A close-up photograph of dried hop cones, showing their characteristic conical shape and green, yellowish, and brownish coloration.

# Sensitivity of North American *Diaporthe humulicola* isolates to chemical control methods in hop

Ross Hatlen



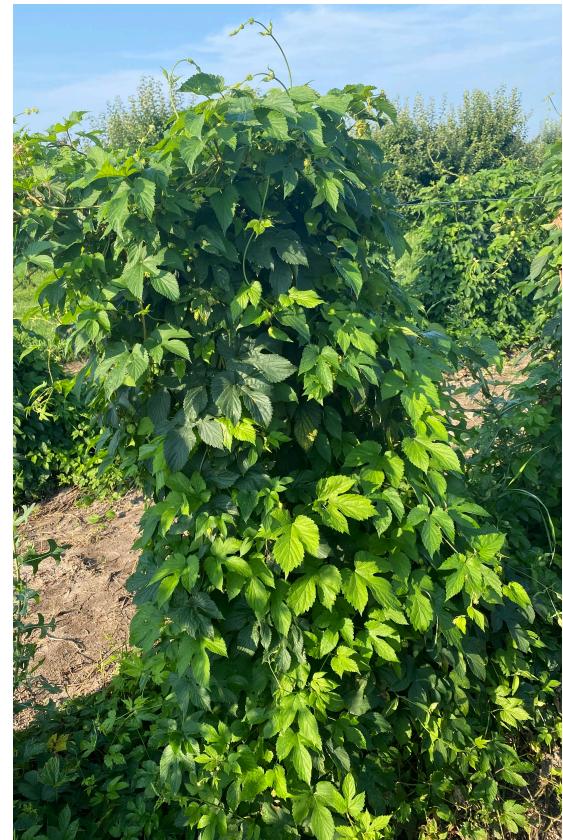
## Outline

- History of halo blight
- Survey of Michigan hop yards
- Fungicide field trials for halo blight of hop
  - ❖ Field trial
  - ❖ Correlation between downy mildew of hops and halo blight of hops
- Poison agar assay and discriminatory dose assay
  - ❖ Poison agar assay to determine EC<sub>50</sub> values and discriminatory dose assay
  - ❖ Fungicide sensitivity of Diaporthe population
  - ❖ Regional differences in fungicide sensitivity



# *Humulus lupulus*: the common hop plant

- Vine growing dioecious plant that grows over 20 feet tall
- Perennial plant that grows from mid spring to late summer
- The flower of the hop (cone) is used for its alpha and beta acids, that are a bittering and aroma agent in beer.
- 59,739 acres of hops are grown in the United States
- Top growers are Washington, Idaho, Oregon, and Michigan



# The Michigan hop industry

- Hop production in Michigan started in the 1800s
  - ❖ disease and insect pressure shifted production to the PNW
- Modern Michigan hop production started approximately in 2007
- The Michigan hop industry covers 700 acres of land
- Top Cultivars: ‘Centennial’, ‘Cascade’, and ‘Chinook’
- The number of harvested acres in Michigan have been decreasing since 2018



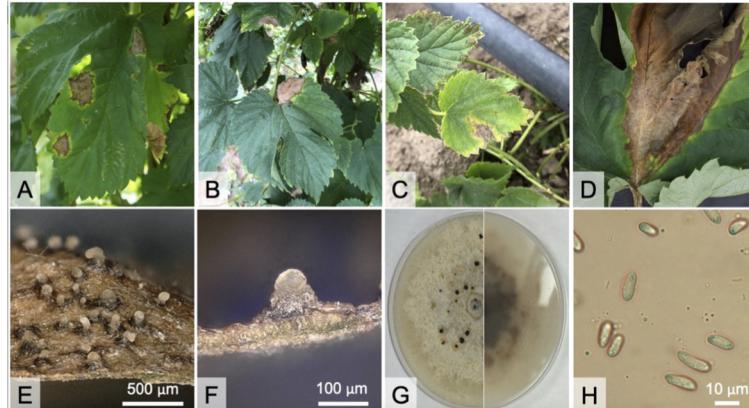
Photo by Bells Brewery, Photo by S.B.C.



# History of halo blight

## Michigan

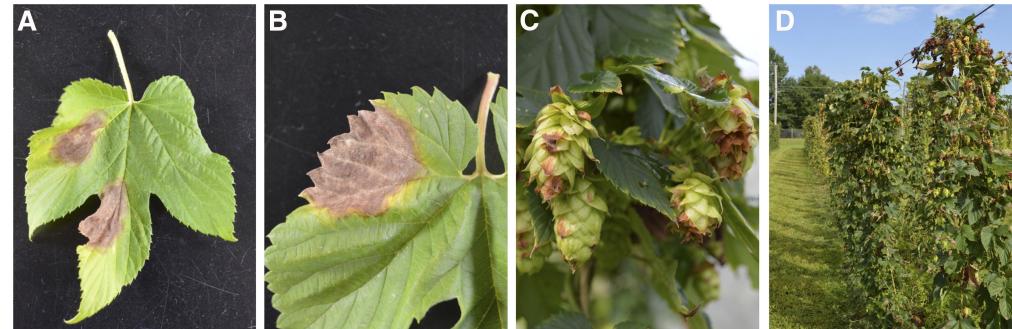
- *Diaporthe* sp 1-MI
- Halo blight
- Southern Michigan
- ITS, TEF, Histone, Beta-tubulin(Tl/Bt-2b)



Higgins et al. 2020

## Connecticut

- *Diaporthe humulicola*
- Diaporthe Leaf Spot
- Hamden, CT
- ITS, TEF, Histone, Beta-tubulin(TUBUF2/TUBUR1, T12/T22)



Allan-Perkins et al. 2020



## Halo blight of hops

- Causes concentric lesions on leaves and premature desiccation of the cone
- Growers can lose up to 60% of their yield from this disease from shattering during sorting
- Lowers the quality of the hop cone and changes the aroma of the hop
- Overwinters on dried hop stems in the field, and colonize dried dead hop tissue



