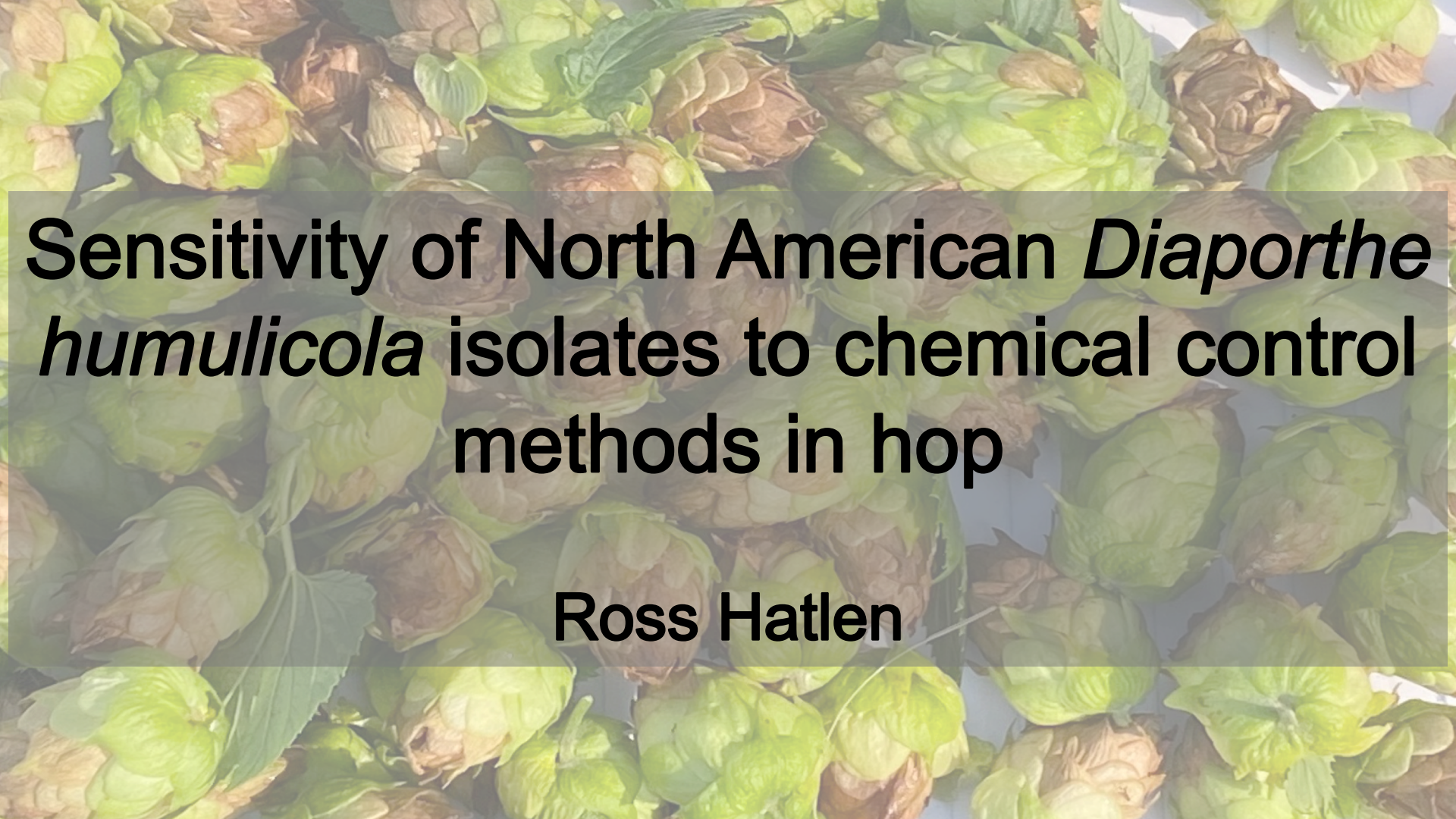




Sensitivity of North American *Diaporthe humulicola* isolates to chemical control methods in hop

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_{50} values and discriminatory dose assay
 - ❖ Fungicide sensitivity of *Diaporthe* population
 - ❖ Regional differences in fungicide sensitivity

