plot. Yield comparisons for each location were made across all study hybrids.

Yield between fungicide treated plots and non-treated control plots was significantly different at two locations: Branch 2017 and Allegan 2018 (Table 2-8). Yields were 0.62 Mg hectare^{-1} greater in Branch 2017 in treated plots compared to non-treated plots. Yields were 0.56 Mg hectare^{-1} greater in Allegan 2018 in treated plots compared to non-treated plots (Figure 2-11). At all other locations, plots were not significantly different from one another (Table 2-8).

Ear rot index (the product of ear rot incidence and severity) did not correlate with yield in either 2017 or 2018 (Figure 2-12). Ear rots did not likely have a great effect on yield in trial locations. Fungicides were found to have no effect on ear rot incidence and only had an effect on ear rot severity at one out of two locations (Chapter 2). Therefore, yield benefits from fungicide use were likely not from fungicide reduction of ear rots. The reduction in yield may have come from a reduction in foliar disease with the use of a fungicide.

Return on investment (ROI) for fungicide application was calculated using an approximated fungicide cost of \$61.75 USD ha^{-1} cost. In most scenarios, fungicide had a negative ROI regardless of maize prices (Table 2-9). At a \$61.75 ha^{-1} application cost the breakeven point for \$118 USD Mg^{-1} maize would be 0.523 Mg ha^{-1}, \$138 maize would be 0.447 Mg ha^{-1}, \$158 maize would be 0.391 Mg ha^{-1}, and \$177 maize would be 0.349 Mg ha^{-1}.

Table 2-8. Analysis of variance results for the effect of fungicide application on maize grain yield from six locations in Michigan in 2017 and seven locations in 2018.

	2017			2018		
	dF[1]	F Value	P-Value	dF[1]	F Value	P-Value
Allegan	1, 54	0.58	0.4510	1, 67	7.13	0.0095
Branch	1, 54	6.62	0.0128	1, 53.32	0.67	0.4159
Cass	1, 47.05	3.89	0.0544	1, 68	0.41	0.5231
Huron	1, 54	2.46	0.1225	1, 64	1.45	0.2331
Ingham	1, 46.71	0.62	0.4346	1, 64	0.07	0.7967
Montcalm	1, 55	0.07	0.7946	1, 68	0.17	0.6847
Washtenaw	-	-	-	1, 68	0.01	0.9288

[1] First number indicates numerator degrees of freedom while second number indicates denominator degrees of freedom.

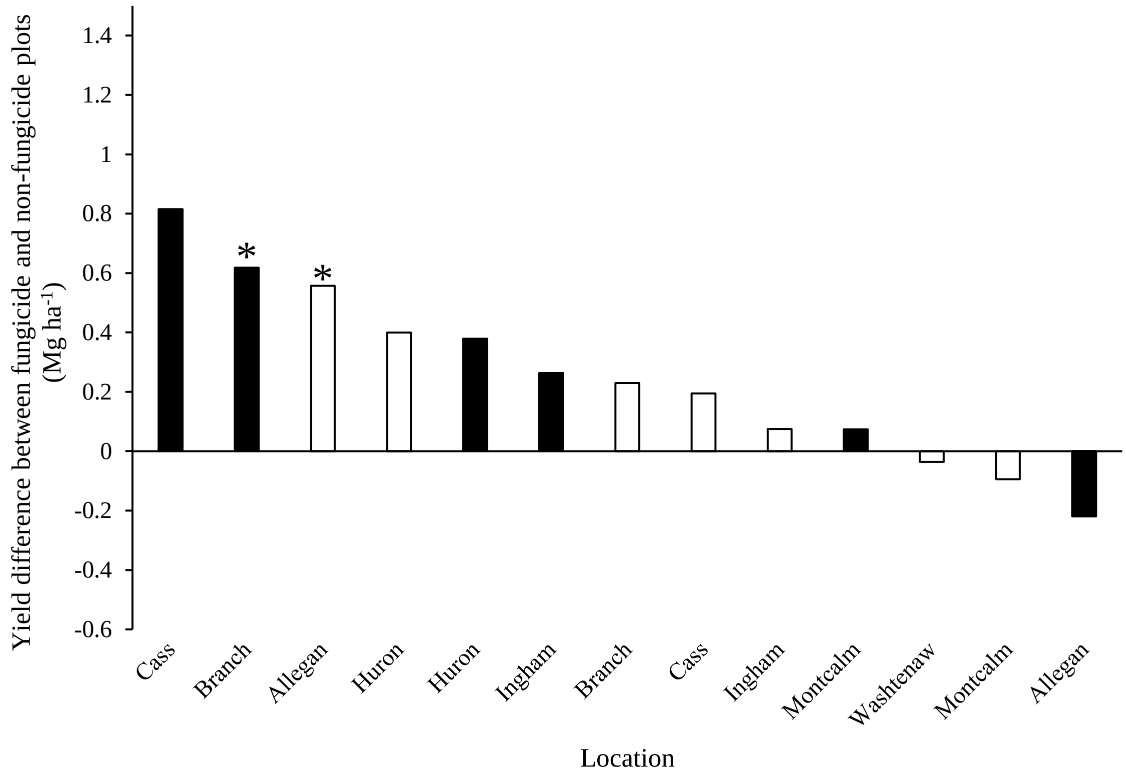

Figure 2-11. Difference in maize grain yield between fungicide treated and non-treated plots at each of 18 site-years in 2017 (black bars) and 2018 (white bars). Asterisk indicates significantly different values between fungicide treated and non-treated plots at a p-value of 0.05. Error bars indicate plus or minus one standard error.

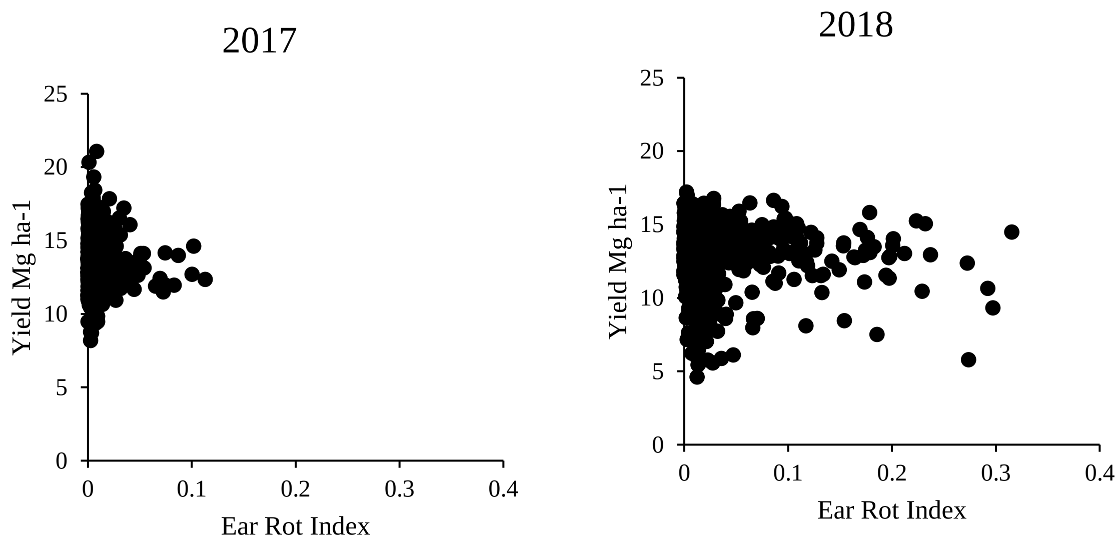

Figure 2-12. Correlation between ear rot index, calculated as the product of the incidence and severity, and maize grain yield (Mg ha^{-1}) at nine locations in each 2017 (left) and 2018 (right) ($p=0.078$ and 0.18 respectively)

Table 2-9. Return on investment of a fungicide at each of 13 locations in 2017 and 2018 at maize grain prices ranging from \$118 Mg^{-1} to \$177 Mg^{-1} and an approximate fungicide cost of \$61.75/ha. Return on investment was calculated using the price per hectare of fungicide application (cost for product and application costs), maize price per Mg, and the change in yield between treated and non-treated plots.

Location	Yield difference between treated and non-treated (Mg)	Maize Price Per Hectare			
		$118.00	$138.00	$158.00	$177.00
Cass 2017	0.8156	$34.49	$50.80	$67.11	$82.61
Branch 2017	0.6185	$11.23	$23.60	$35.97	$47.72
Allegan 2018	0.5571	$3.99	$15.13	$26.27	$36.86
Huron 2018	0.3996	-$14.60	-$6.61	$1.39	$8.98
Huron 2017	0.3783	-$17.11	-$9.54	-$1.98	$5.21
Ingham 2017	0.2635	-$30.66	-$25.39	-$20.12	-$15.11
Branch 2018	0.2297	-$34.65	-$30.05	-$25.46	-$21.09
Cass 2018	0.1946	-$38.79	-$34.90	-$31.00	-$27.31
Ingham 2018	0.0747	-$52.94	-$51.44	-$49.95	-$48.53
Montcalm 2017	0.0742	-$52.99	-$51.51	-$50.03	-$48.62
Washtenaw 2018	-0.0361	-$66.01	-$66.73	-$67.45	-$68.14
Montcalm 2018	-0.0943	-$72.88	-$74.76	-$76.65	-$78.44
Allegan 2017	-0.2191	-$87.60	-$91.99	-$96.37	-$100.53

*Price docking from mycotoxin contamination was not included in ROI calculations because in instances where DON levels are high enough for price docking to occur, fungicides were not found to lower mycotoxin levels (Chapter 2).

