

Introduction

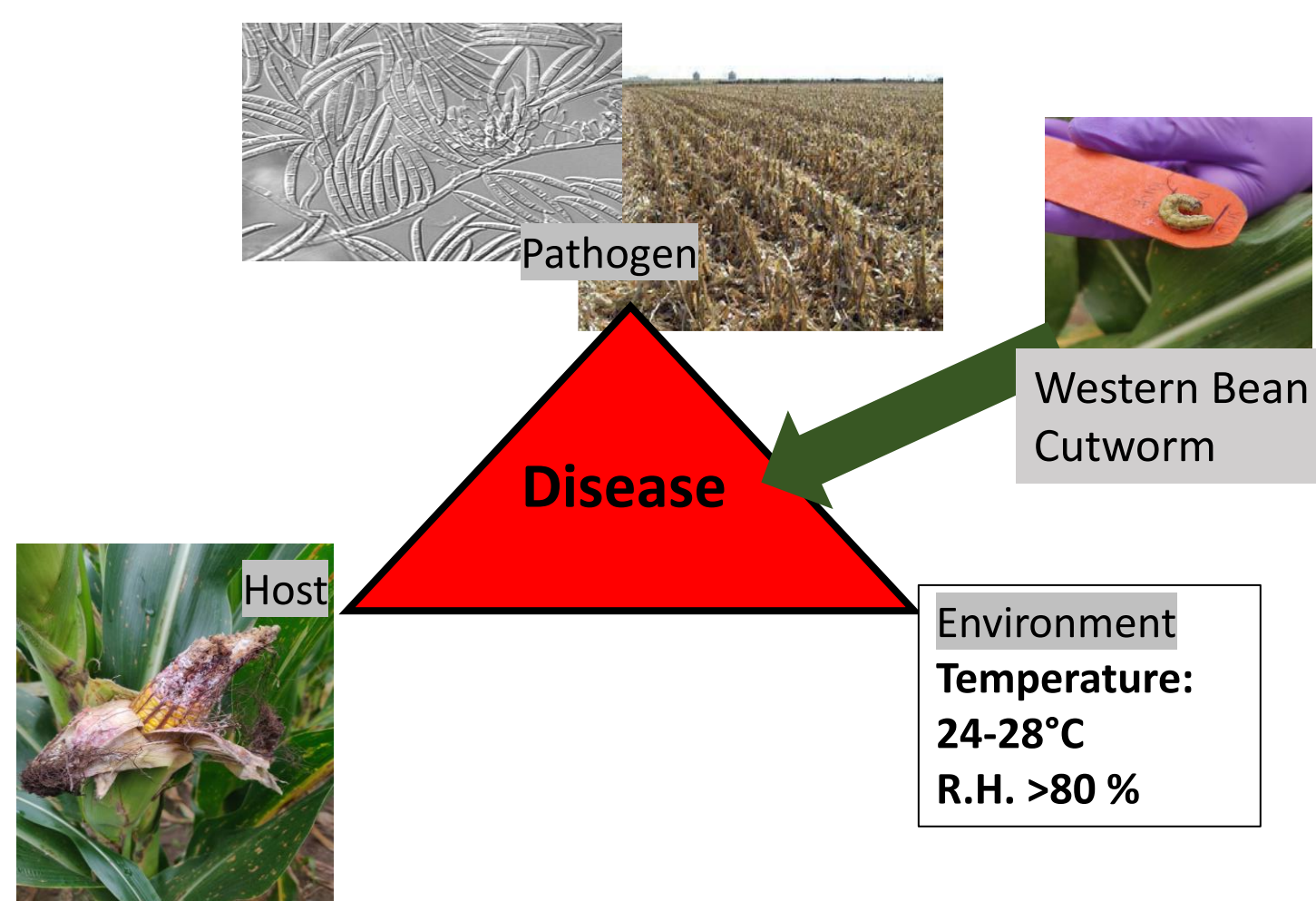


Fig. 1: Disease triangle showing the interaction of various biotic and abiotic factors responsible for ear rot development in corn silage.