Humulus lupulus: the common hop plant

- Vine growing diecious 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



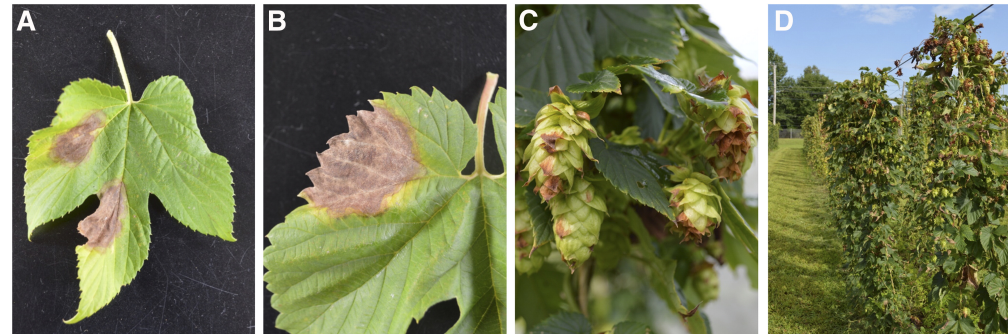
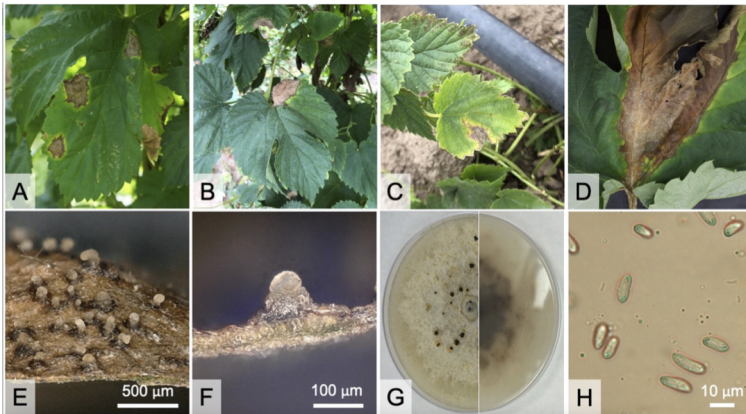
History of halo blight