LITERATURE CITED

LITERATURE CITED

Abendroth, L., R. Elmore, M. Boyer and S. Marlay. 2011. Corn Growth and Development. Iowa State University Extension, Ames, IA.

Anderson, N.R., M.P.R. Luna, J.D. Ravellette and K.A. Wise. 2017. Impact of foliar fungicides on Gibberella ear rot and deoxynivalenol levels in Indiana corn. Plant Health Progress: PHP-01-17-0010-RS. doi:10.1094/php-01-17-0010-rs.

Bayer Crop Science. 2016. Bayer CropScience LP Proline® 480 SC Fungicide. Retrieved October 21, 2019. (http://www.cdms.net/ldat/ld89K001.pdf)

Blandino, M., Reyneri, A., Vanara, F., Pascale, M., Haidukowski, M., & Saporiti, M. 2008. Effect of sowing date and insecticide application against European corn borer (Lepidoptera: Crambidae) on fumonisin contamination in maize kernels. Crop Protection, 27(11), 1432-1436. doi:10.1016/j.cropro.2008.06.005

Bowers, E., R. Hellmich and G. Munkvold. 2013. Vip3Aa and Cry1Ab proteins in maize reduce Fusarium ear rot and fumonisins by deterring kernel injury from multiple Lepidopteran pests. World Mycotoxin Journal 6: 127-135. doi:10.3920/wmj2012.1510.

Catangui, M.A. and R.K. Berg. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera : Noctuidae), as a potential pest of transgenic Cry1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environmental Entomology 35: 1439-1452. doi:10.1093/ee/35.5.1439.

Chungu, C., D.E. Mather, L.M. Reid and R.I. Hamilton. 1996. Inheritance of kernel resistance to *Fusarium graminearum* in maize. Journal of Heredity 87: 382-385. doi:10.1093/oxfordjournals.jhered.a023019.

Dai, S.W., M.D. Shulski, K.G. Hubbard and E.S. Takle. 2016. A spatiotemporal analysis of Midwest US temperature and precipitation trends during the growing season from 1980 to 2013. International Journal of Climatology 36: 517-525. doi:10.1002/joc.4354.

DiFonzo, C.D. and R. Hammond. 2008. Range expansion of western bean cutworm, *Striacosta albicosta* (Noctuidae), into Michigan and Ohio. Crop Management 7.

DiFonzo, C.D. and P. Porter. 2019. The Handy Bt Trait Table for U.S. Corn Production. Retrieved December 5, 2019. (https://lubbock.tamu.edu/files/2018/11/BtTraitTableNov2018.pdf)

Dill-Macky, R. and R.K. Jones. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease 84: 71-76. doi:10.1094/pdis.2000.84.1.71.

Dutzmann, S. and A. Suty-Heinze. 2004. Prothioconazole: a broad spectrum demethylation-inhibitor (DMI) for arable crops. Pflanzenschutz-Nachrichten Bayer 57: 249-264.

Folcher, L., M. Delos, E. Marengue, M. Jarry, A. Weissenberger, N. Eychenne, et al. 2010. Lower mycotoxin levels in Bt maize grain. Agronomy for Sustainable Development 30: 711-719. doi:10.1051/agro/2010005.

Fuentes, R.G., H.R. Mickelson, R.H. Busch, R. Dill-Macky, C.K. Evans, W.G. Thompson, et al. 2005. Resource allocation and cultivar stability in breeding for Fusarium head blight resistance in spring wheat. Crop Science 45: 1965-1972. doi:10.2135/cropsci2004.0589.

Greig, J. 2019. Deep Discount Questions. Farmtario. Retreieved October 30, 2019. (https://farmtario.com/news/ontario-farmers-took-deep-discounts-on-mouldy-corn-in-2018/)

Groth, J.V., E.A. Ozmon and R.H. Busch. 1999. Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries. Plant Disease 83: 1033-1038. doi:10.1094/pdis.1999.83.11.1033.

Haidukowski, M., A. Visconti, G. Perrone, S. Vanadia, D. Pancaldi, L. Covarelli, et al. 2012. Effect of prothioconazole-based fungicides on Fusarium head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions. Phytopathologia Mediterranea 51: 236-246.

Hammond, B.G., K.W. Campbell, C.D. Pilcher, T.A. Degooyer, A.E. Robinson, B.L. McMillen, et al. 2004. Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United States in 2000-2002. Journal of Agricultural and Food Chemistry 52: 1390-1397. doi:10.1021/jf030441c.

Khonga, E.B. and J.C. Sutton. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 10: 232-239. doi:10.1080/07060668809501730.

Mansfield, M.A., E.D. De Wolf and G.A. Kuldau. 2005. Relationships between weather conditions, agronomic practices, and fermentation characteristics with deoxynivalenol content in fresh and ensiled maize. Plant Disease 89: 1151-1157. doi:10.1094/pd-89-1151.

Miller, S.S., L.M. Reid and L.J. Harris. 2007. Colonization of maize silks by *Fusarium graminearum*, the causative organism of Gibberella ear rot. Canadian Journal of Botany-Revue Canadienne De Botanique 85: 369-376. doi:10.1139/b07-027.

Mueller, D.S., K.A. Wise, A.J. Sisson, T.W. Allen, G.C. Bergstrom, D.B. Bosley, et al. 2016. Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. Plant Health Progress 17: 211-222.

Munkvold, G.P. 2003. Epidemiology of Fusarium diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705-713. doi:10.1023/a:1026078324268.

Munkvold, G.P., R.L. Hellmich and L.G. Rice. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Disease 83: 130-138. doi:10.1094/pdis.1999.83.2.130.

Ostrem, J.S., Z. Pan, J.L. Flexner, E. Owens, R. Binning and L.S. Higgins. 2016. monitoring susceptibility of western bean cutworm (lepidoptera: noctuidae) field populations to B*acillus thuringiensis* Cry1F protein. Journal of Economic Entomology 109: 847-853. doi:10.1093/jee/tov383.

Parker, N.S., N.R. Anderson, D.S. Richmond, E.Y. Long, K.A. Wise and C.H. Krupke. 2017. Larval western bean cutworm feeding damage encourages the development of Gibberella ear rot on field corn. Pest Management Science 73: 546-553. doi:10.1002/ps.4313.

Prelusky, D.B., B.A. Rotter and R.G. Rotter. 1994. “Toxicology of mycotoxins”. Pp. 359-403 in Mycotoxins in grain: Compounds other than aflatoxin, edited by J.D. Miller and H.L. Trenholm. Minnestota: Eagan Press.

Reid, L.M., D.E. Mather, R.I. Hamilton and A.T. Bolton. 1992. Genotypic differences in the resistance of maize silk to *Fusarium graminearum*. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 14: 211-214. doi:10.1080/07060669209500877.

Rice, M.E. 2000. Western bean cutworm hits northwest Iowa. Integrated Crop Management News.

Schaafsma, A.W., R.W. Nicol and L.M. Reid. 1997. Evaluating commercial maize hybrids for resistance to Gibberella ear rot. European Journal of Plant Pathology 103: 737-746. doi:10.1023/a:1008629629069.

Smith, J.L., DiFonzo, C.D. Baute, T.S., Michel, A.P., & Krupke, C.H. 2019. Ecology and Management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans- revision with focus on the great lakes region. Journal of Integrated Pest Management, 10(1). Doi:10.1093/jipm/pmz025.

Smith, J.L., M.D. Lepping, D.M. Rule, Y. Farhan and A.W. Schaafsma. 2017. Evidence for field-evolved resistance of *Striacosta albicosta* (Lepidoptera: Noctuidae) to Cry1F *Bacillus thuringiensis* protein and transgenic corn hybrids in Ontario, Canada. Journal of Economic Entomology 110: 2217-2228. doi:10.1093/jee/tox228.

Smith, J.L., V. Limay-Rios, D.C. Hooker and A.W. Schaafsma. 2018. Fusarium graminearum mycotoxins in maize associated with *Striacosta albicosta* (Lepidoptera: Noctuidae) injury. Journal of Economic Entomology 111: 1227-1242. doi:10.1093/jee/toy005.

Tschanz, A., R. Horst and P.E. Nelson. 1976. The effect of environment on sexual reproduction of *Gibberella zeae*. Mycologia 68: 327-340.

Unglesbee, E. 2016. Cry1F Crisis: Herculex trait fails against western bean cutworm. DTN/ The Progressive Farmer, October 5, 2016. Retrieved December 4, 2019.

(https://www.dtnpf.com/agriculture/web/ag/crops/article/2016/10/05/herculex-trait-fails-western-bean-4)

Unglesbee, E. 2017. Cry1F failure confirmed: companies no longer claim Herculex trait controls western bean cutworm. DTN/The Progressive Farmer, September 20, 2017. Retreived December 4, 2019. (https://www.dtnpf.com/agriculture/web/ag/crops/article/2017/09/20/companies-longer-claim-herculex-bean)

US Food and Drug Administration. 2010. Guidance for Industry and FDA: Advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. US FDA. Silver Spring, MD.