- Ear and stalk rot infections by *Fusarium verticillioides*, *Gibberella zeae*, *Aspergillus flavus*, and *Cladosporium spp* in corn (*Zea mays* L.) silage can cause accumulation of toxic secondary metabolites; called mycotoxins in plant biomass^{1,3}.
- Mycotoxins deteriorate silage quality and cause feed rejections, reduced appetite, diarrhea, low milk production, impaired fertility, and even death of livestock in severe cases³.
- Favorable environmental conditions for fungal infection and damage by ear-feeding insects increase mycotoxin risk¹.
- In Michigan, the main ear damaging insects are *Striacosta albicosta* (Western Bean Cutworm, WBC) and *Ostrinia nubilalis* (European Corn Borer, ECB)

Mycotoxins in Michigan

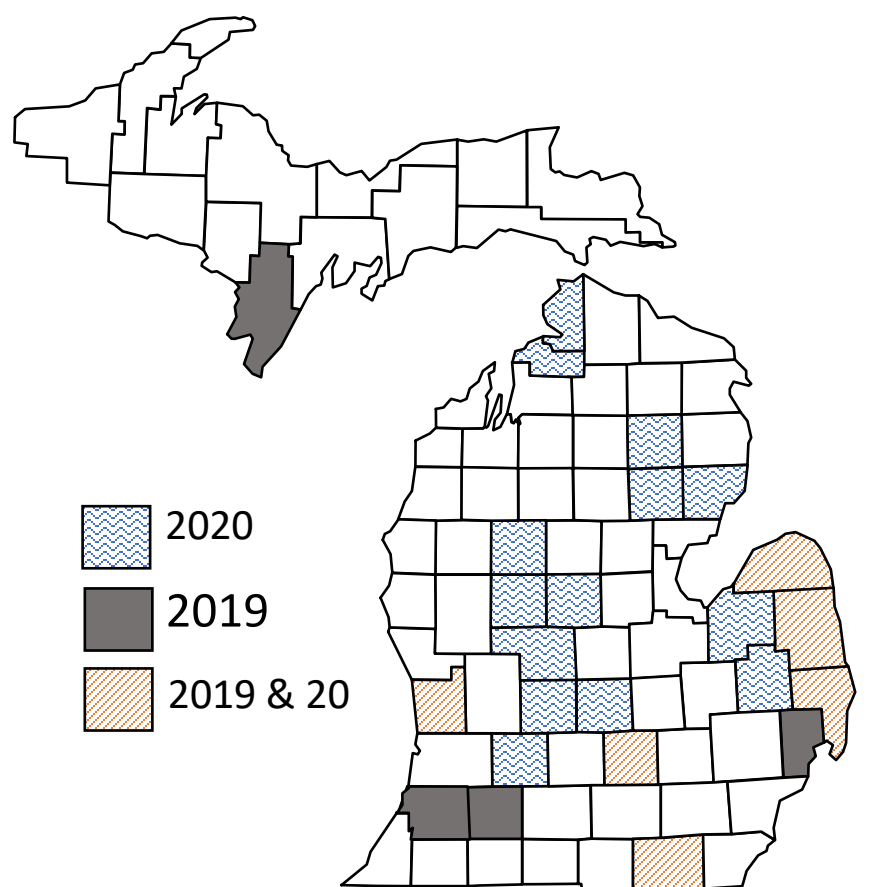


Fig. 2: Michigan counties submitting silage samples for analysis over years

- A silage survey across Michigan in 2019-20 corroborated the presence of mycotoxins. Growers from more than 20 counties submitted samples for mycotoxin over two years (Fig. 2).
- All the samples tested positive for at least one mycotoxin. Deoxynivalenol (DON) was positive in 100% of samples; second most frequent mycotoxin was Zearalenone (ZON) in 2019 and Fumonisin (FB) in 2020.
- DON levels were higher than the threshold limit (1 ppm)⁴ for 50% of samples in 2019 and 12% in 2020. Mycotoxins were also found to coexist.
- Presence of these toxins make the silage unfit for livestock. Therefore, it is important to explore strategies to help growers manage mycotoxins to improve the quality of corn silage.

Objectives

- To investigate the use of hybrid insect protection and fungicide application for alleviating ear rot infections and eventually, mycotoxin accumulation in corn silage.
- To evaluate the relation between ear damage (insect and disease) and mycotoxins.
- To quantify the impact of these management strategies on yield and feed quality of corn silage.