Michigan

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

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



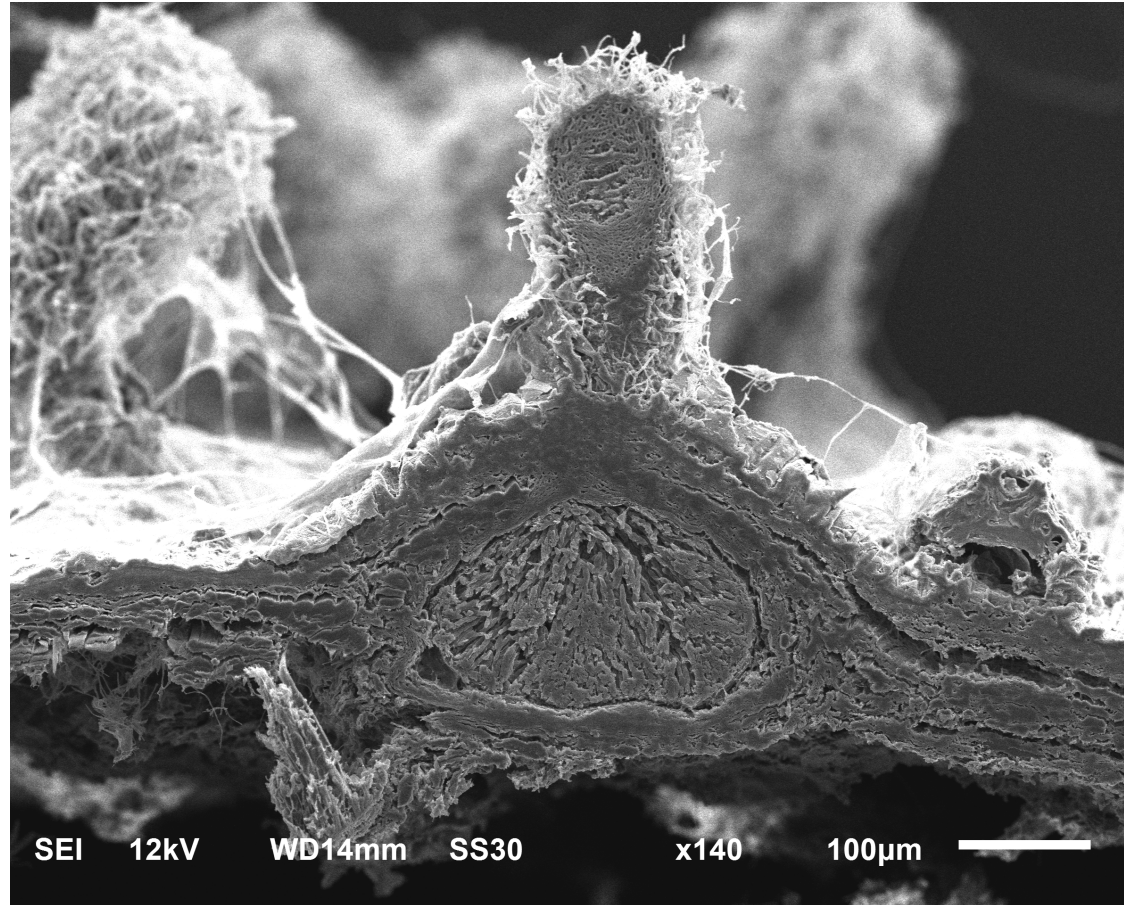
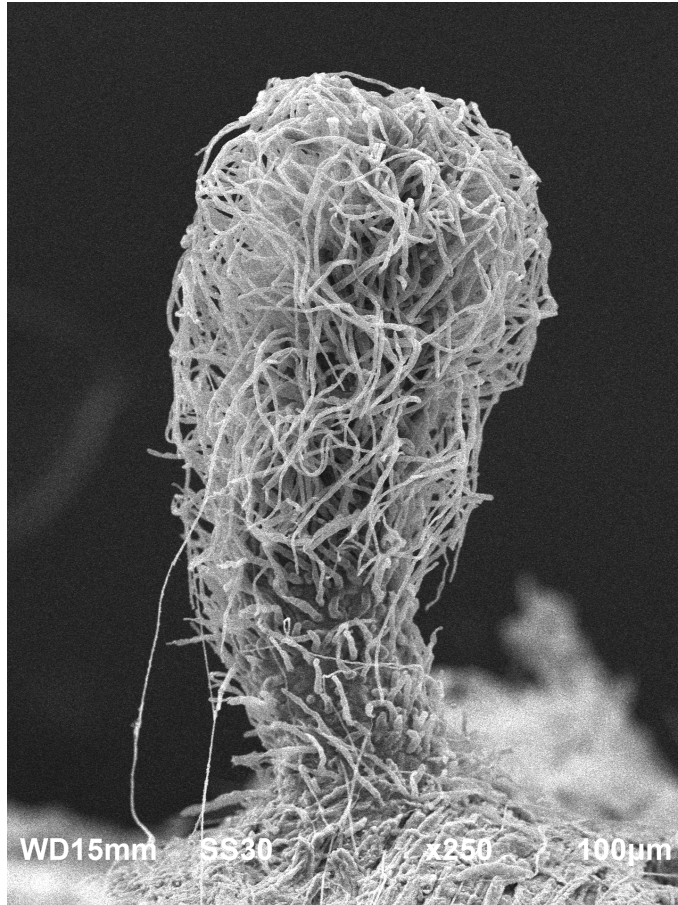
Pycnidia are formed on desiccated hop tissue

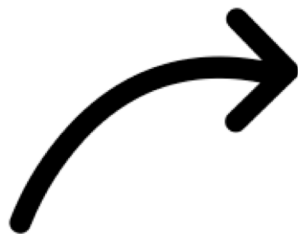


Photo by Ross Hatlen and Amy Albin

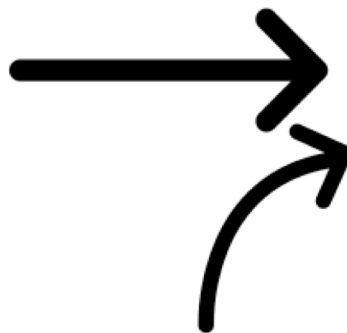


Photo by Ross Hatlen



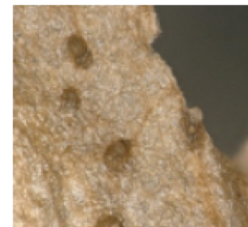


Conidia
germinate and
cause lesions
with concentric
circles on hop
leaves.