Healthy



Diseased





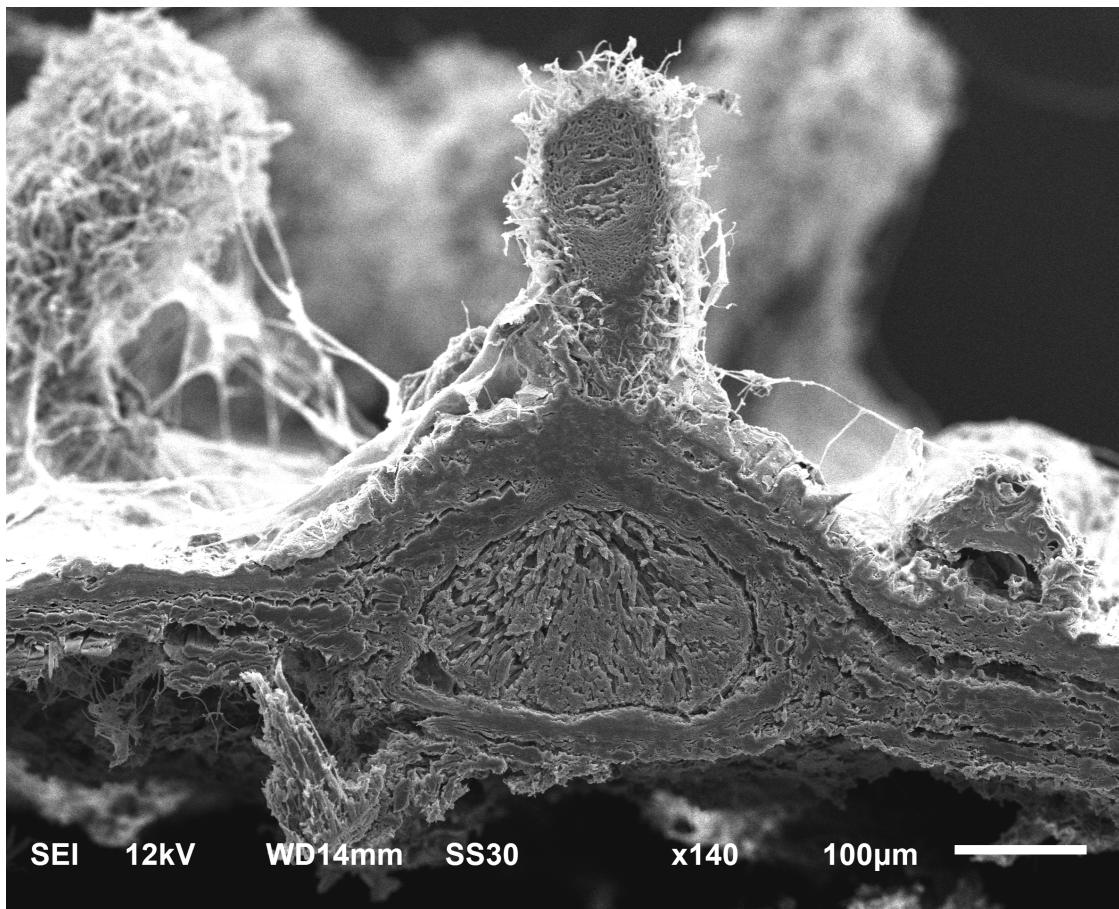
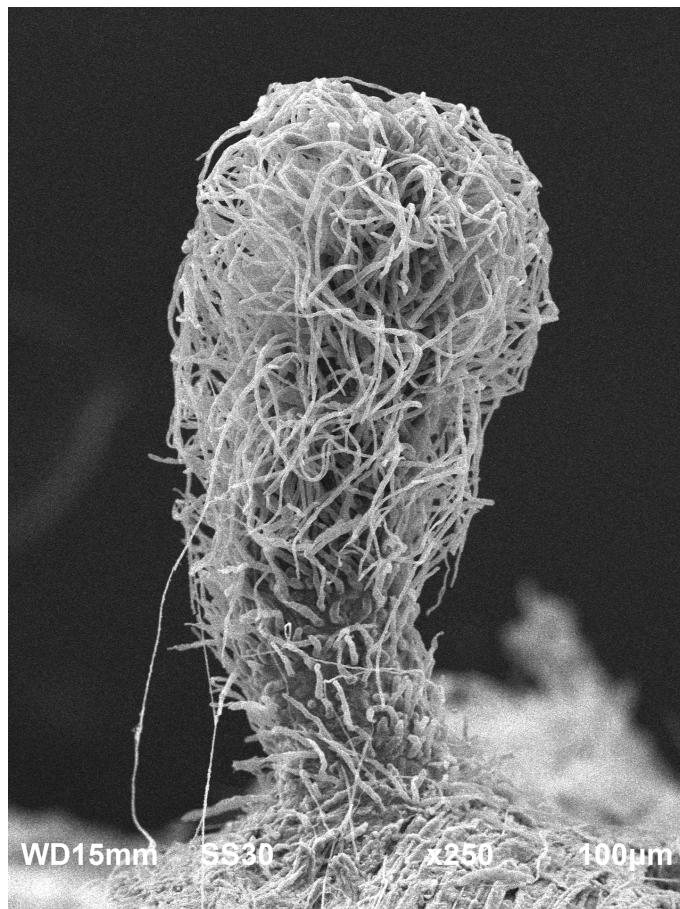
# Pycnidia are formed on desiccated hop tissue

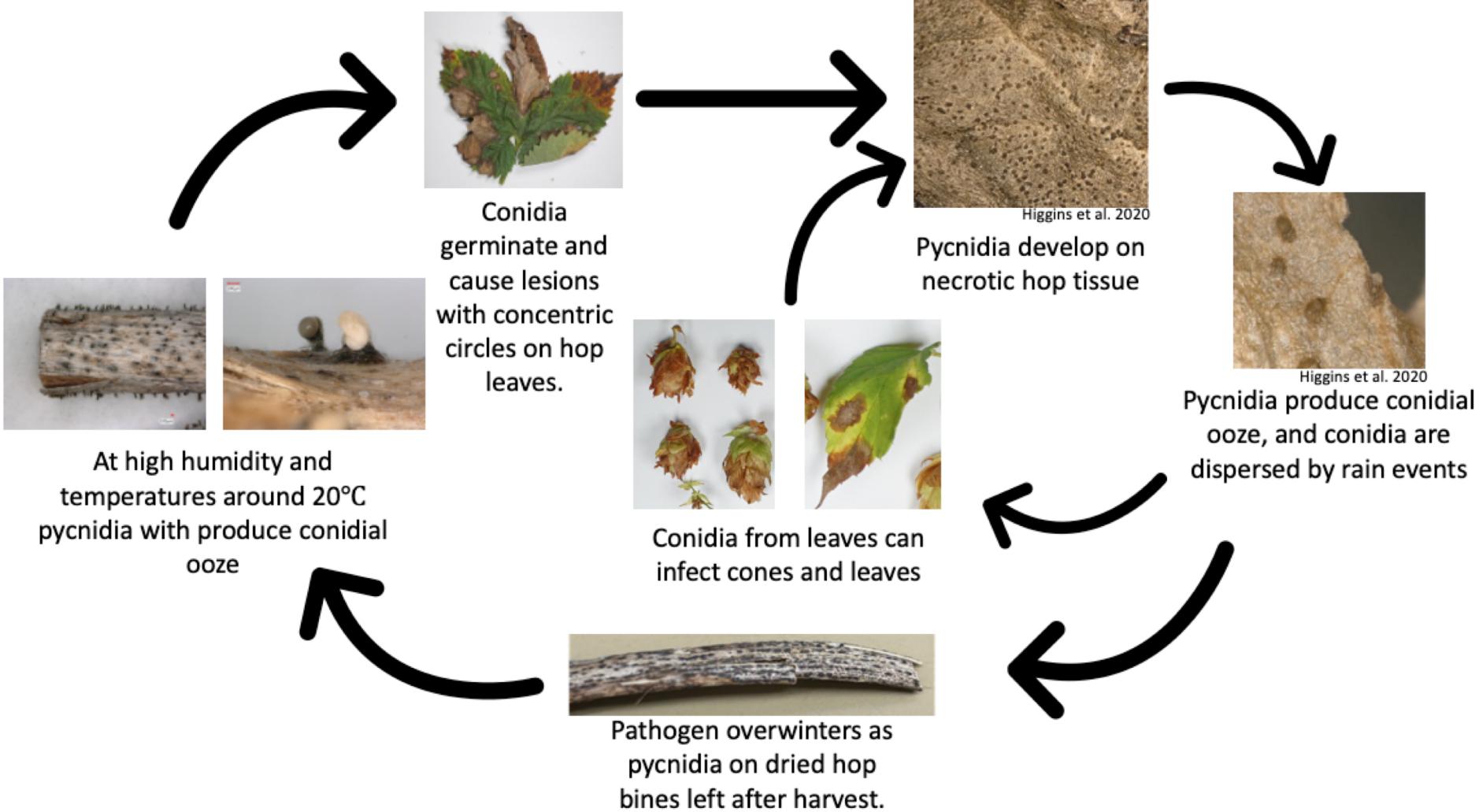


Photo by Ross Hatlen and Amy Albin



Photo by Ross Hatlen

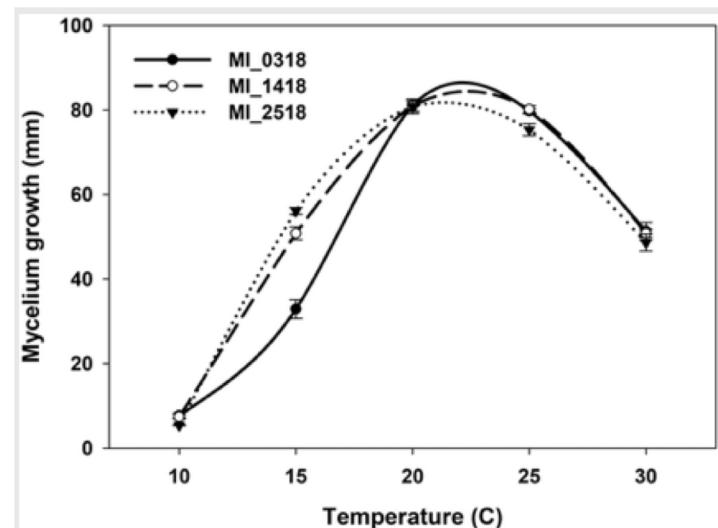






## Current literature on *Diaporthe* on hops

- Higher growth rates at 20-25 degrees Celsius and at high humidity (Higgins et al. 2020)
- Able to infect at least 12 cultivars: Centennial, Cascade, and Chinook (Allan-Perkins et al. 2020 and Higgins et al. 2020)
- Fungicides pyraclostrobin and boscalid will decrease the growth of this pathogen *in vitro* (Allan-Perkins et al. 2020)