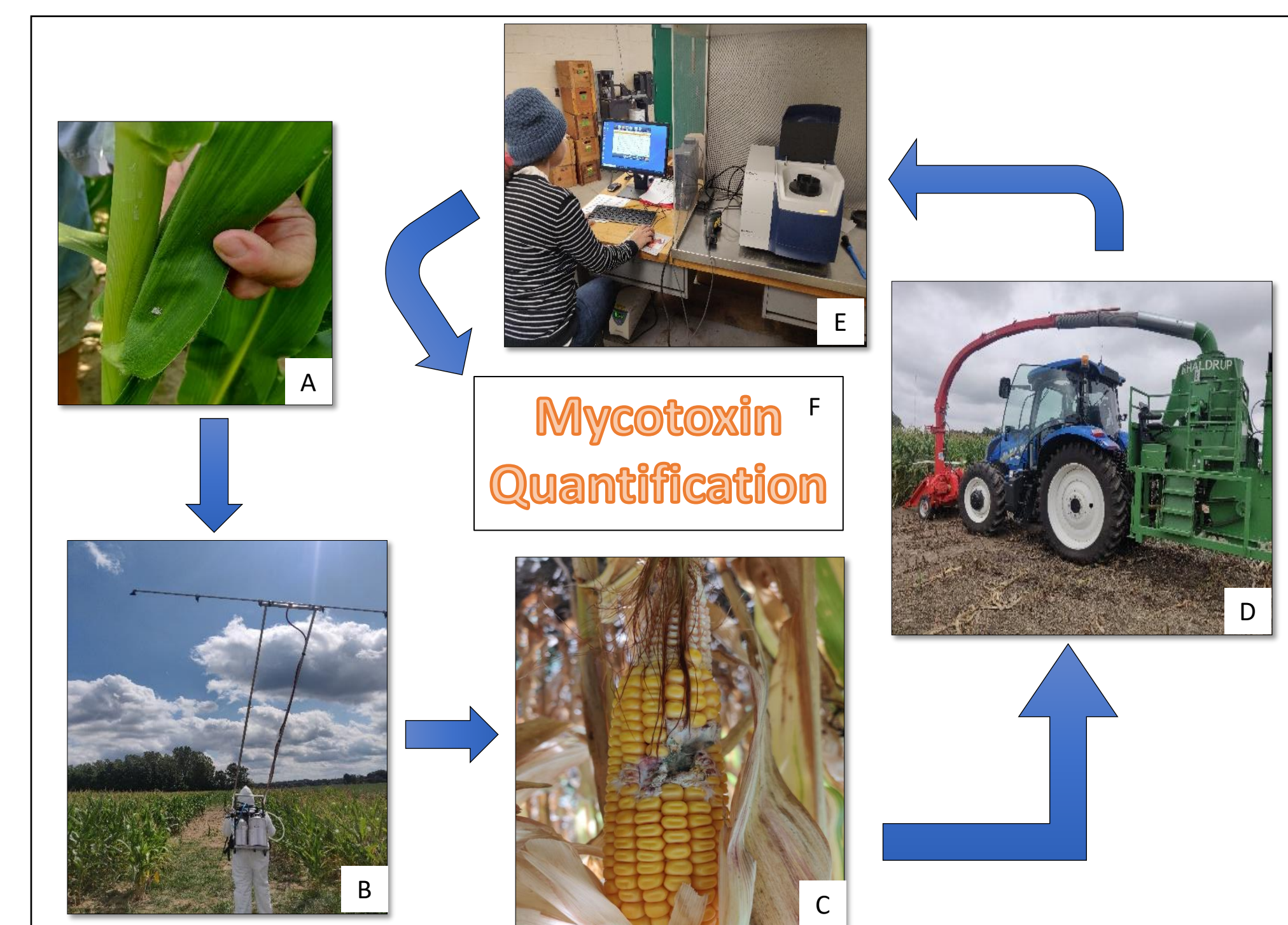
Materials and Methods

- Multi-location field trials were conducted from 2019-21 in Michigan in randomized complete block design with five replications (Fig. 3).
- Treatments included three hybrids insect protection levels (Table 1) and fungicide application of prothioconazole @ 416.5 ml ha⁻¹ at silking^(2,5).

Table 1. Hybrids planted in the study had three levels of insect resistance (two hybrids in each level)

Hybrid Insect Protection Trait	Protein	Insect Protection
Bt _N	none	No Insect protection
Bt _E	Cry1F	ECB
Bt _{EW}	Cry1F + Vip3A	ECB & WBC

- At silage harvest, 10 ears from center rows of four-row plots were rated for disease and insect damage where, **incidence** = % of total ears with damage and **severity** = average amount of damage per ear



- After harvest, representative samples were collected and ground to 1mm sieve size and then analyzed for quality, mycotoxin type, and concentration (Fig. 4).
- Data was analyzed using Proc Glimmix in SAS 9.4 using Tukey's adjustment at $\alpha = 0.1$.