Higgins et al. 2020

Pycnidia develop on
necrotic hop tissue



Higgins et al. 2020

Pycnidia produce conidial
ooze, and conidia are
dispersed by rain events



At high humidity and
temperatures around 20°C
pycnidia will produce conidial
ooze



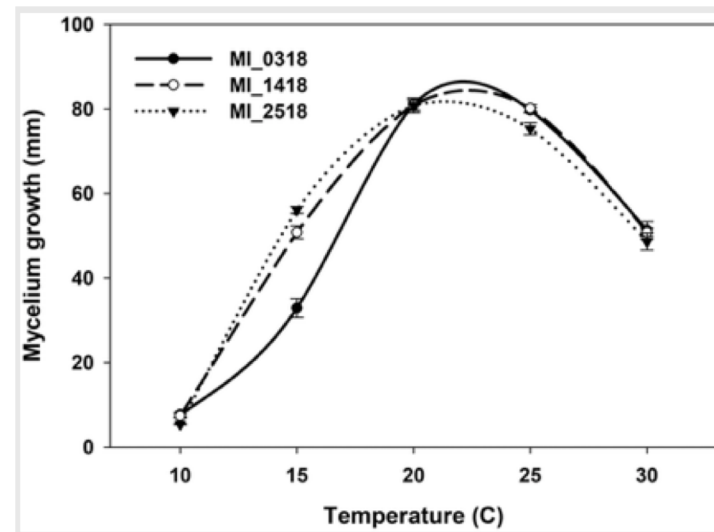
Conidia from leaves can
infect cones and leaves



Pathogen overwinters as
pycnidia on dried hop
bines left after harvest.

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 ①
Sustainability

Follow

Daily News

New Fungus Causes Hop Disease Across Lower Peninsula

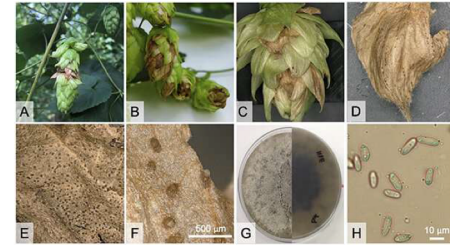
A new species of fungus is threatening Michigan's hop industry, according to researchers at Michigan State University in East Lansing. While craft brewers in the state are not facing a hop shortage, they prefer to use locally grown hops.