Higgins et al. 2020



# Disease so severe it made national news

## Beer Heroes Battle Hops Disease In Great Lakes State



**Jeff Kart** Contributor  
Sustainability

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Daily News

## New Fungus Causes Hop Disease Across Lower Peninsula

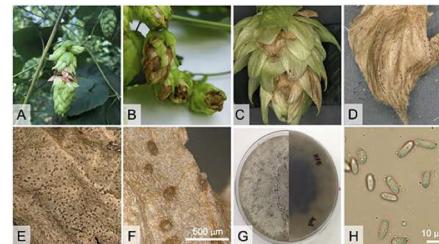
A new species of fungus is threatening Michigan's hop industry, according to researchers at Michigan State University in East Lansing. While craft brewers in the state are not facing a hop shortage, they prefer to use locally grown hops.

By **Grace Turner** - October 12, 2020

JUNE 8, 2021

### A new disease called halo blight threatens Michigan hop production

by American Phytopathological Society



Signs and symptoms of a *Diaporthe* sp. 1-MI on hop cones. Credit: Douglas S. Higgins, Ross J. Hatlen,...



## Summary of introduction

- Halo blight when not controlled causes high amounts of yield loss and is polycyclic
- The pathogen can overwinter on dead hop material
- Some fungicides decrease the growth of the fungi but have not been tested in a hopyard
- Halo blight changes the chemical profile of the hop cone



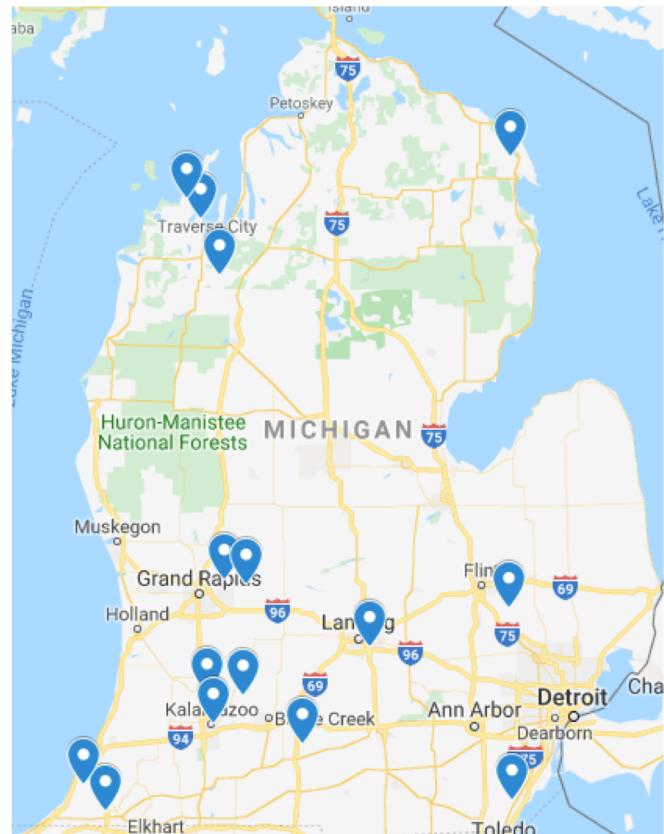


## Objectives

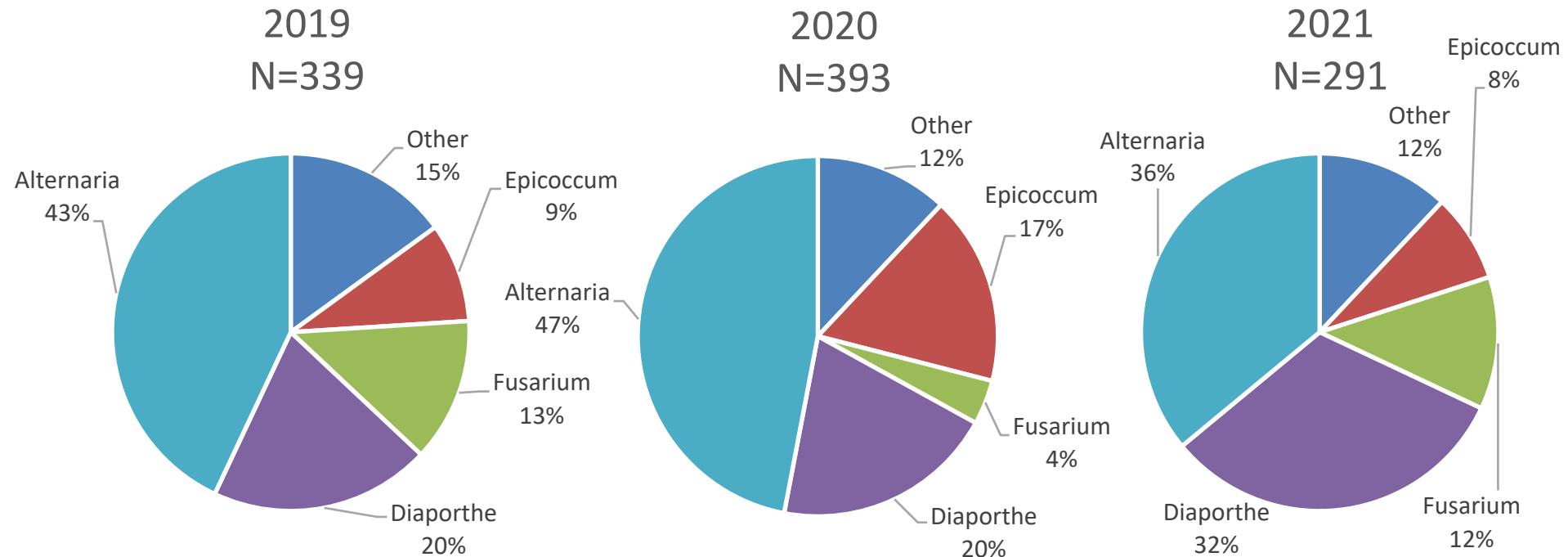
1. Determine what pathogens are greatly affecting hop yards in the Michigan
2. Identify fungicides that are already registered for hops that can decrease the severity of halo blight caused by *Diaporthe humulicola* through fungicide field testing
3. Test fungicides for the ability to decrease the growth rate of *Diaporthe humulicola* in a poison agar assay and test a population of *D. humulicola* for fungicide sensitivity

# Objective 1: Fungal cone disease survey

- 3 years (2019-2021) of surveying Michigan hop cone diseases
- Samples collected close to harvest, favoring fully developed cones
- Cone lesions were surface sterilized and then transferred to PDA amended with antibiotics
- Halo blight is in each hopyard sampled in Michigan