Fig. 4. Field operations and data collection during the growing season
 A. Insect egg mass scouting to get an estimate of possible insect flight at V12
 B. Fungicide application at R1 (silking)
 C. Insect and disease damage ratings on the day of harvest
 D. Silage harvest at 65% moisture (mid-dent stage) using a two-row Champion C1200 Kemper forage harvester with a rear mounted Haldrup M-63 weight system to measure fresh biomass yield.
 E. Quality parameter analysis using Near Infrared Spectroscopy (Parameters analyzed, NDF: Neutral Detergent Fiber, NDFD: NDF Digestibility, ADF: acid detergent fiber), starch, crude protein (CP)
 F. Mycotoxin concentration quantified using Liquid Chromatography / Mass Spectrometry (LC/MS).

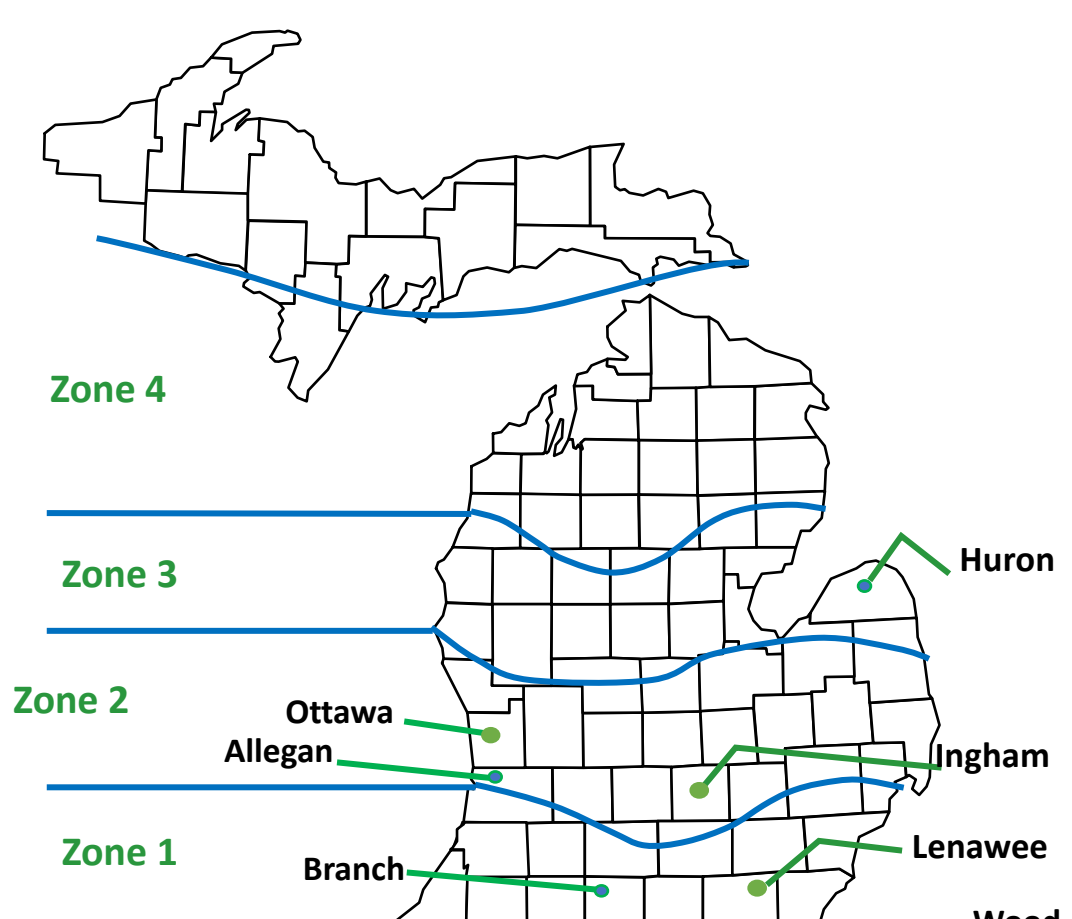


Fig. 3. Field trials locations in Michigan (zones were based on normal growing degree days)