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

① 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

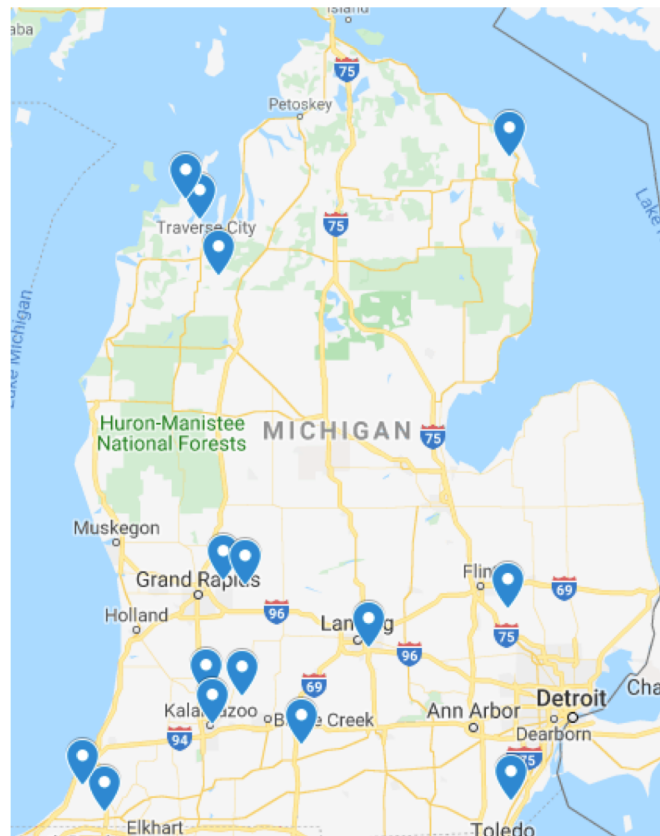


Objectives

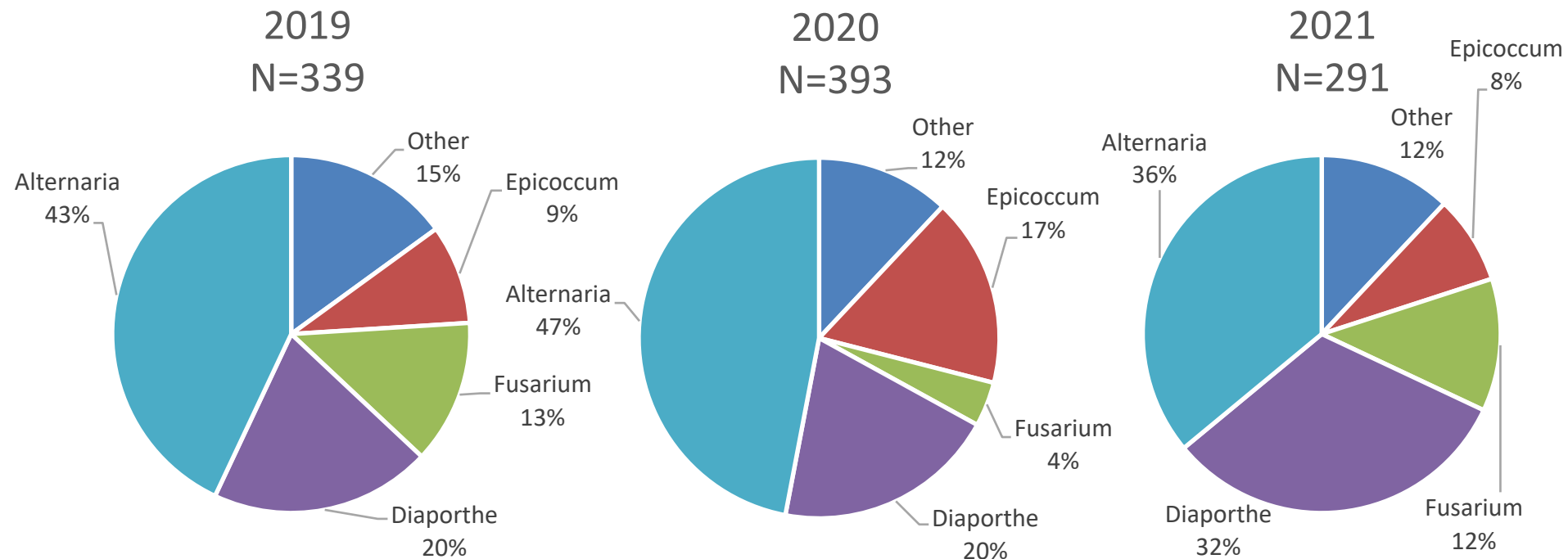
1. Determine what pathogens are greatly affecting hop yards in the Michigan
2. Identify fungicides that are already registered for hops that can decrease the severity of halo blight caused by *Diaporthe humulicola* through fungicide field testing
3. Test fungicides for the ability to decrease the growth 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!!



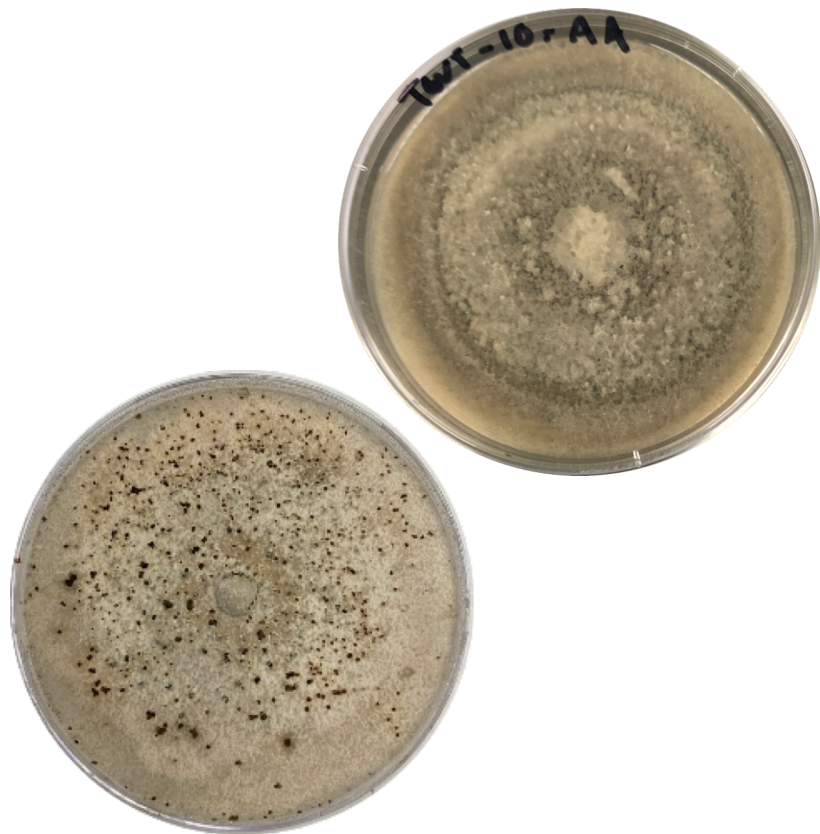
Halo blight of hops is not just in Michigan!