# *Diaporthe* populations in Michigan are increasing!!





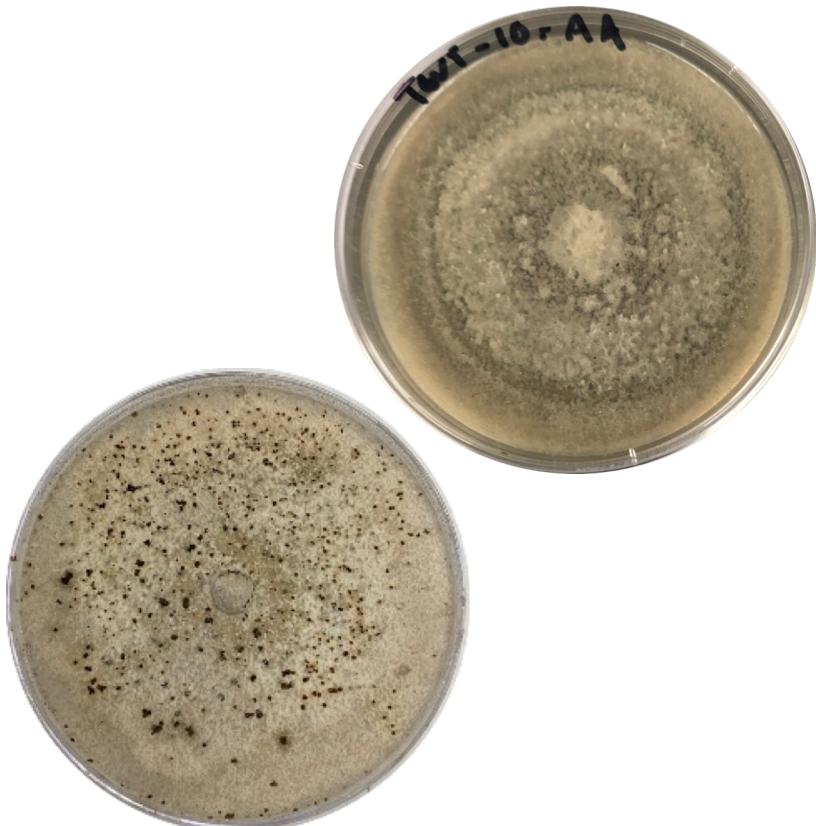
# Halo blight of hops is not just in Michigan!

- Growers throughout the Americas have sent samples to our lab
- Isolates are hyphal tipped transferred to produce pure cultures
- Isolates are stored in glycerol at -80°C
- **No isolates of *D. humulicola* have been discovered in the PNW**

Location	Number of isolates
Michigan	118
New York	47
Canada	24
<b>Minnesota</b>	11
Arkansas	4
Indiana	2
Connecticut	2

## Summary of hop disease survey

- *Alternaria* is the most prevalent pathogen, but is not the most economically important pathogen
- *Diaporthe* is now in every growing region in Michigan and seems to be spreading throughout the state.
- Halo blight has been found in 6 states and 2 countries



## Objective 2: Fungicide field trial

- Fungicide efficacy trial on short trellised hops
- 8 different fungicides that were already registered for hops for downy or powdery mildew control
- Random block trial with 4 replicates for each fungicide
- Repeated for 3 years
- Recorded disease severity and incidence

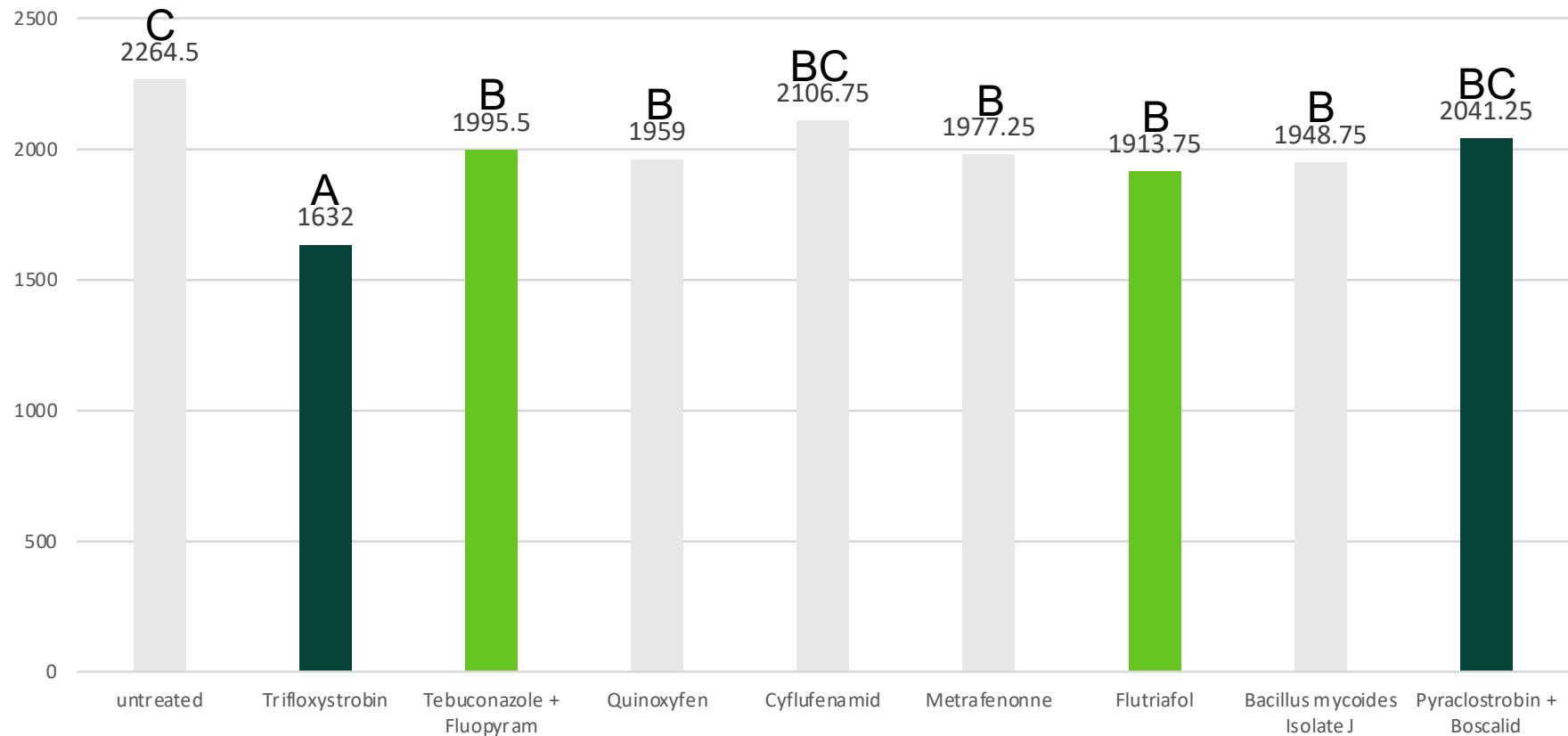


# Fungicides tested for control of halo blight

Active ingredient	FRAC group	Treatment	Manufacturer	Activity
Quinoxyfen	13	Quintec	Dow AgroSciences	Powdery mildew
Flutriafol	3	Rhyme	FMC	Broad spectrum
Trifloxystrobin	11	Flint extra	Bayer	Broad spectrum
Metrafenone	U8	Vivando	BASF Ag Products	Powdery mildew
Tebuconazole				Broad spectrum
Fluopyram	7/3	Luna experience	Agrian	
Pyraclostrobin				Broad spectrum
Boscalid	7/11	Pristine	BASF Ag Products	
Cyflufenamid	U6	Torino	Gowan	Powdery mildew
<i>Bacillus mycoides</i>				
Isolate J	P6	Lifegard WG	Certis	?? (Includes SAR)
Untreated				