Results and Discussion

Weather Patterns

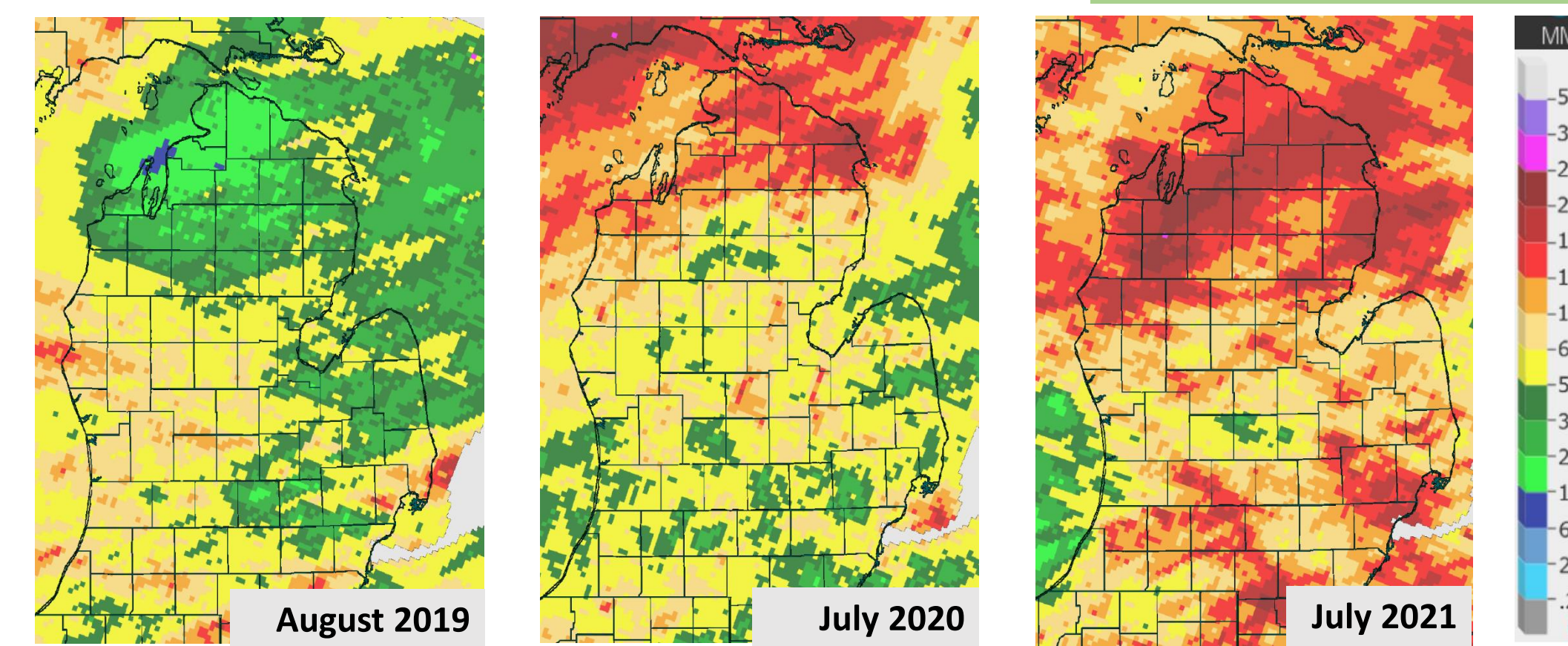


Fig. 5: Average rainfall (mm) in Michigan while corn was at silking stage in 2019, 2020 and 2021

- A wet spring in 2019 delayed planting. Lenawee and Ottawa locations were dropped due to poor emergence and low plant stand. Planting progressed on time in 2020 and 2021.
- Rainfall around silking (August 2019, July 2020, and 2021) was lower than average in 2019 and 2020, however, 2021 was closer to a normal year with 70-80 mm average precipitation (Fig. 5).
- Dry spell during silking window in 2019 and 2020 resulted in low disease pressure as most of ear rot fungi prefer high humidity. Although 2021 was closer to an average year, a lot of variation was seen in the disease levels.

Western Bean Cutworm Damage

Table 2. Mean percent insect damage for hybrid insect protection trait (Bt_N: No insect protection; Bt_E: protection only against ECB; Bt_{EW}: protection against both ECB and WBC). ECB: European Corn Borer; WBC: Western Bean Cutworm. Values with same letter within a site year are not different (p = 0.1).

Ear Damage	Insect Protection	Site Year			
		Ingham 2019	Huron 2020	Wood 2020	Ingham 2021
Western Bean Cutworm Severity Incidence	Bt _N	19.5 a	15.5 ab	13.0 a	12.0 a
	Bt _E	8.0 b	19.5 a	2.0 b	6.0 ab
	Bt _{EW}	4.5 b	7.5 b	1.1 b	2.5 b
	Bt _N	11.6 a	5.8 ab	3.1 a	7.1 a
	Bt _E	5.9 ab	15.2 a	0.4 b	2.2 a
	Bt _{EW}	0.6 b	0.8 b	0.4 b	0.4 b

- WBC damage was at least 80% less in Bt_{EW} hybrids than Bt_N hybrids (Table 2), due to dual insect protection in these hybrids.
- No impact of hybrid insect protection was observed at other site years, probably due to low insect pressure.