- 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
Minnesota	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

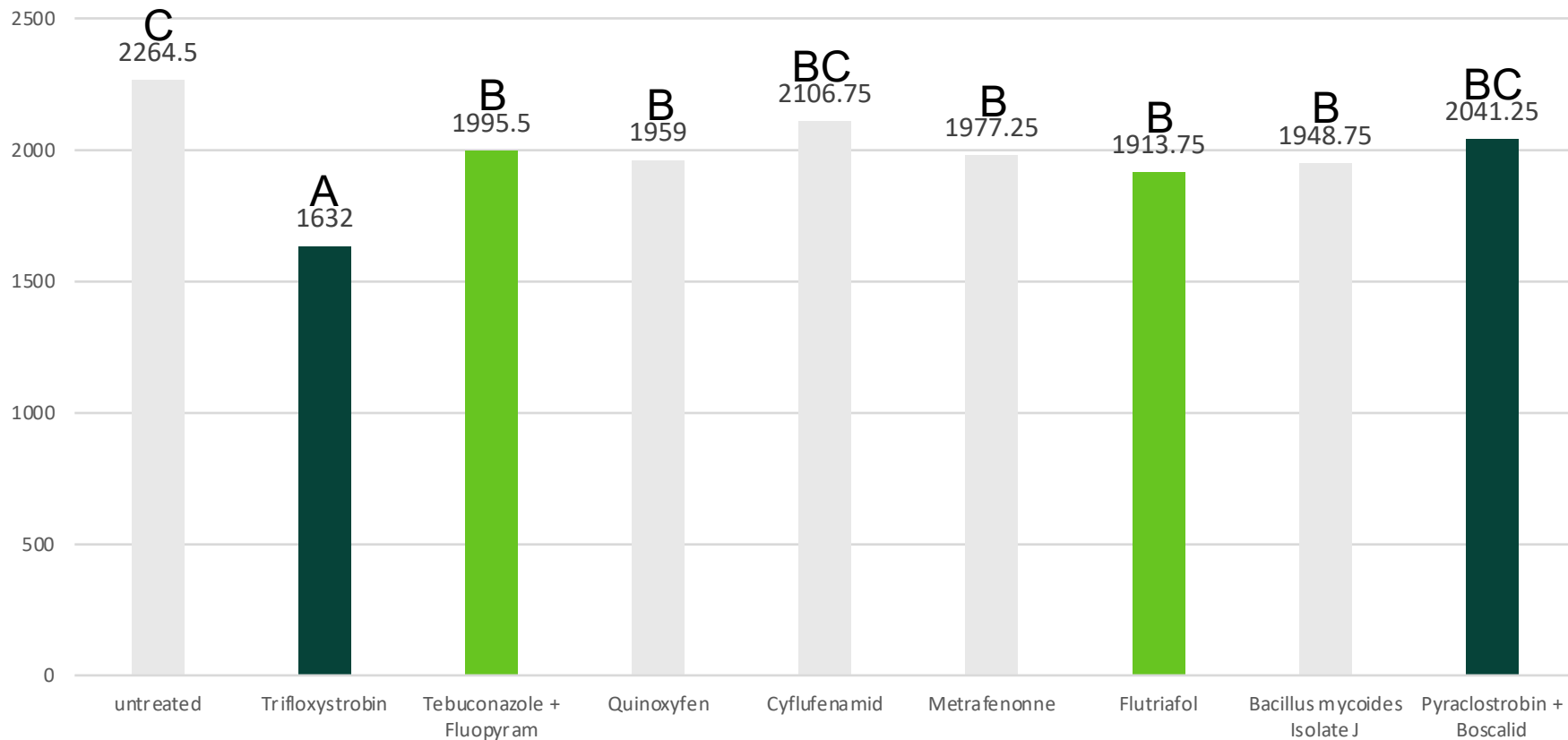
- Fungicide efficacy trial on short trellised hops
- 8 different fungicides that were already registered for hops for downy or powdery mildew control
- Random block trial with 4 replicates for each fungicide
- Repeated for 3 years
- Recorded disease severity and incidence