Vigier, B., L.M. Reid, L.M. Dwyer, D.W. Stewart, R.C. Sinha, J.T. Arnason, et al. 2001. Maize resistance to Gibberella ear rot: symptoms, deoxynivalenol, and yield. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 23: 99-105. doi:10.1080/07060660109506915.

Vigier, B., L.M. Reid, K.A. Seifert, D.W. Stewart and R.I. Hamilton. 1997. Distribution and prediction of Fusarium species associated with maize ear rot in Ontario. Canadian Journal of Plant Pathology 19: 60-65.

Wise, K., D. Mueller, A. Sisson, D. Smith, C. Bradley and A. Robertson. 2016. A farmers guide to corn diseases. APS Press, The American Pytopathological Society, St. Paul, MN.

Wuebbles, D.J., Fahey, D.W., & Hibbard, K.A. 2017. Climate science special report: fourth national climate assessment, volume 1.

Yu, C.G., M.A. Mullins, G.W. Warren, M.G. Koziel and J.J. Estruch. 1997. The Bacillus thuringiensis vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Applied and Environmental Microbiology 63: 532-536.

CHAPTER 3

MYCOTOXIN CO-OCCURENCE IN MICHIGAN MAIZE GRAIN

Introduction

Mycotoxins are secondary metabolites produced by specific fungal species, particularity in the genera *Alternaria*, *Aspergillus*, *Fusarium*, or *Penicillium* (Grenier and Oswald, 2011, Lee, et al., 2015). These toxins can be harmful to both humans and animals. Acute, short term, exposure can lead to varied effects depending on the type of mycotoxin present (Fink-Gremmels, 1999). Although acute exposure is important, grain with high levels of mycotoxin usually does not enter the channel of trade. More often, undetected chronic exposure to low doses of mycotoxins is found. This chronic exposure can lead to reduced weight gain, diminished productivity, and increased susceptibility to infections in animals (Fink-Gremmels, 1999).

In maize grain (*Zea mays* L), mycotoxin contamination occurs through fungal ear rot infections. Because mycotoxin producing fungi are usually able to produce more than one type of mycotoxin, and grain can become infected with multiple fungal species at a time, it is important to understand the frequency of mycotoxin co-occurrence (Grenier and Oswald, 2011). When mycotoxins co-occur, they can interact and have antagonistic, additive, less than additive, or synergistic effects (Grenier and Oswald, 2011). However, limited information on incidences, severities, and effects of multiple mycotoxin contamination is available in maize grain.

Although there are thousands of mycotoxins currently identified, only a few are regulated across the world. These regulated mycotoxins are the aflatoxins, ochratoxin A, zearalenone (ZON), the fumonisins, and select trichothecenes including deoxynivalenol (DON), HT-2 and T-2 (Kovalsky, et al., 2016, Smith, et al., 2016). Although these are often the most important for the health and safety of humans and animals, other masked or emerging mycotoxins are also important. Masked mycotoxins are plant metabolites of other mycotoxins. Emerging mycotoxins are a group of mycotoxins with no current regulations that are very chemically diverse from one another (Kovalsky, et al., 2016). Masked and emerging mycotoxins are important as they may interact with regulated mycotoxins present in maize grain.

To regulate the amount of mycotoxin entering grain markets, the United States (U.S.) and other countries set limits in grain sold in the marketplace. The U.S. Food and Drug Administration (FDA) has set action levels for aflatoxins and advisory levels for DON and fumonisins (US Food and Drug Administration, 2001, 2010, 2019). In the U.S. these mycotoxins are regularly tested for in food and feed due to their regulated status. Outside of the U.S., all countries with set mycotoxin regulations have limits for aflatoxin B1 or the total aflatoxin level in food and/or feed (van Egmond, et al., 2007). Other mycotoxins regulated in various countries include aflatoxin M1, diacetoxyscirpenol, T-2, HT-2, agaric acid, the ergot alkaloids, ochratoxin A, patulin, phomopsins, sterigmatocystin, and zearalenone (van Egmond, et al., 2007). These mycotoxins are regulated in other countries due to health concerns but can remain undetected in the U.S. maize supply as they are not regularly tested for.

Due to limited information available on multiple mycotoxins in maize grain in Michigan and across the northern Corn Belt, the objectives of this study were to determine the type of mycotoxins present in Michigan maize, their level, and frequency of occurrence and co-occurrence with one another in relation to environmental variability.

Methods

Samples were obtained from a larger experiment at nine locations in Michigan in both the 2017 and 2018 growing seasons for a total of 18 unique site-years (Figure 3-1). Locations were selected to represent the variability of environmental conditions across three growing zones in

Michigan. All plots were planted on commercial farms and all fungal infection was from natural sources. Plots were planted with a four-row Almaco packet planter (Almaco, Nevada, IA) with row spacing of 0.76 m. Plots measured 6.71 m by 3.05 m with 0.91 m alleyways between plots. The center two rows of each plot were used for data collection with the outer two rows acting as a buffer. Plots were managed according to grower standards for the area. One location (Cass 2018) received a blanket fungicide application of Delaro® fungicide via overhead irrigation (prothioconazole and trifloxystrobin) at 365.56 ml ha^{-1}. Delaro® is not labeled for the control of ear rots in maize grain. All other locations in the study were not treated with a fungicide. Agronomic details for each site-year are presented in Table 3-1.

Samples were collected from five replicated plots of one hybrid typical to Michigan growers at each of the 18 site-years. This resulted in 90 samples across two years of the study period. The hybrid selected required 2600 GDD to black layer. This hybrid was chosen because it had “average” tolerance to Gibberella ear rot, Aspergillus ear rot, and Fusarium ear rot as rated by the company. The Bt trait package had the Lepidoptera proteins Cry1A.105, Cry1Ab2, and Cry1F which provide protection against several ear feeding Lepidoptera insects. This includes black cutworm (*Agrotis ipsilon*), corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), stalk borer (*Papaipema nebris*) (DiFonzo and Porter, 2019). Western bean cutworm, the most important ear feeding insect in Michigan was not controlled by the Bt hybrid. Ten consecutive ears from each of the middle two rows (20 ears total) were hand-harvested once ears reached physiological maturity (Abendroth, et al., 2011). Husks were removed and ears were shelled using a Haban Husker-Sheller (Haban Manufacturing Co, Racine, WI). The shelled grain from the 20 ears was combined into one bulk sample and mixed thoroughly for a representative sample. A 500 g sample was taken from the bulk plot sample for storage. From this 500 g subsample, a 50 g sample was obtained for mycotoxin analysis. This was ground using a cyclone sample mill (UDY Corporation, Fort Collins, CO) with a 1 mm screen and submitted for mycotoxin analysis.

Samples were sent to the University of Guelph, Ridgetown Campus, Ridgetown, Ontario, Canada for testing of 26 different mycotoxins (Table 3-2) using HPLC-ESI-MS/MS (Limay-Rios and Schaafsma, 2018). Limit of detection (LOD) and limit of quantification (LOQ) (Table 3-3 and Table 3-4) were calculated according to Limay-Rios and Schaafsma (2018). All values lower than the LOD were considered negative.

Mycotoxin data was analyzed using PROC GLIMMIX in SAS software (SAS Institute Inc., version 9.4) at a p-value of 0.05. Data analysis was conducted separately by year due to large environmental differences between 2017 and 2018. Location was considered as a fixed effect and replication as a random effect in the model. Locations were considered significantly different at $p<0.05$ and all pairwise comparisons were made using the lsmeans statement with Tukey's adjustment.

Results

Frequency of Multiple Mycotoxin Occurrence

Co-contamination by multiple mycotoxins was found to be highly prevalent in Michigan maize grain. Contamination with more than one mycotoxin was observed in all samples tested. Each sample in the study was contaminated with at least four different mycotoxins in 2017 and six different mycotoxins in 2018. The maximum number of contaminates per sample was 13 in 2017 and 16 in 2018. The majority of samples were contaminated with 10 different mycotoxins in both 2017 and 2018. The average number of individual contaminates per sample was nine in 2017 and 11 in 2018 (Figure 3-2).

Mycotoxin incidence and severity

Results from this study indicate that the overall incidence of mycotoxin contamination in Michigan is relatively high (Table 3-3 and Table 3-4). Every sample in the study was contaminated with mycotoxins. Several mycotoxins had particularly high incidences, showing up in a large number of the samples tested. In 2017, DON, ENNA, ENNB, FB1, and FB2 were found in more than 80% of samples tested, whereas BEAU, DON, D3G, 15ADON, FB1, FB2, FB3, and ZON were found in greater than 80% of samples in 2018.

Concentrations varied greatly with mycotoxin type (Figure 3-3). Several mycotoxins were found at concentrations higher than 1000 $\mu g\ kg^{-1}$. In 2017, these mycotoxins were BEAU, DON, D3G, FB1, FB2, FB3, and ZON. In 2018, the mycotoxins with concentrations greater than 1000 $\mu g\ kg^{-1}$ were BEAU, DON, D3G, 15ADON, FB1, FB2, FB3, MON, and ZON.