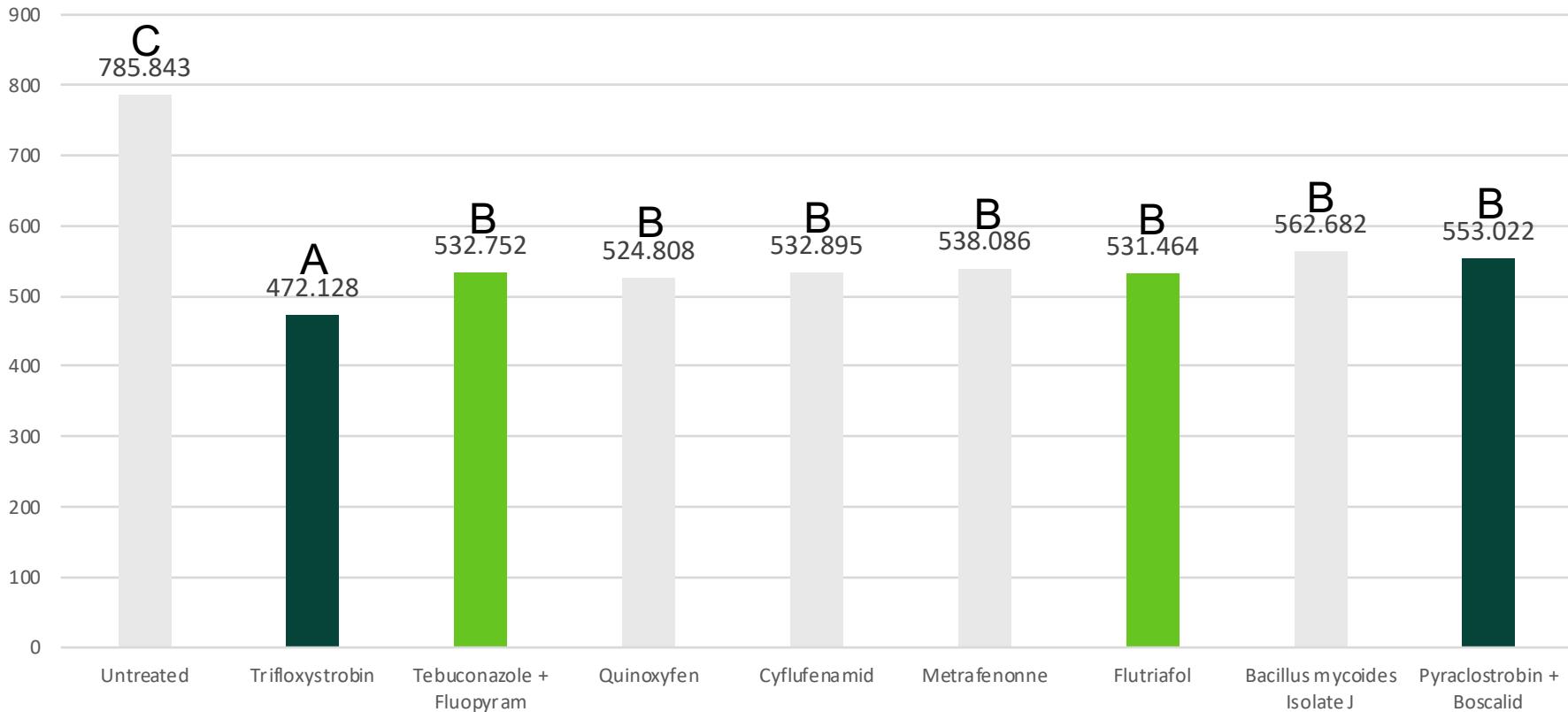


FRAC groups **11** and **3** can consistently decrease the **incidence** of halo blight infections





Frac groups **11** and **3** can consistently decrease the **severity** of halo blight infections



# Relationship of downy mildew and halo blight severity

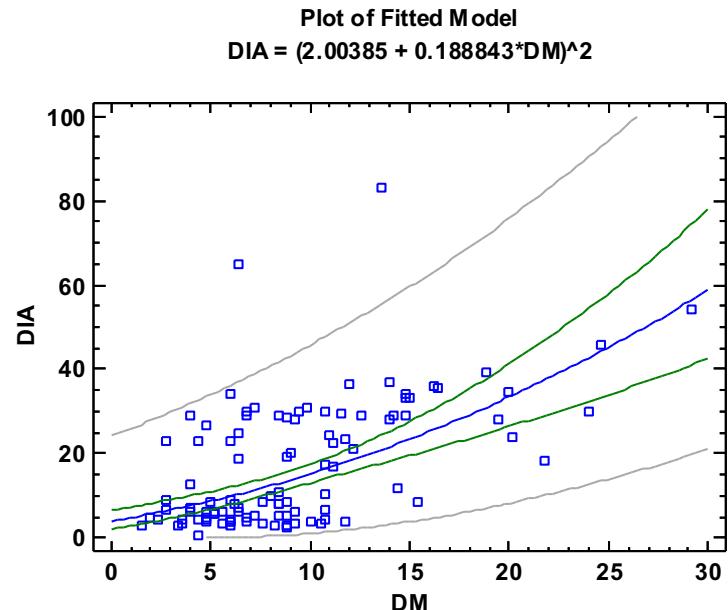
- Fungicide efficacy trial on short trellised hops repeated for two years
- Hops are inoculated with hop downy mildew and then halo blight of hops
- Plants were then sprayed with a variety of fungicides
- Hops are rated for downy mildew for the first half of the season and then halo blight





# There is a relationship between early season downy mildew infections and late season halo blight of hop infections

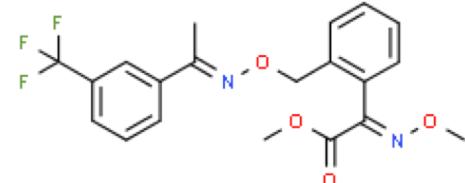
correlation between downy mildew and halo blight of hops			
Model	Correlation	R-Squared	ANOVA p value
Square root-Y	0.5676	32.22%	<0.002
Double square root	0.5642	31.83%	<0.002
Linear	0.5546	30.76%	<0.002
Logarithmic-Y square root-X	0.5469	29.92%	<0.002
Square root-X	0.5454	29.75%	<0.002
Exponential	0.5442	29.61%	<0.002
Square root-Y logarithmic-X	0.5394	29.09%	<0.002
Multiplicative	0.5289	27.97%	<0.002
Square root-Y squared-X	0.5281	27.89%	<0.002
Squared-X	0.5277	27.85%	<0.002



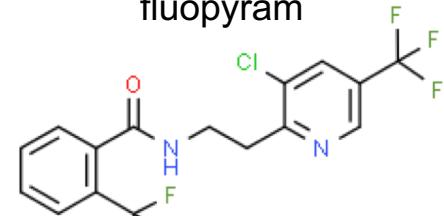
## Summary of fungicide field trials

- FRAC 3 and 11 chemicals can reduce the severity and incidence of halo blight
- Downy mildew infections in the early season can lead to higher amounts of halo blight lesions before harvest
- Some powdery mildew products (Quinoxyfen, Metrafenone, and Cyflufenamid) all seem to have some effect on the severity of halo blight lesions

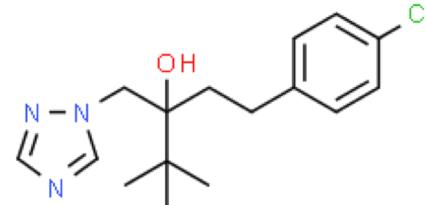
trifloxystrobin



fluopyram



tebuconazole



## Objective 3: Fungicide poison agar test

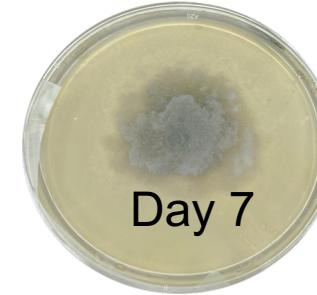
- Poison agar assay was conducted to produce EC50 values from the fungicides tested in the field experiment.
- Isolates 3M, 21M, CT, and CD6C isolates were used in this experiment.
- Isolates were grown on media with 0.001, 0.001, 0.01, 0, 1, 10, and 100ppm of the selected fungicide in triplicate
- Isolates were grown for seven days before measurements were conducted