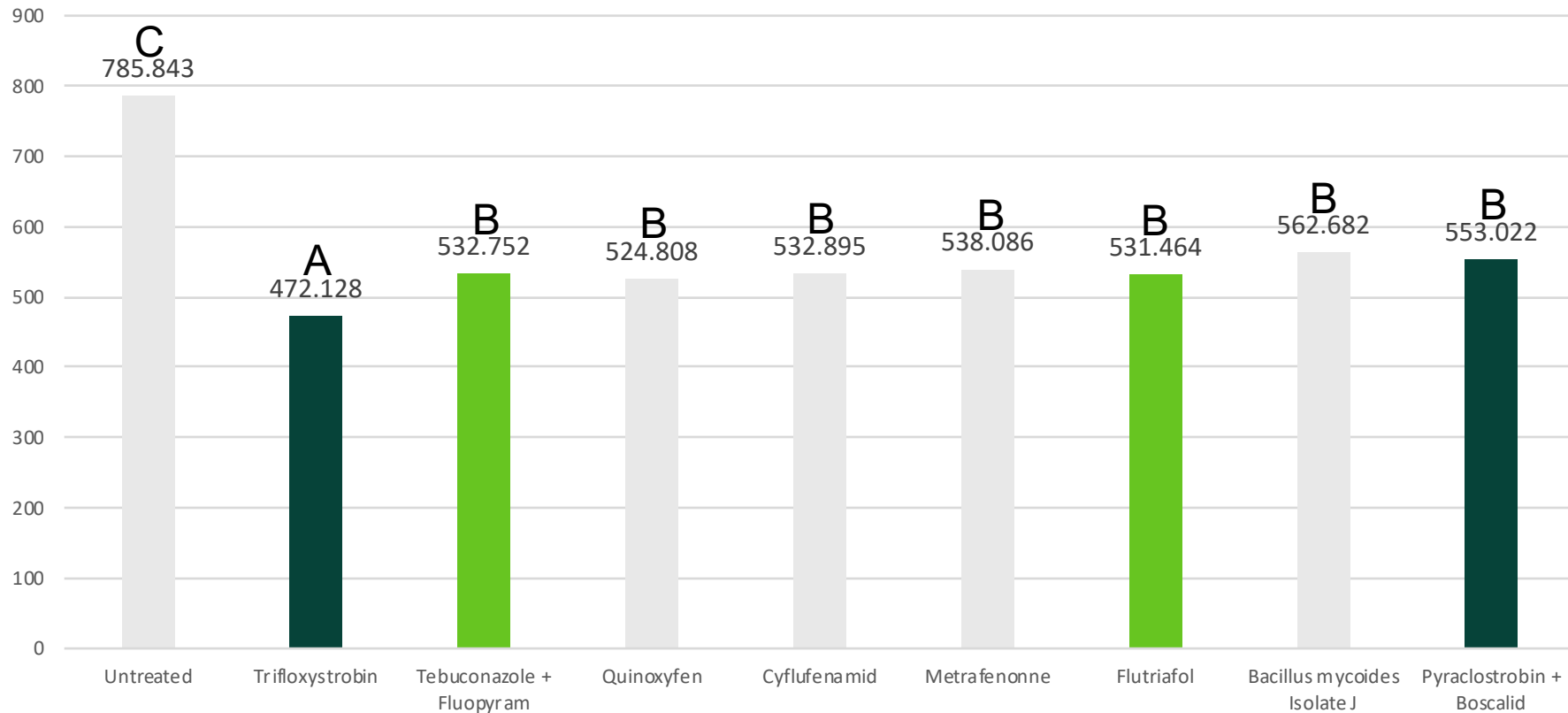
Fungicides tested for control of halo blight

Active ingredient	FRAC group	Treatment	Manufacturer	Activity
Quinoxifen	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	7/3	Luna experience	Agrian	Broad spectrum
Fluopyram				
Pyraclostrobin	7/11	Pristine	BASF Ag Products	Broad spectrum
Boscalid				
Cyflufenamid	U6	Torino	Gowan	Powdery mildew
<i>Bacillus mycoides</i>	P6	Lifegard WG	Certis	?? (Includes SAR)
Isolate J				
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