Regulated Mycotoxins

Deoxynivalenol, one of the regulated mycotoxins, was the most commonly occurring mycotoxin in the study with 93% of samples contaminated in 2017 (Table 3-3) and 100% contaminated in 2018 (Table 3-4). Deoxynivalenol was found at a high level in many of the samples. Average DON values in both 2017 and 2018 were greater than 1000 $\mu g\ kg^{-1}$. In the US, DON is regulated by the FDA through advisory levels. In the current study many samples were found to be above FDA advisory levels at 1000 $\mu g\ kg^{-1}$ for grain and grain by products for humans and 5000 $\mu g\ kg^{-1}$ for some animals, including swine (US Food and Drug Administration, 2010). Deoxynivalenol was found over 1000 $\mu g\ kg^{-1}$ in 42.2% of samples in 2017 and 80% of samples in 2018. It was also found to be over 5000 $\mu g\ kg^{-1}$ in 2.2% of samples in 2017 and 37.8% of samples in 2018.

Fumonisins are also an important mycotoxin regulated by the FDA. The fumonisins; fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3) are regulated in the U.S. as the total fumonisin concentration of all three analogs (US Food and Drug Administration, 2001). One sample was over the FDA limit of 20,000 μg kg^{-1} for total fumonisin concentration for swine (US Food and Drug Administration, 2001). This sample was from Huron in 2018 and had a total fumonisin concentration of 82,236 μg kg^{-1}.

The fumonisins FB1, FB2, and FB3 are often found occurring together (Murphy, Rice, & Ross, 1993). Contamination of samples by all three analogs, FB1, FB2, and FB3 was found in both years. All three analogs were found in 56% and 89% of samples in 2017 and 2018 respectively. Strong correlations were found between the concentrations of FB1 and FB2 in 2017 and 2018 (R^2= 0.97 and 0.97 respectively) and between FB1 and FB3 in 2018 (R^2=0.98).

Zearalenone (ZON) is considered a regulated mycotoxin although it is not regulated in the U.S or in Canada. Zearalenone was found in 69% of samples in 2017 and 100% of samples in 2018. At least 16 countries have ZON regulations in place with limits for ZON in maize and other cereals ranging from 50 to 1,000 μg kg^{-1} (Zinedine, et al., 2007). In 2017, 16 samples (35.6%) were over 50 μg kg^{-1} and 3 samples (6.7%) were over 1,000 μg kg^{-1}. During the 2018 growing season, 29 samples (64.4%) were over 50 μg kg^{-1} while 7 samples (15.6%) were over 1,000 μg kg^{-1}.

Like ZON, T-2 and HT-2 are not regulated in the U.S. In 2017, only T-2 was found in samples while in 2018 both T-2 and HT-2 were present. Certain countries regulate for T-2 toxin individually, while others regulate the total concentration of T-2 and HT-2. The European Union's lowest limit for T-2 and HT-2 is 15 μg kg^{-1} (Adhikari, et al., 2017). In both 2017 and 2018, 9% of samples were found to be above 15 μg kg^{-1}.

Masked Mycotoxins

Deoxynivalenol 3-ß-D-glycoside (D3G) is considered a masked mycotoxin and was present in a high number of samples with 73% and 100% of samples contaminated in 2017 (Table 3-3) and 2018 respectively (Table 3-4). DON and D3G were correlated in both years of the study with R^2 values of 0.82 and 0.63 in 2017 and 2018 respectively.

Emerging Mycotoxins

Emerging mycotoxins found in this study included beauvericin (BEAU), 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON), enniatin A (ENNA), enniatin A1 (ENNA1), enniatin B (ENNB), enniatin B1 (ENNB1), moniliformin (MON), and neoxolaniol (NEO). Out of these mycotoxins, BEAU was found most often with 78% contamination in 2017 (Table 3-3) and 100% contamination in 2018 (Table 3-4).

The mycotoxins 15ADON and 3ADON are derivatives of DON (Logreico, et al., 2002). The mycotoxin 15ADON was found in both 2017 and 2018 while 3ADON was only found in 2018. The correlation between DON and 15ADON had an R^2 value of 0.91 in both 2017 and 2018. The mycotoxin 3ADON was had a correlation R^2 value of 0.59 with DON.

Variability across locations

In 2017, DON, D3G, ZON, ENNA, and AME levels varied significantly by location (Table 3-5). Washtenaw had the highest DON levels but was not significantly different from Cass, Allegan, Branch or Montcalm. Likewise, D3G followed a similar pattern as DON with Washtenaw having the highest levels of D3G followed by Allegan, Cass, and Branch. Only four out of the nine sites had AME in 2017. The mycotoxin ENNA was found at all nine sites with Saginaw, Washtenaw, and Ingham having the highest levels of ENNA. Zearalenone was found to be the highest in Washtenaw while only very low levels were found in Huron, Ingham, Mason, Montcalm, and Saginaw.

In 2018, DON, D3G, HT-2, T-2, and NEO varied significantly by location in 2018 (Table 3-6). Huron had the highest DON levels but was not significantly different from Ingham, Mason, Montcalm, or Saginaw. In addition, D3G levels were found to be the highest in Saginaw and lowest in Branch and Washtenaw. Significantly, higher levels of T-2 were found in Huron than all other locations. HT-2 only occurred in two out of nine locations in 2018 while NEO was only present in Branch, Huron, and Mason counties.

Environmental Conditions

In 2017, average temperatures July through September at each location ranged between 17.7° C and 20.1° C. Temperatures were fairly consistent between locations in 2017 but rainfall differed greatly by location. July through September precipitation totals ranged from 75.8 mm to 199.8 mm in 2017. In 2017, all locations had less than 25 mm of rainfall in the seven days following R1 (silking). Allegan, Huron, and Ingham counties had less than 5 mm of rainfall (Figure 3-4).

Environmental conditions also varied between locations in 2018. Temperatures for July through September ranged between 19.7° C and 21.5° C in 2018. At the majority of locations in the study, temperatures trended higher in 2018 throughout most of July and August compared to 2017. Though temperatures were higher through July and August, temperatures in 2018 were lower during the last week of September compared to temperatures in 2017. Between locations, the temperature was less variable in 2018 than in 2017. Average temperatures ranged between 19.66° C and 21.48° C for the months of July through September. Precipitation levels were also generally higher in 2018 when compared to 2017. July through September precipitation totals ranged between 157.82 mm and 325.76 mm in 2018. Rainfall around silking was highly variable in 2018. Branch, Huron, and Washtenaw counties had greater than 30 mm of rain in the seven days following silking while Cass, Ingham, and Montcalm counties had less than 0.5 mm of rain following silking (Figure 3-4).

Discussion

Overall in this study, a large number of different mycotoxins were found across the state of Michigan. Not only was the incidence of these mycotoxins high but the concentration of mycotoxin contamination was high in certain scenarios. Along with this, the levels of mycotoxin co-occurrence was found to be extremely high across the two years of the study.

High levels of multiple mycotoxin contamination were shown to occur in this study with all samples testing positive for at least four different mycotoxins. Results from a worldwide mycotoxin survey between 2009 and 2011 found that North, Central, and South America had 40% of finished feed samples testing positive for contamination from multiple mycotoxins through either ELISA or HPLC testing for Aflatoxins, ZON, DON, Fumonisins, and Ochratoxins (Rodrigues and Naehrer, 2012). Studies from maize in Tanzania reported that 87% of samples were contaminated with more than one mycotoxin when tested using HPLC/TOFMS (Kamala, et al., 2015). Based on this information multiple mycotoxin co-occurrence is common in corn and other feedstuffs across many geographic areas. The implication of this high level of contamination by multiple mycotoxins on human and animal health is currently unknown. Data looking at the *in vivo* effects of multiple mycotoxins on humans and animals is limited. Because of this, researchers are unable to determine an accurate health risk for humans and animals from exposure to multiple mycotoxins (Grenier and Oswald, 2011).

Both the incidence and severity of mycotoxins influences the levels of contamination. The mycotoxins DON, BEAU, FB1, D3G, ZON, FB2, and FB3 were all found in greater than 80% of the samples surveyed across the two years of study. This is similar to findings from a neighboring region in Ontario, Canada where mycotoxins with the highest incidence were DON, FB1, and ZON (Schaafsma, et al., 2008). Mycotoxins found in both regions are produced by *Fusarium spp*. (Logrieco, et al., 2002, Marin, et al., 2004, Zinedine, et al., 2007) indicating that mycotoxin production by *Fusarium spp.* in maize grain may be the most common issue in the Great Lakes region. Rate of mycotoxin production can vary with the fungal genera and within strains of a fungal species (Fink-Gremmels, 1999). Ranges of mycotoxin contamination are especially important to consider when concentrations reach levels that cause harm to human and animal health.

Understanding the role of environmental factors on ear rot occurrence and associated mycotoxin accumulation is important in efforts to reduce the co-contamination of mycotoxins. Infection through maize silks is an important point of entry for many ear rot causing fungal pathogens (Munkvold, 2003). Environmental conditions before, during, and after the silking period are important for fungal infection (Munkvold, 2003). Differences in environmental conditions around silking between years and locations may factor into which mycotoxins are present and at what concentrations. Between locations, weather patterns were variable around silking times causing concentrations of mycotoxins to vary by location. Each fungal species grows under certain ideal conditions. Knowing the weather patterns around and following silking can help producers to predict which mycotoxins may be present in their fields and at what concentrations. This study demonstrates the variability of mycotoxins along with the variability of environmental conditions.