Day 0



Day 3

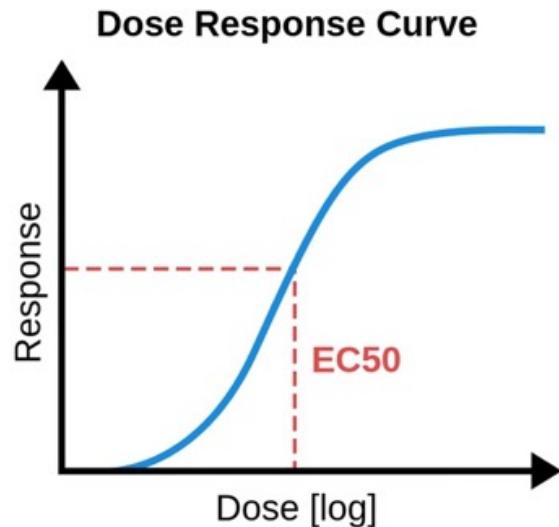


Day 7



## EC<sub>50</sub> Value

Concentration of a fungicide where the growth is reduced by 50% when compared to the control



<https://theory.labster.com/ec50/>

## Discriminatory Dose

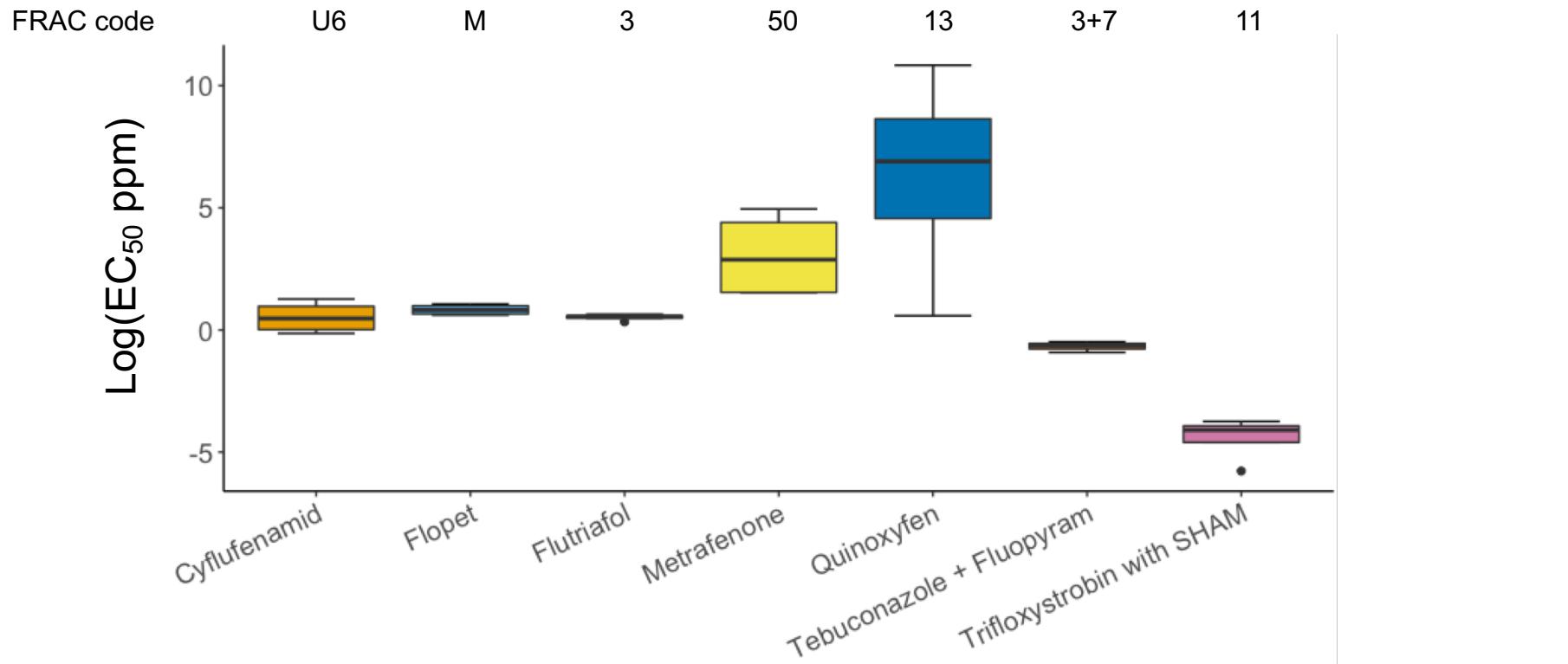
A test dosage to determine if an isolate is resistant to a specific chemical



Bradley et al. 2019

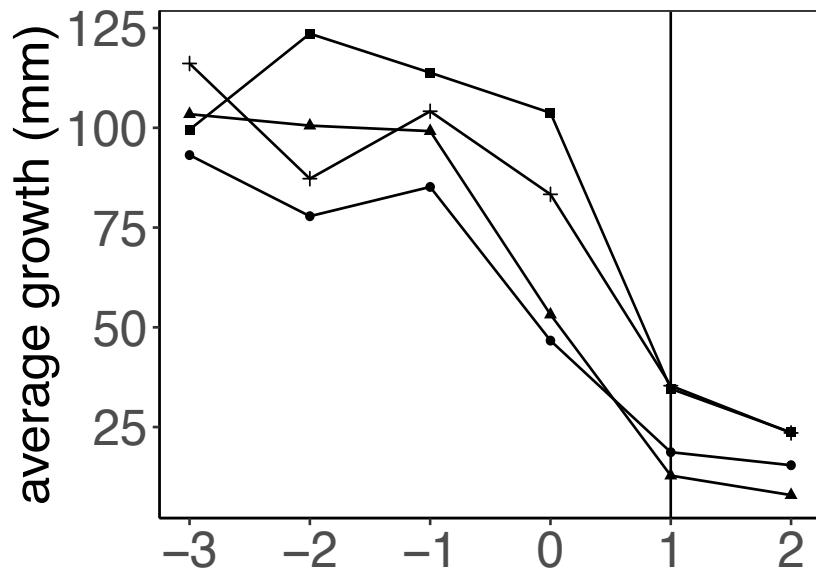


Chemicals metrafenone and quinoxyfen reduce severity of infections in the field, but don't affect the growth of *Diaporthe humulicola*

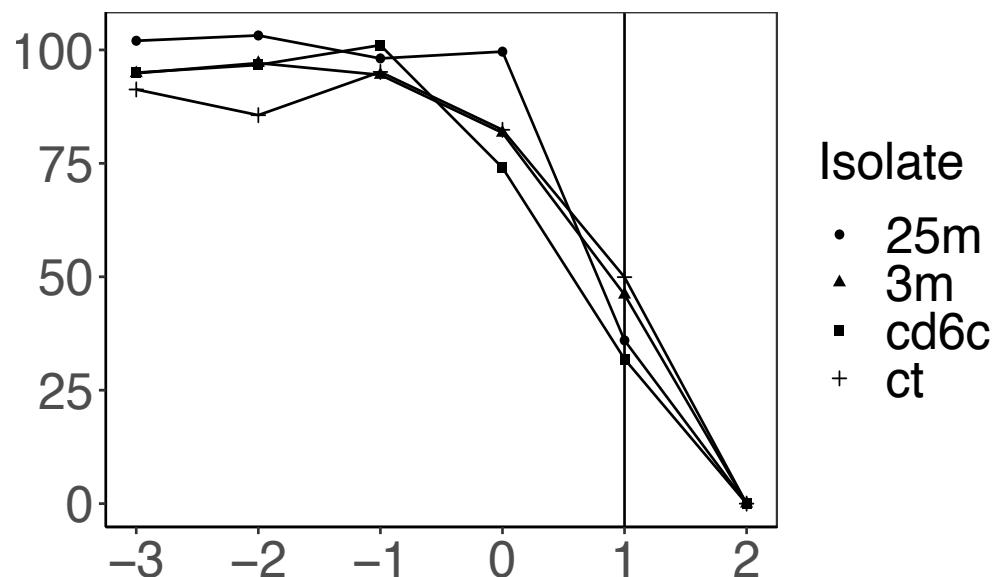


Chemicals cyflufenamid and flutriafol were tested with a discriminatory dose of 10ppm