Ear Rot Damage

Table 3. Mean percent ear rot damage for hybrid insect protection trait (Bt_N: No insect protection; Bt_E: protection only against ECB; Bt_{EW}: protection against both ECB and WBC). ECB: European Corn Borer; WBC: Western Bean Cutworm. Values with same letter within a site year are not different (p = 0.1).

Ear Damage	Insect Protection	Site Year			
		Ingham 2019	Ingham 2020	Huron 2020	Wood 2020
Ear Rot Severity Incidence	Bt _N	18.0 a	26.0 a	25.0 a	14.5 a
	Bt _E	11.0 a	20.0 a	23.0 a	4.5 b
	Bt _{EW}	12.5 a	18.0 a	17.0 a	4.3 b
	Bt _N	17.1 a	21.1 a	15.8 a	5.8 a
	Bt _E	4.7 b	15.7 a	15.5 a	0.6 b
	Bt _{EW}	0.1 b	1.3 b	3.9 b	2.8 b

- No interaction was observed between hybrid insect protection trait and fungicide treatment.
- Ear rot severity was at least 50% less in Bt_{EW} hybrids than Bt_N hybrids (Table 3). Trends were very similar to those seen in insect damage.
- Fungicide application reduced ear rot incidence only at Lenawee and Branch 2020 when disease incidence was < 20% (data not shown).
- Strong positive correlations were seen between ear rot and WBC damage at Huron in 2020 (Fig. 6), emphasizing importance of insect damage on fungal infection.
- Weak or no correlations observed for other site years, indicating that other factors such as temperature and humidity are also crucial for ear rot infections.

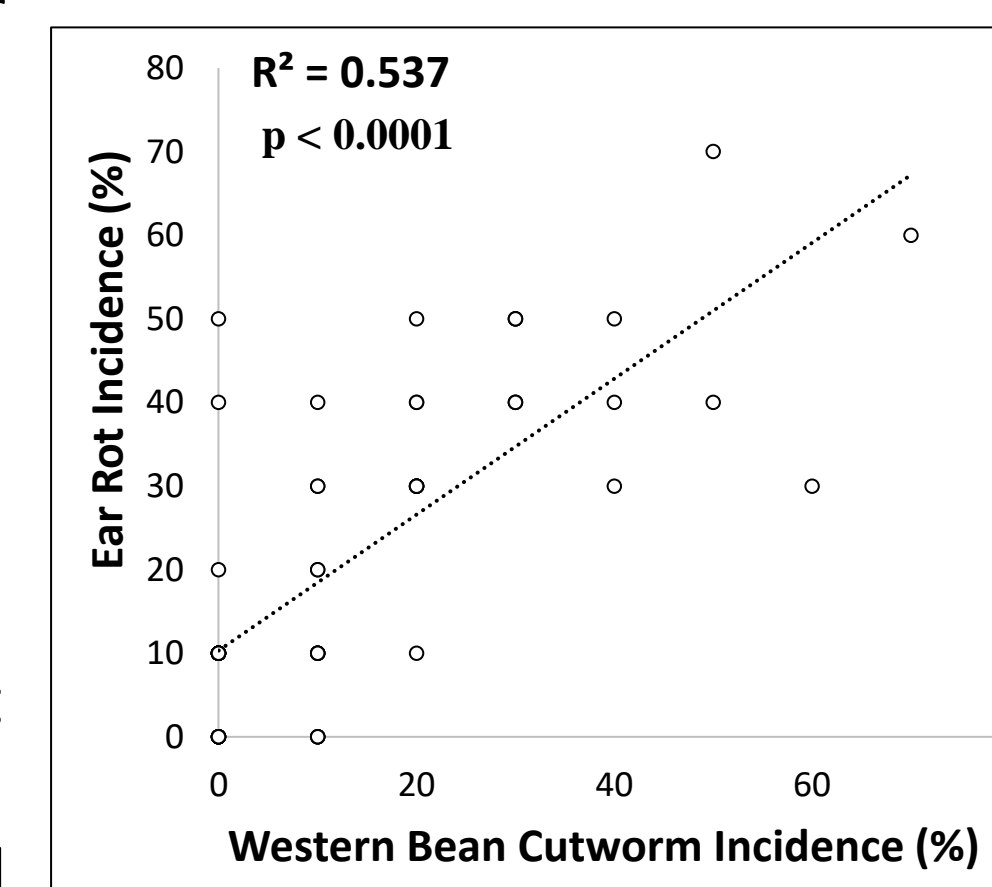


Fig. 6. Relationship between western bean cutworm incidence and ear rot incidence and Huron 2020