Overall, this study has determined that multiple mycotoxin contamination is present at high levels in Michigan and likely throughout the northern U.S. Corn Belt. Toxins not regularly tested for in the U.S. are present in maize grai at concerning levels, which may be affected by environmental conditions. A broader survey should be conducted to increase knowledge on the occurrence of multiple mycotoxin contamination in maize grain throughout the varied growing environments found in the U.S. Corn Belt. Along with this, these findings emphasize the importance of research into the effects of mycotoxin co-occurrence on human and animal health. Though concentrations of some detected mycotoxins were low, these should be taken into consideration due to the possible synergistic or additive effects with other mycotoxins occurring in Michigan maize grain.

APPENDIX

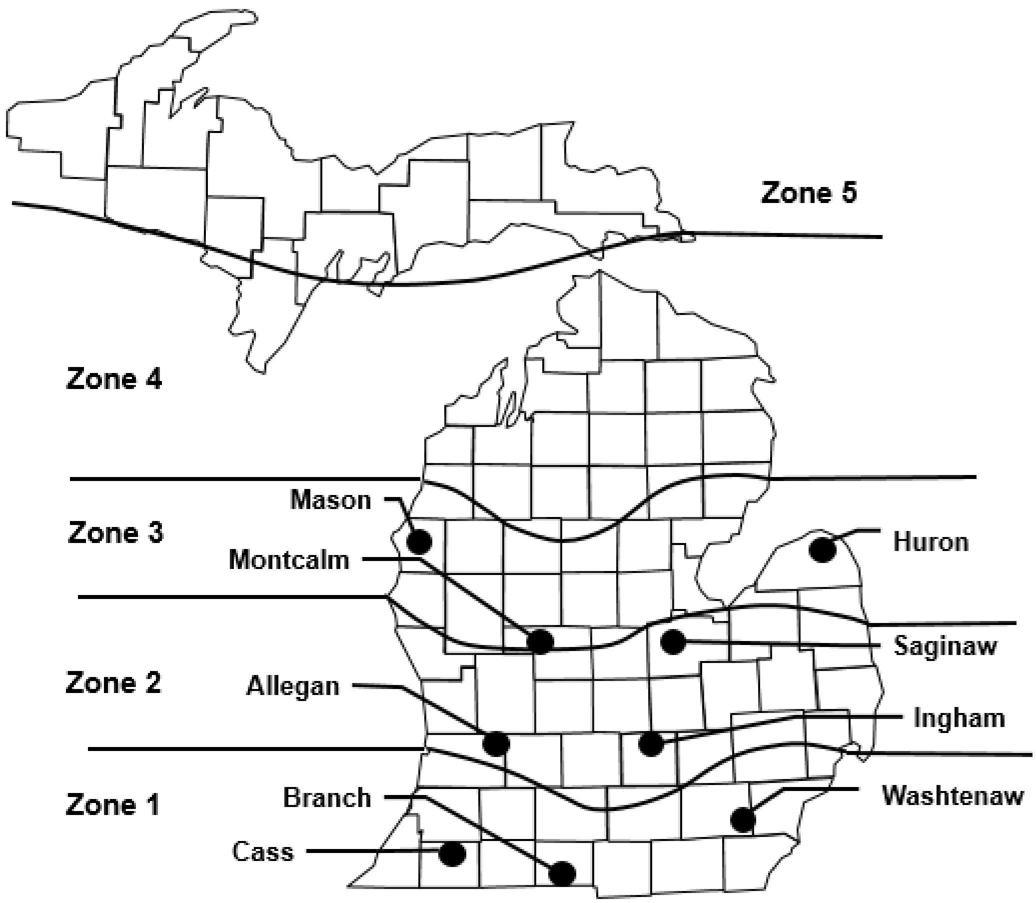

Figure 3-1. Maize grain sampling locations spread throughout lower Michigan in 2017 and 2018 with three locations in each of the lower three zones. Zones reflect 30 year (1981-2010) normal accumulated GDD from May 1 to October 31, 2557, 2478, and 2342 for zones 1, 2, and 3 respectively. Maize samples were obtained from the same hybrid (2600 growing degree units to black layer) at each location in both years of the study.

Table 3-1. Agronomic management details at each of nine locations in 2017 and 2018.

Location	Town	Previous Crop	Irrigation	Soil Classification	Soil Test Values pH	P	K	Planting Date	Average Population (plants hectare^{-1})[1]	Fertilizer N-P-K	Fungicide Application Date	Harvest Date
2017												
Allegan	Martin	Soybean	Dryland	Ockley loam	6.05	86	218	May 12	80,956	109-9-3 +manure	July 21	October 20
Branch	Coldwater	Soybean	Irrigated	Oshtemo sandy loam	6.4	115	117	May 31	85,586	221-9-3	August 1	October 17
Cass	Vandalia	Soybean	Irrigated	Kalamazoo loam	6.7	50	142.5	May 14	83,890	240-9-3	July 21	October 17
Huron	Bad Axe	Maize	Dryland	Kilmanagh loam	7.5	61	118	May 17	84,917	127-9-3 +manure	August 3	October 11
Ingham	East Lansing	Soybean	Dryland	Conover loam	6.85	52	197.5	May 23	83,646	190-9-3	July 26	October 13
Mason	Scottville	Carrots w/ rye cover	Irrigated	Fern-Marlette complex	6.1	139	153	May 10	81,575	109-9-3 +manure	-	October 27
Montcalm	Greenville	Soybean	Dryland	McBride and Isabella sandy loam	5.85	73	111.5	May 16	82,863	154-9-3	July 28	October 31
Saginaw	New Lothrop	Soybean	Dryland	Conover loam	6.8	44	118	May 29	85,325	154-9-3	-	October 15
Washtenaw	Milan	Soybean	Dryland	Pella silt loam	6.8	41.5	164.5	May 15	81,445	184-9-3	-	October 24
2018												
Allegan	Martin	Soybean	Dryland	Ockley loam	6.1	95	170	May 18	77,219	160-9-3 +manure	July 30	October 18
Branch	Coldwater	Maize	Irrigated	Oshtemo sandy loam	6.2	80	110	May 29	83,314	190-9-3	August 6	November 1
Cass	Vandalia	Maize	Irrigated	Kalamazoo loam	6.4	25	198	May 9	82,796	245-9-3	July 24	October 19
Huron	Bad Axe	Maize	Dryland	Kilmanagh loam	7.9	77.5	236	May 16	83,509	160-9-3 +manure	July 31	October 22
Ingham	East Lansing	Soybean	Dryland	Conover loam	6.0	49	196	May 8	78,351	160-9-3	July 23	October 17

Table 3-1 (cont'd)

Mason	Scottville	Soybean	Irrigated	Ithica-Arkona complex	6.6	47	121	May 23	82,671	160-9-3 +manure	-	October 23
Montcalm	Greenville	Maize	Dryland	McBride and Isabella sandy loams	5.95	96	198	May 18	82,153	160-9-3	July 30	October 25
Saginaw	New Lothrop	Soybean	Dryland	Conover loam 61% Brookston loam 39%	6.75	47	219	May 30	82,671	160-9-3	-	October 30
Washtenaw	Milan	Sweet Corn	Dryland	Boyer loamy sand 64% Wasepi sandy loam 36%	5.95	107	325.5	June 13	81,580	160-9-3	August 15	November 20

[1] In the first year of the experiment, plots were thinned to a maximum population of 87,053 plants hectare $^{-1}$. In the second year, plots were not thinned due to the use of a newer more precise planter.

Table 3-2. Complete list of mycotoxins tested along with abbreviations, fungal species that produce each particular mycotoxin, and regulatory limits worldwide for each mycotoxin across crops.

Mycotoxin	Abbreviation	Produced by:	Regulations
Aflatoxin	AB1	*Aspergillus flavus*, *A. parasiticus*, and *A. nominus* (Puschner, 2002)	Food Aflatoxin B1: 61 countries; 1 μg kg^{-1} to 20 μg kg^{-1} (van Egmond and Jonker, 2004) Total Aflatoxins: 76 countries; 0 μg kg^{-1} to 35 μg kg^{-1} (van Egmond and Jonker, 2004) Feed Aflatoxin B1: 39 countries; 5 μg kg^{-1} to 50 μg kg^{-1} (van Egmond and Jonker, 2004) Total Aflatoxins: 21 countries; 5 μg kg^{-1} to 50 μg kg^{-1} (van Egmond and Jonker, 2004)
Alternariolmethylether	AME	*Alternaria* spp. (Torres, et al., 1998)	
Alternariol	AOH	*Alternaria* spp. (Torres, et al., 1998)	
Beauvericin	BEA	*Beauveria bassiana* and *Fusarium* spp. (Jestoi, 2008, Logrieco, et al., 2002, Wang and Xu, 2012)	
Citrinin	CTN	*Monascus* spp. (Blanc, et al., 1995)	
Diacetoxyscirpenol	DIAS	*Fusarium* spp. esp. *F. poae, F. equiseti*, *F. sambucinum*, and *F. sporotrichioides* (Logrieco, et al., 2002, Mirocha, et al., 1976)	
Deoxynivelenol	DON	*Fusarium graminearum* and *F.culmorum* (Logrieco, et al., 2002)	37 countries; 300 μg kg^{-1} to 2000 μg kg^{-1} (van Egmond and Jonker, 2004)
deoxynivalenol 3-β-D - glucoside	D3G	*Fusarium* spp. (Nagl, et al., 2014)	
15-acetyl-deoxynivalenol	15-ADON	*Fusarium graminearum* and *F. culmorum* (Logrieco, et al., 2002)	
3-acetyl-deoxynivalenol	3-ADON	*Fusarium graminearum* and *F. culmorum* (Logrieco, et al., 2002)	
Enniatin A	ENNA	*Fusarium* spp. (Jestoi, 2008)	