Cyflufenamid

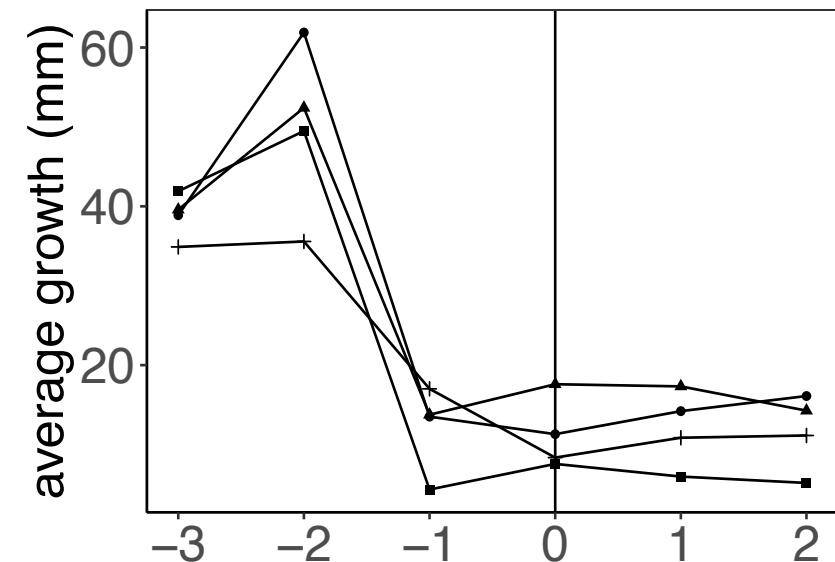


Flutriafol

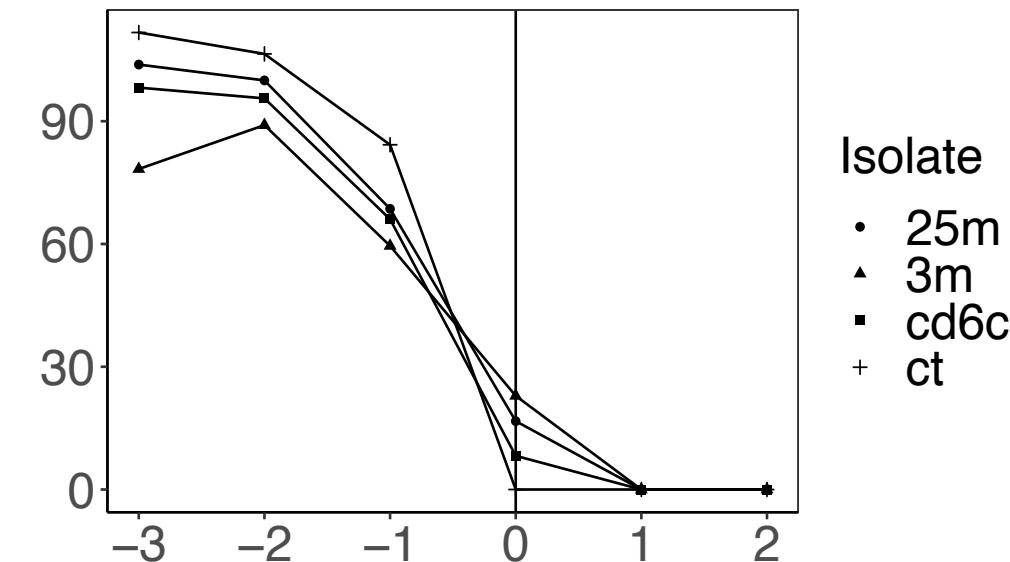


Chemicals trifloxystrobin with SHAM and tebuconazole + fluopyram were tested with a discriminatory dose of 1ppm

Trifloxystrobin



Tebuconazole + Fluopyram



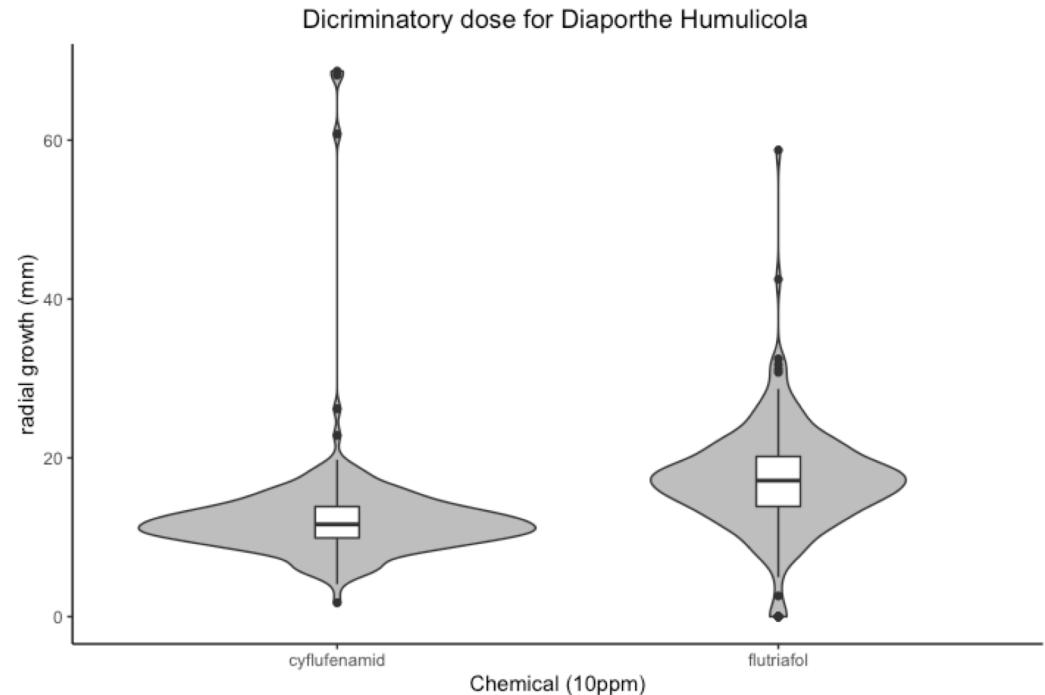
## Objective 3: Discriminatory dose assay

- Discriminatory dose assay of trifloxystrobin with sham, tebuconazole + fluopyram, cyflufenamid, and flutriafol
- Used isolate growth from  $EC_{50}$  value experiment to determine discriminatory dose testing values
- Plates were measured 7 days after subculture

Location	Number of isolates
Michigan	118
New York	47
Canada	24
Minnesota	11
Arkansas	4
Indiana	2