Mycotoxin Accumulation

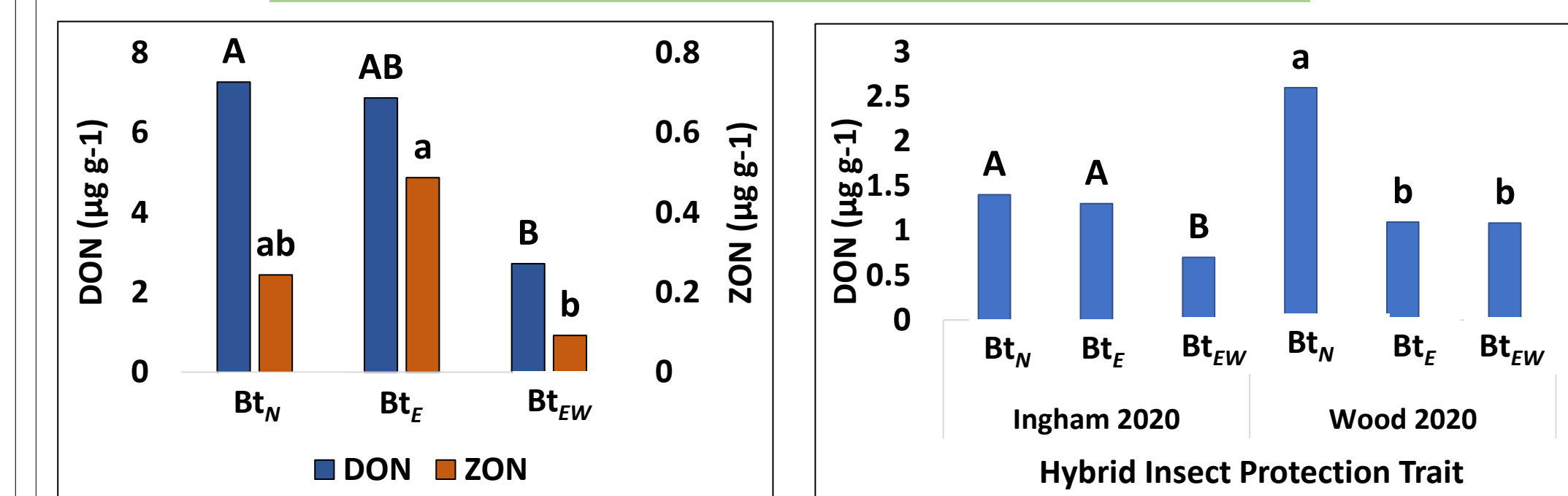


Fig. 7: Deoxynivalenol (DON) and Zearalenone (ZON) levels at Ingham 2019 for hybrid insect protection trait (Bt_N: No insect protection; Bt_E: protection only against ECB; Bt_{EW}: protection against both ECB and WBC). ECB: European Corn Borer and WBC: Western Bean Cutworm. Bars with same letter do not differ from each other significantly at p = 0.1.
 Fig. 8: Deoxynivalenol (DON) levels at Ingham and Wood 2020 for hybrid insect protection trait (Bt_N: No insect protection; Bt_E: protection only against ECB; Bt_{EW}: protection against both ECB and WBC). ECB: European Corn Borer and WBC: Western Bean Cutworm. Bars with same letter do not differ from each other significantly at p = 0.1.

- Highest concentrations were seen for DON and ZON while all the other toxins were at very low levels (mostly < 1 µg g⁻¹).
- Lowest DON and ZON were found in hybrids with dual insect protection (Fig. 7).
- Deoxynivalenol was lowest for Bt_{EW} hybrids at Ingham and Wood 2020 (Fig. 8).
- Fungicide reduced mycotoxins only at Lenawee, Ingham, Allegan, and Branch in 2020; up to 70% reductions in treated compared to non-treated plots (data not shown).
- Mycotoxins and ear damage (insect and disease) were poorly correlated (data not shown), suggesting that toxins may come from stalk infections as well.