Table 3-2 (cont'd)

Enniatin A1	ENNA1	*Fusarium* spp. (Jestoi, 2008)	
Enniatin B	ENNB	*Fusarium* spp. (Jestoi, 2008)	
Enniatin B1	ENNB1	*Fusarium* spp. (Jestoi, 2008)	
Fumonisin B1	FB1	*Fusarium* spp. (Logrieco, et al., 2002, Marin, et al., 2004)	Total Fumonisins: 6 countries; 1000 μg kg^{-1} to 3000 μg kg^{-1} (van Egmond and Jonker, 2004)
Fumonisin B2	FB2	*Fusarium* spp. (Logrieco, et al., 2002, Marin, et al., 2004)	
Fumonisin B3	FB3	*Fusarium* spp. (Logrieco, et al., 2002, Marin, et al., 2004)	
Fusarenon-X	FUSX	*Fusarium* spp. (Pronk, et al., 2002)	
HT-2 Toxin	HT-2	*Fusarium* spp. esp. *F. sporotrichioides, F. acuminatum,* and *F. poae* (Logrieco, et al., 2002)	T-2 + HT-2: EU; 15 μg kg^{-1} to 2000 μg kg^{-1} (Adhikari, et al., 2017)
Moniliformin	MON	*Fusarium* spp. esp. *F. subglutinans, F. groliferatum, F. avenaceum,* and *F. tricinctum* (Jestoi, 2008, Logrieco, et al., 2002)	
Neosolaniol	NEO	*Fusarium* spp. esp. *F. sporotrichioides, F. poae*, and *F. acuminatum* (Logrieco, et al., 2002)	
Nivelenol	NIV	*Fusarium cerealis, F. poae, F. graminearum,* and *F. culmorum* (Logrieco, et al., 2002, Pronk, et al., 2002)	
Ochratoxin A	OTA	*Aspergillus* spp. and *Penicillium* spp. (Bayman and Baker, 2006, Wang, et al., 2016)	37 countries; 3 μg kg^{-1} to 50 μg kg^{-1} (van Egmond and Jonker, 2004)
Ochratoxin B	OTB	*Aspergillus* spp. and *Penicillium* spp. (Bayman and Baker, 2006, Wang, et al., 2016)	

Table 3-2 (cont'd)

Penitrm A	PENA	*Aspergillus* spp., *Penicillium* spp., *Claviceps* spp. (Bunger, et al., 2004, Puschner, 2002)	
Roquefortine C	ROC	*Aspergillus* spp., *Penicillium* spp., *Claviceps* spp. (Bunger, et al., 2004, Puschner, 2002)	
Sterigmatocystin	STER	*Aspergillus* spp., *Penicillium* spp., *Bipolaris* spp., *Chaetomium* spp., *Emiricella* spp. (Versilovskis and De Saeger, 2010)	
T-2 Toxin	T-2	*Fusarium* spp. esp. *F. sporotrichioides, F. acuminatum,* and *F. poae* (Logrieco, et al., 2002)	T-2 + HT-2: China, Iran, Canada, EU; 15 μg kg^{-1} to 2000 μg kg^{-1} (Adhikari, et al., 2017)
Zearalenone	ZON	*Fusarium* spp. esp. *F. graminearum, F. culmorum, F. cerealis, F. equiseti, F. crookwellense*, and *F. semitectum* (Logrieco, et al., 2002, Zinedine, et al., 2007).	16 countries; 50 μg kg^{-1} to 1000 μg kg^{-1} (van Egmond and Jonker, 2004)

Table 3-3**.** Statistics of mycotoxin concentrations found in 45 maize grain samples in 2017 across nine locations in lower Michigan. Percentage of positive samples, the limit of detection (LOD), the limit of quantification (LOQ), mean, standard deviation, minimum, and maximum concentrations were calculated for each mycotoxin across all locations.

Mycotoxin	2017						
	% Positive[a]	LOD[b]	LOQ[c]	Mean	SD	Min	Max
		----------------------------μg kg^{-1}------------------------					
Alternariolmethylether	20	0.01	0.02	0.74	2.81	<LOD	16.57
Alternariol	-	-	-	-	-	-	-
Beauvericin	78	0.04	0.10	0.11	0.03	<LOD	3217.50
Deoxynivalenol	93	14.34	30.13	1228.65	1657.95	<LOD	8288.60
Deoxynivalenol 3-β-D-glucoside	73	1.44	3.30	1195.70	1631.45	<LOD	6266.49
15-acetyl-deoxynivalenol	47	23.24	39.88	137.23	193.50	<LOD	927.64
3-acetyl-deoxynivalenol	-	-	-	-	-	-	-
Enniatin A	100	0.01	0.02	0.11	0.03	0.05	0.19
Enniatin A1	-	-	-	-	-	-	-
Enniatin B	84	0.00	0.01	0.28	0.14	<LOD	0.68
Enniatin B1	4	0.00	0.01	0.02	0.11	<LOD	0.65
Fumonisin B1	80	0.19	0.49	299.37	737.82	<LOD	3686.44
Fumonisin B2	80	0.97	2.47	984.53	2310.07	<LOD	22538.63
Fumonisin B3	71	0.58	1.55	642.24	1737.81	<LOD	8733.03
HT-2 toxin	-	-	-	-	-	-	-
Moniliformin	47	0.12	0.26	45.50	104.90	<LOD	420.45
Neosolaniol	9	0.23	0.56	0.44	1.66	<LOD	8.01
T-2 toxin	11	0.30	0.69	3.42	11.70	<LOD	64.00
Zearalenone	69	0.03	0.07	196.60	451.59	<LOD	2204.13

[a] Samples below the limit of detection were considered negative reads.

[b] Limit of Detection. Calculated as three times the standard deviation around the analyte retention time.

[c] Limit of Quantification. Calculated as ten times the standard deviation around the analyte retention time.

Table 3-4**.** Statistics of mycotoxin concentrations found in 45 maize grain samples in 2018 across nine locations in lower Michigan. Percentage of positive samples, the limit of detection (LOD), the limit of quantification (LOQ), mean, standard deviation, minimum, and maximum concentrations were calculated for each mycotoxin across all locations.

Mycotoxin	2018						
	% Positive[a]	LOD[b]	LOQ[c]	Mean	SD	Min	Max
		---------------------------μg kg^{-1}------------------------					
Alternariolmethylether	38	0.01	0.03	3.84	13.06	<LOD	71.06
Alternariol	31	0.13	0.23	3.83	9.59	<LOD	50.25
Beauvericin	100	0.02	0.07	588.58	1442.40	1.04	7,446.21
Deoxynivalenol	100	58.49	139.66	5143.06	4910.49	173.82	20,475.00
Deoxynivalenol 3-β-D-glucoside	100	0.73	1.77	757.88	845.09	7.44	3,249.36
15-acetyl-deoxynivalenol	100	11.64	22.77	451.20	1787.60	38.93	1,787.60
3-acetyl-deoxynivalenol	64	2.51	6.06	11.63	13.36	<LOD	63.04
Enniatin A	11	0.01	0.03	0.52	3.25	<LOD	21.84
Enniatin A1	9	0.05	0.10	0.65	4.06	<LOD	27.28
Enniatin B	20	0.01	0.01	0.11	0.45	<LOD	2.34
Enniatin B1	9	0.01	0.02	0.21	1.19	<LOD	7.94
Fumonisin B1	96	0.21	0.50	2179.62	6926.47	<LOD	45,145.82
Fumonisin B2	89	0.09	0.23	730.75	2861.31	<LOD	19,118.06
Fumonisin B3	89	0.09	0.25	700.72	2693.38	<LOD	17,972.72
HT-2 toxin	9	1.65	0.80	14.07	52.63	<LOD	276.74
Moniliformin	73	0.06	0.13	141.25	267.99	<LOD	1,160.35
Neosolaniol	11	0.09	0.24	1.27	4.91	<LOD	30.05
T-2 toxin	27	0.30	0.69	7.18	26.47	<LOD	156.65
Zearalenone	100	0.03	0.05	592.84	984.60	0.56	4,148.75

[a] Samples below the limit of detection were considered negative reads.
[b] Limit of Detection. Calculated as three times the standard deviation around the analyte retention time.
[c] Limit of Quantification. Calculated as ten times the standard deviation around the analyte retention time.