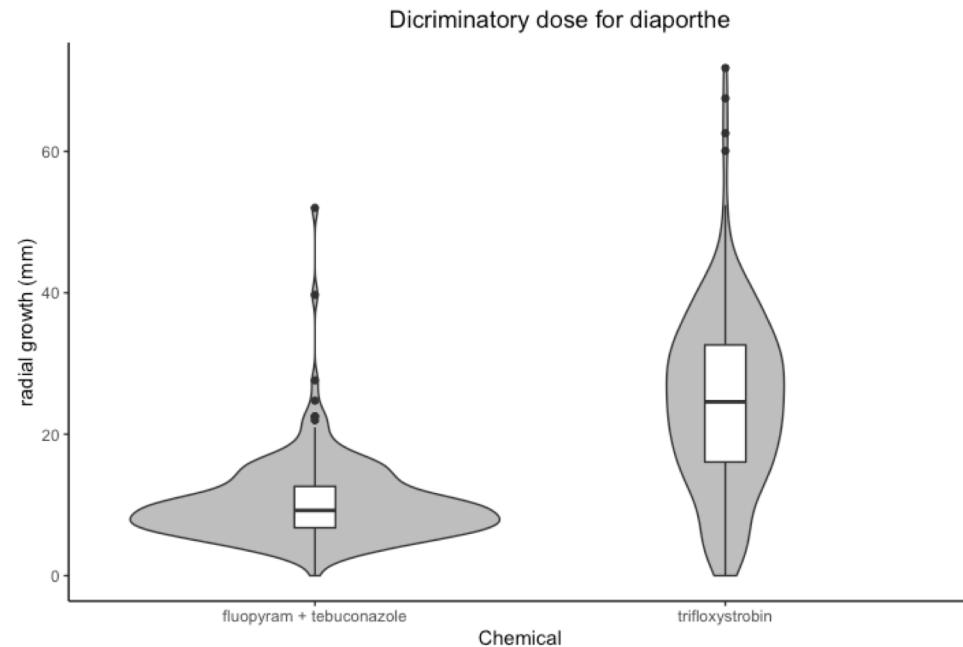
# There is more variation in size when exposed to flutriafol

- Violin plots are a combination of box plots and kernel density plots are used to visualize distribution of numerical data
- Cyflufenamid has lower amount of growth compared to flutriafol and growth was consistent across different isolates
- Flutriafol had more variety in size after 7 days of growth



# Some isolates have lessened sensitivity to trifloxystrobin with SHAM

- Fluopyram + tebuconazole seem to have the lowest average growth size of all fungicides
- Over half the isolates grown on trifloxystrobin with SHAM were able to grow bigger than the median size
- Trifloxystrobin is in two different products labeled for hops.

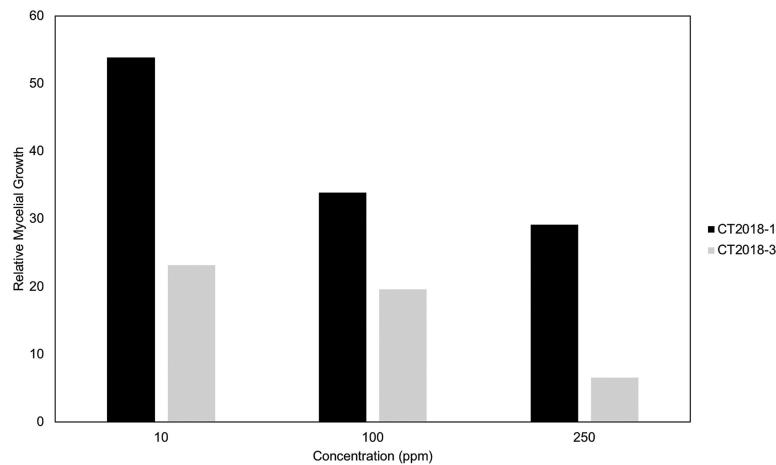


# Differences in fungicide sensitivity based on regionality

ANOVA DD and location				
Fungicide				
Locations	Tebuconazole + Floupyram	Flutriafol	Cyflufenamid	Trifloxystrobin + SHAM
		Measurements (mm)		
Canada	7.266 A	14.8025 BC	11.4752 A	23.6469 A
Michigan	9.86 B	17.4773 BC	12.2369 A	25.2744 A
New York	10.332 BC	19.111 C	13.459 A	22.0535 A
Minnesota	13.389 CD	15.8844 BC	13.5611 A	24.5374 A
Indiana	14.652 BCD	11.2342 ABC	14.74167 A	24.6625 AB
Arkansas	21.962 D	10.3775 A	16.8183 A	44.1467 B
Location <i>P</i> value	<0.01	0.0149	0.3895	0.0268

## Summary of poison agar and discriminatory dose assay

- Quinoxyfen and metrafenone seem to decrease severity in field experiments, but do not decrease the growth of *D. humulicola* in petri dishes
- While trifloxystrobin works in the field and has a low EC<sub>50</sub> value there seems to be parts of the *D. humulicola* population that have lowered sensitivity, and there seems to be different levels of sensitivity in a single yard
- There are slight differences based on regionality for fluopyram + tebuconazole, and flutriafol



From: Allan-Perkins et al 2020

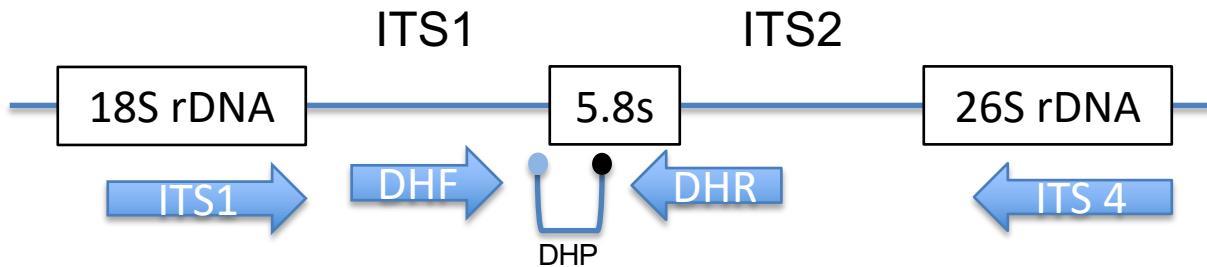
## Discussion

- FRAC 3 and 11 are our best options for the treatment of halo blight of hops
- Some *D. humulicola* isolates are less sensitive to trifloxystrobin, and sensitivity dose not seem to be consistent across a single yard
- Fluopyam + tebuconazole (Luna Experience) has a low EC50 value, a sensitive population, and can control the severity and incidence of the pathogen, thus is one of our top recommendations for control of halo blight

# Future directions for halo blight of hops research

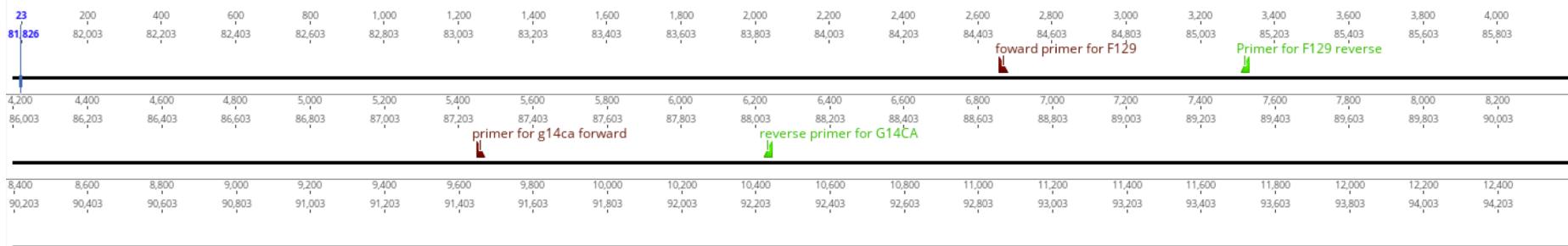


## Primers and probes for identification of halo blight



- ITS region of *D. humulicola* was used to produce a qPCR assay for identification
- Still in validation stage
- Will be multiplexed with downy mildew and powdery mildew qPCR assays

# Screening for Qol fungicide resistance



- Used *D. humulicola* genome to piece together cytochrome b (8 exons)
- Developed PCR primers to screen for known mutations F129, G137, G143
- Screen population from previous experiments and use this to determine isolates of interest for low coverage genome sequencing



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## Committee members:

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Dr. Marty Chilvers  
Dr. Mary Hausbeck  
Dr. Timothy Miles

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Dr. Kerri Neugubauer  
Lexi Heger  
Nancy Sharma  
Shay Szymanski  
Sam Thompson  
Jerri Gillett  
Roger Sysak  
And all Miles' undergrads



**Biggest thanks to  
Michigan hop  
growers**

QUESTIONS?

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