Mycotoxin Co-occurrence

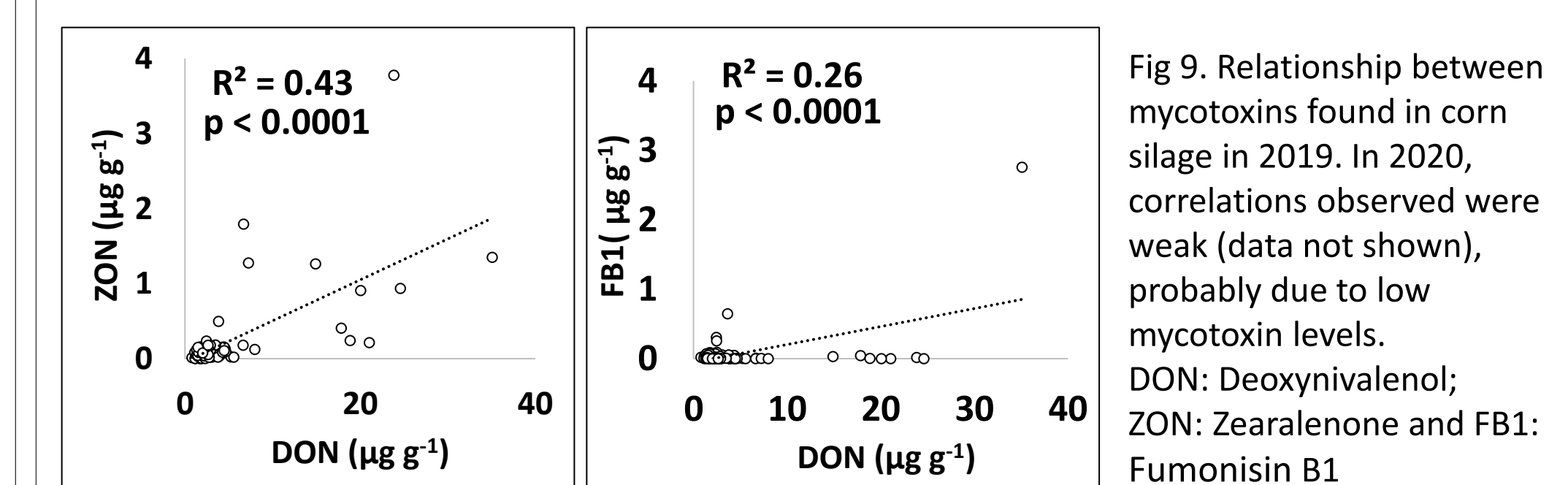


Fig. 9. Relationship between mycotoxins found in corn silage in 2019. In 2020, correlations observed were weak (data not shown), probably due to low mycotoxin levels. DON: Deoxynivalenol; ZON: Zearalenone and FB1: Fumonisin B1

- Co-occurrence of multiple mycotoxins was observed in most samples (Fig. 9).
- This can lead to ambiguity in issuing guidelines for toxin levels and may also result in complex reactions in livestock metabolism.
- Neither hybrid insect protection nor fungicide application impacted dry yield.
- Quality parameters such as CP and NDFD were impacted by hybrid class, with CP highest for Bt_N hybrids, while NDFD was highest for Bt_{EW} hybrids.
- Fungicide did not impact any quality parameters, indicating minimal benefit of fungicide application on quality in this study.

Dry Yield and Quality

Conclusions and Future Directions

- Functional hybrid insect protection traits play a crucial role in preventing insect damage to corn ears and restrict the entry of pathogen into host, and eventually suppression of mycotoxin accumulation.
- Weak correlations between insect damage, ear rots, and mycotoxins indicate that benefits of insect management strategies in minimizing mycotoxins in corn silage might be limited to locations with high insect pressure or lack of favorable conditions for disease development at silking.
- Fungicide application showed minimal impact on ear rots, mycotoxin accumulation, and silage feed quality at all locations in this study. Previous research has shown variable results based on field conditions and application methods. More research is needed to evaluate the return on investment of fungicide application across wide environments in corn silage.
- Mycotoxins were found to co-exist and therefore, it is important to explore their correlations and possibly using the presence of some toxins as an indicator for others in order to reduce post-harvest testing expenses.
- Overall, an integrated in-field management approach must be used to minimize mycotoxins in corn silage including continued research at regional level on ear vs stalk rots, harvest and plant timing, hybrid selection, crop rotation, residue management, and tillage practices.

Acknowledgements

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