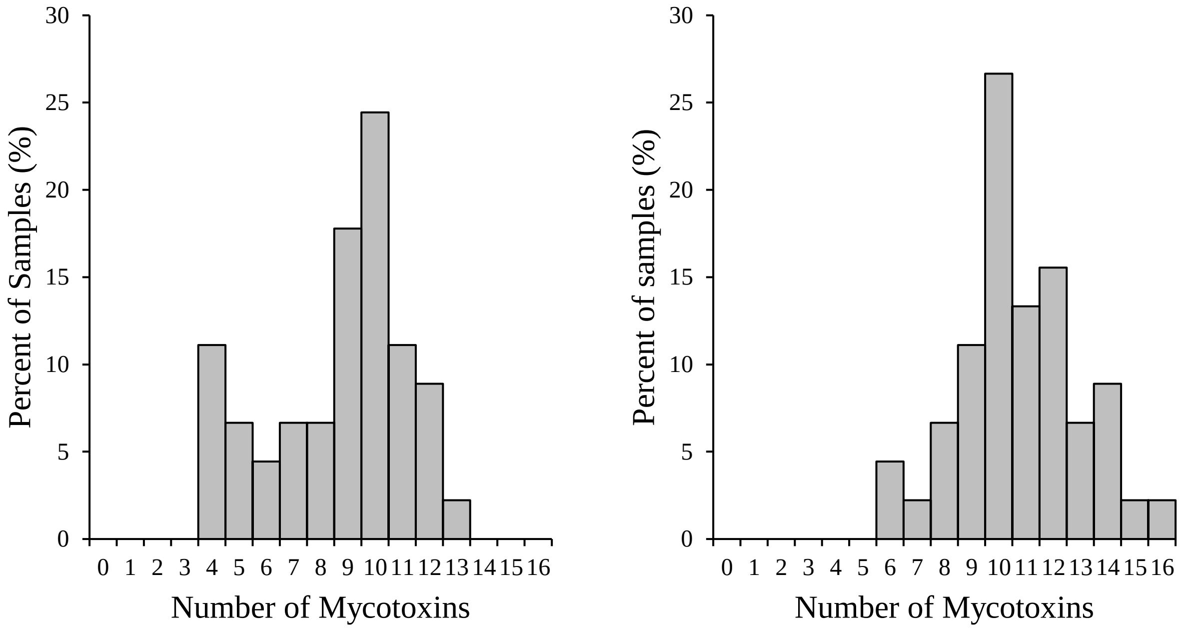

Figure 3-2**.** Distribution of multiple mycotoxin occurrence in 45 samples in each of nine locations throughout Michigan during the 2017 (left) and 2018 (right) growing seasons. Bars indicate the percentage of samples with a certain number of mycotoxins present.

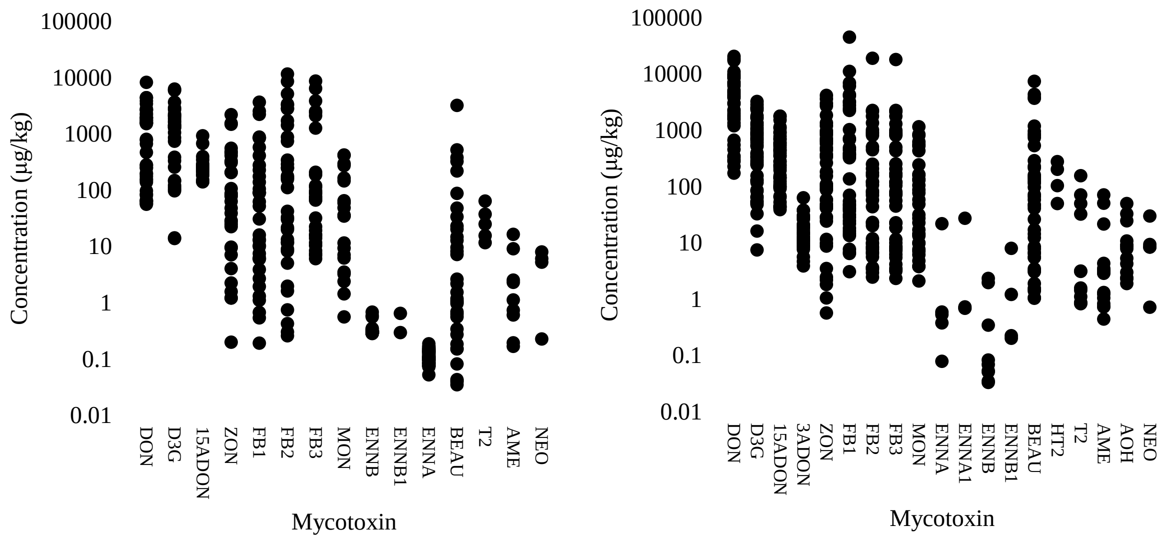

Figure 3-3**.** Range of concentrations (µg kg^{-1}) of mycotoxins found in samples during the 2017 (left) and 2018 (right) growing seasons. The concentration range is presented on the log scale. Refer to Table 2-1 for a list of acronyms.

Table 3-5. Effect of location on Deoxynivalenol (DON), deoxynivalenol 3-β-D –glucoside (D3G), Zearalenone (ZON), Enniatin A (ENNA), and Alternariolmethylether (AME) contamination levels in 2017.

Location	Mycotoxin									
	DON		D3G		ZON		ENNA		AME	
	---$\mu g\ kg^{-1}$---									
Allegan	1900	ab	2400	ab	386	ab	0.08	b	0	b
Branch	1780	ab	1570	ab	191	ab	0.1	ab	2.53	a
Cass	1950	ab	2220	ab	143	ab	0.09	b	0.29	ab
Huron	130	b	106	b	0.49	b	0.1	ab	0.04	ab
Ingham	205	b	171	b	0.36	b	0.14	a	0	b
Mason	485	b	336	b	112	b	0.12	b	0	b
Montcalm	1320	ab	769	b	63.9	ab	0.12	ab	0.17	ab
Saginaw	126	b	51.5	b	3.39	b	0.14	a	0	b
Washtenaw	3690	a	3110	a	869	a	0.14	a	0	b
p-value	0.002		0.002		0.03		0.0006		0.02	

[1]Letters indicate significance within columns. Means followed by different letters indicate significant differences at p=0.05. All other mycotoxins did not differ between locations.

Table 3-6. Effect of location on Deoxynivalenol (DON), deoxynivalenol 3-β-D –glucoside (D3G), HT-2 Toxin (HT-2), T-2 Toxin (T-2), and Neosolaniol (NEO) contamination levels in 2018.

Location	Mycotoxin									
	DON		D3G		HT-2		T-2		NEO	
	---μg kg^{-1}-------------------------------------									
Allegan	1820	bcd	361	ab	0	b	0.169	b	0	b
Branch	969	d	130	b	0	b	0.619	b	0.144	b
Cass	3040	bcd	252	ab	0	b	0.166	b	0	b
Huron	10800	a	1150	ab	117	a	55.5	a	9.56	a
Ingham	3870	abcd	741	ab	0	b	0.221	b	0	b
Mason	5870	abcd	148	ab	10.03	b	7.27	b	1.68a	b
Montcalm	9190	abc	1000	ab	0	b	0.314	b	0	b
Saginaw	9480	ab	1600	a	0	b	0	b	0	b
Washtenaw	1250	d	103	b	0	b	0.289	b	0	b
p-value	0.0001		0.006		0.001		0.005		0.02	

[1] Letters indicate significance within columns. Means followed by different letters indicate differences at p=0.05. All other mycotoxins did not differ between locations.

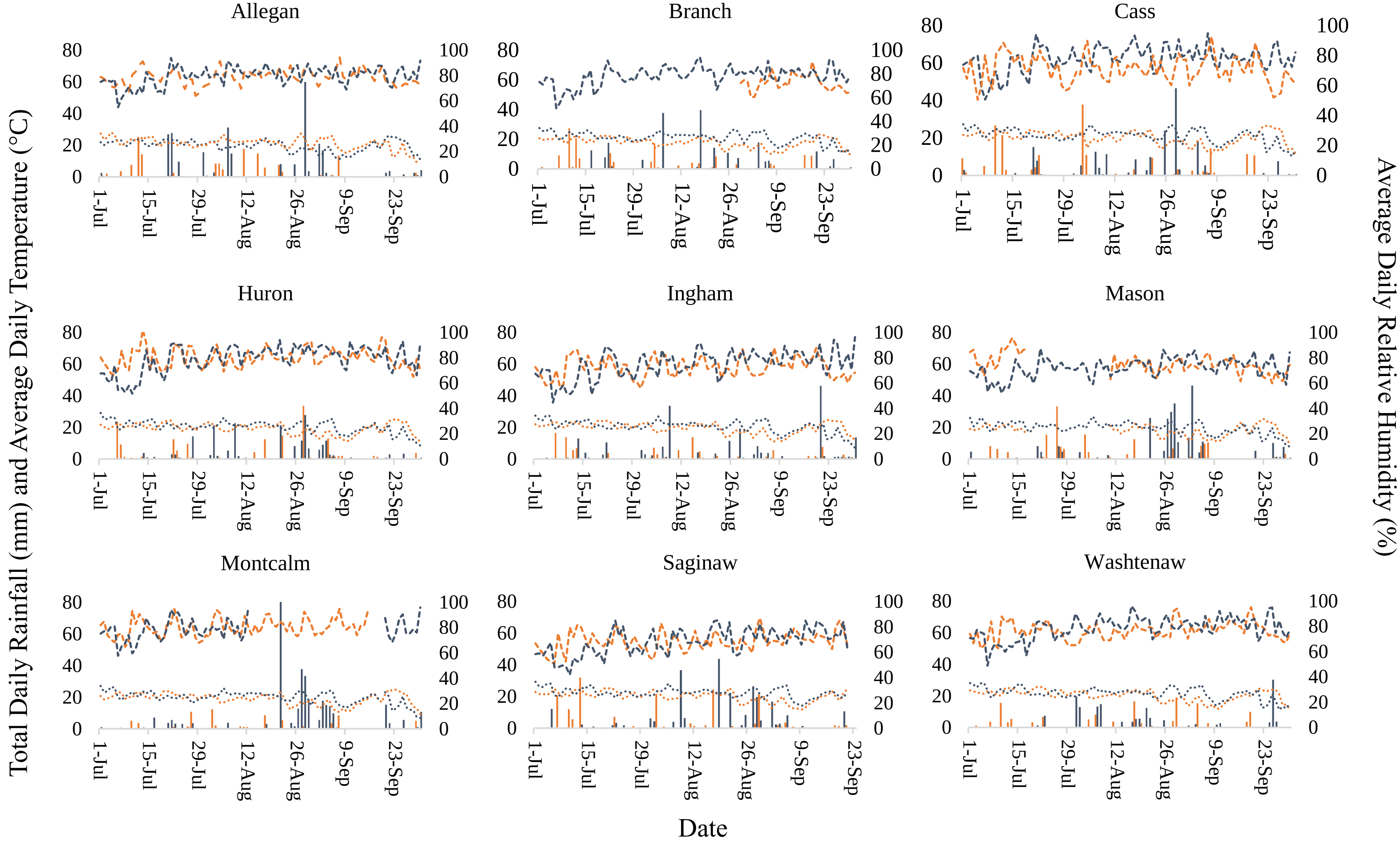

Figure 3-4. Weather patterns during the 2017 and 2018 growing season at nine locations throughout lower Michigan. Bars indicate total daily precipitation (mm) with orange bars indicating precipitation in 2017 and blue bars indicating precipitation in 2018. Dotted orange lines indicate the average daily temperature (°C) in 2017 and blue dotted lines indicate the temperature in 2018. Dashed lines indicate average daily relative humidity (%).

LITERATURE CITED

LITERATURE CITED

Abendroth, L., R. Elmore, M. Boyer and S. Marlay. 2011. Corn Growth and Development. Iowa State University Extension, Ames, IA.

Adhikari, M., B. Negi, N. Kaushik, A. Adhikari, A.A. Al-Khedhairy, N.K. Kaushik, et al. 2017. T-2 mycotoxin: toxicological effects and decontamination strategies. Oncotarget 8: 33933-33952. doi:10.18632/oncotarget.15422.

Alassane-Kpembi, I., M. Kolf-Clauw, T. Gauthier, R. Abrami, F.A. Abiola, I.P. Oswald, et al. 2013. New insights into mycotoxin mixtures: The toxicity of low doses of Type B trichothecenes on intestinal epithelial cells is synergistic. Toxicology and Applied Pharmacology 272: 191-198. doi:10.1016/j.taap.2013.05.023.

Bayman, P. and J.L. Baker. 2006. Ochratoxins: A global perspective. Mycopathologia 162: 215-223. doi:10.1007/s11046-006-0055-4.

Blanc, P.J., M.O. Loret and G. Goma. 1995. Production of citrinin by various species of *Monascus*. Biotechnology Letters 17: 291-294. doi:10.1007/bf01190639.

Bunger, J., G. Westphal, A. Monnich, B. Hinnendahl, E. Hallier and M. Muller. 2004. Cytotoxicity of occupationally and environmentally relevant mycotoxins. Toxicology 202: 199-211. doi:10.1016/j.tox.2004.05.007.

DiFonzo, C.D. and P. Porter. 2019. The Handy Bt Trait Table for U.S. Corn Production. Retrieved December 5, 2019. (https://lubbock.tamu.edu/files/2018/11/BtTraitTableNov2018.pdf)

Fink-Gremmels, J. 1999. Mycotoxins: Their implications for human and animal health. Veterinary Quarterly 21: 115-120. doi:10.1080/01652176.1999.9695005.

Grenier, B. and I.P. Oswald. 2011. Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. World Mycotoxin Journal 4: 285-313. doi:10.3920/wmj2011.1281.

Jestoi, M. 2008. Emerging Fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin - A review. Critical Reviews in Food Science and Nutrition 48: 21-49. doi:10.1080/10408390601062021.

Kamala, A., Ortiz, J., Kimanya, M., Haesaert, G., Donoso, S., Tiisekwa, B., & De Meulenaer, B. (2015). Multiple mycotoxin co-occurrence in maize grown in three ago-ecological zones of Tanzania. Food Control, 54, 208-215. doi:10.1016/j.foodcont.2015.02.002

Kovalsky, P., G. Kos, K. Nahrer, C. Schwab, T. Jenkins, G. Schatzmayr, et al. 2016. Co-Occurrence of Regulated, Masked and Emerging Mycotoxins and Secondary Metabolites in Finished Feed and Maize-An Extensive Survey. Toxins 8. doi:10.3390/toxins8120363.

Lee, H.B., A. Patriarca and N. Magan. 2015. Alternaria in Food: Ecophysiology, Mycotoxin Production and Toxicology (vol 43, pg 93, 2015). Mycobiology 43: 371-371. doi:10.5941/myco.2015.43.3.371.

Limay-Rios, V. and A.W. Schaafsma. 2018. Effect of prothioconazole application timing on Fusarium mycotoxin content in maize grain. Journal of Agricultural and Food Chemistry 66: 4809-4819. doi:10.1021/acs.jafc.8b00788.

Logrieco, A., G. Mule, A. Moretti and A. Bottalico. 2002. Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe. European Journal of Plant Pathology 108: 597-609. doi:10.1023/a:1020679029993.

Marin, S., N. Magan, A.J. Ramos and V. Sanchis. 2004. Fumonisin-producing strains of Fusarium: A review of their ecophysiology. Journal of Food Protection 67: 1792-1805. doi:10.4315/0362-028x-67.8.1792.

Mirocha, C., S. Pathre, B. Schauerhamer and C. Christensen. 1976. Natural occurrence of Fusarium toxins in feedstuff. Appl. Environ. Microbiol. 32: 553-556.

Munkvold, G.P. 2003. Epidemiology of Fusarium diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705-713. doi:10.1023/a:1026078324268.

Murphy, P.A., L.G. Rice and P.F. Ross. 1993. Fumonisin-b1, fumonisin-b2, and fumonisin-b3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. Journal of Agricultural and Food Chemistry 41: 263-266. doi:10.1021/jf00026a024.

Nagl, V., B. Woechtl, H.E. Schwartz-Zimmermann, I. Hennig-Pauka, W.D. Moll, G. Adam, et al. 2014. Metabolism of the masked mycotoxin deoxynivalenol-3-glucoside in pigs. Toxicology Letters 229: 190-197. doi:10.1016/j.toxlet.2014.06.032.

Pronk, M., R. Schothorst and H. Van Egmond. 2002. Toxicology and occurrence of nivalenol, fusarenon X, diacetoxyscirpenol, neosolaniol and 3-and 15-acetyldeoxynivalenol; a review of six trichothecenes.

Puschner, B. 2002. Mycotoxins. Veterinary Clinics of North America-Small Animal Practice 32: 409-+. doi:10.1016/s0195-5616(01)00011-0.

Rodrigues, I. and K. Naehrer. 2012. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins 4: 663-675. doi:10.3390/toxins4090663.

Schaafsma, A.W., V. Limay-Rios and L. Tamburic-Illincic. 2008. Mycotoxins and Fusarium species associated with maize ear rot in Ontario, Canada. Cereal Research Communications 36: 525-527.

Smith, M.C., S. Madec, E. Coton and N. Hymery. 2016. Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins 8. doi:10.3390/toxins8040094.

Torres, A., H.H.L. Gonzalez, M. Etcheverry, S.L. Resnik and S. Chulze. 1998. Production of alternariol and alternariol mono-methyl ether by isolates of *Alternaria spp*. from Argentinian maize. Food Additives and Contaminants 15: 56-60.

US Food and Drug Administration. 2001. Guidance for Industry: Fumonisin Levels in Human and Animal Feeds. US FDA. Silver Springs, MD.

US Food and Drug Administration. 2010. Guidance for Industry and FDA: Advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. US FDA. Silver Spring, MD.

US Food and Drug Administration. 2019. Action Levels for Aflatoxins in Animal Food: Compliance Policy Guide. US FDA. Silver Springs, MD.

van Egmond, H.P. and M. Jonker. 2004. Worldwide regulations for mycotoxins in food and feed in 2003. Food and Agriculture organization of the United Nations.

van Egmond, H.P., R.C. Schothorst and M.A. Jonker. 2007. Regulations relating to mycotoxins in food. Analytical and Bioanalytical Chemistry 389: 147-157. doi:10.1007/s00216-007-1317-9.

Versilovskis, A. and S. De Saeger. 2010. Sterigmatocystin: Occurrence in foodstuffs and analytical methods - An overview. Molecular Nutrition & Food Research 54: 136-147. doi:10.1002/mnfr.200900345.

Wang, Q.G. and L.J. Xu. 2012. Beauvericin, a Bioactive Compound Produced by Fungi: A Short Review. Molecules 17: 2367-2377. doi:10.3390/molecules17032367.

Wang, Y., L.Q. Wang, F. Liu, Q. Wang, J.N. Selvaraj, F.G. Xing, et al. 2016. Ochratoxin A producing fungi, biosynthetic pathway and regulatory mechanisms. Toxins 8. doi:10.3390/toxins8030083.

Zinedine, A., J.M. Soriano, J.C. Molto and J. Manes. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. Food and Chemical Toxicology 45: 1-18. doi:10.1016/j.fct.2006.07.030.