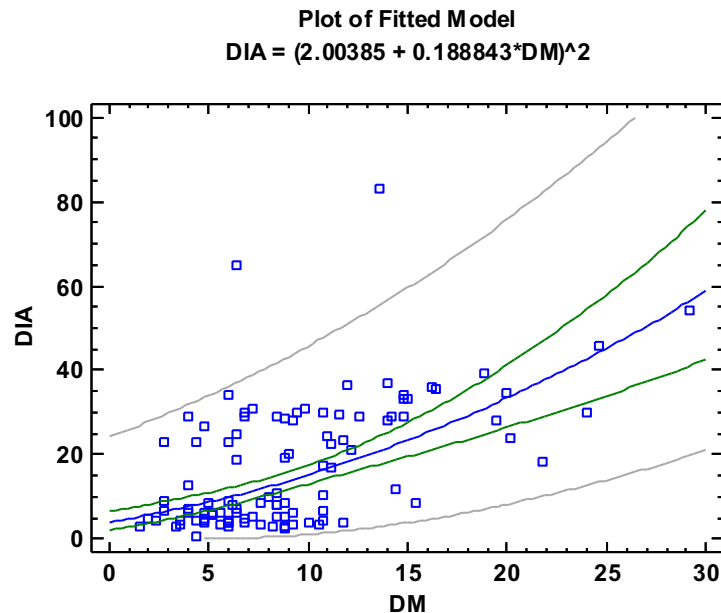
Relationship of downy mildew and halo blight severity

- 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

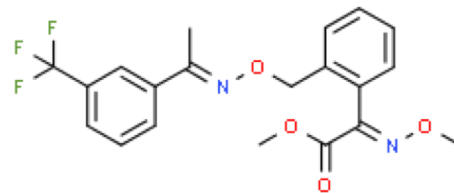
correlation between downy mildew and halo blight of hops			
Model	Correlation	R-Squared	ANOVA p value
Square root-Y	0.5676	32.22%	<0.002
Double square root	0.5642	31.83%	<0.002
Linear	0.5546	30.76%	<0.002
Logarithmic-Y square root-X	0.5469	29.92%	<0.002
Square root-X	0.5454	29.75%	<0.002
Exponential	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



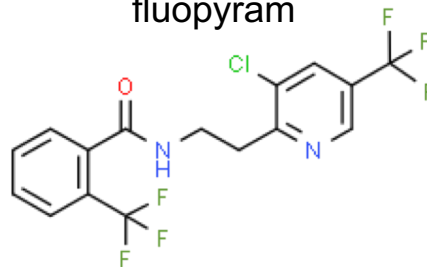
Summary of fungicide field trials

- 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 (Quinoxifen, Metrafenone, and Cyflufenamid) all seem to have some effect on the severity of halo blight lesions

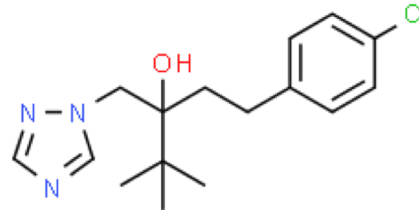
trifloxystrobin



fluopyram

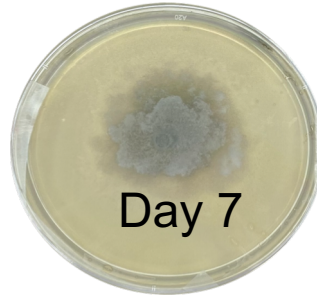
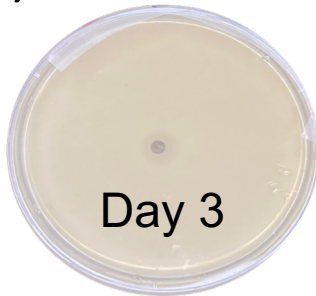
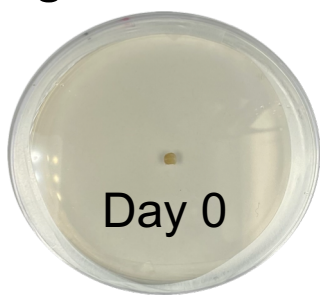


tebuconazole



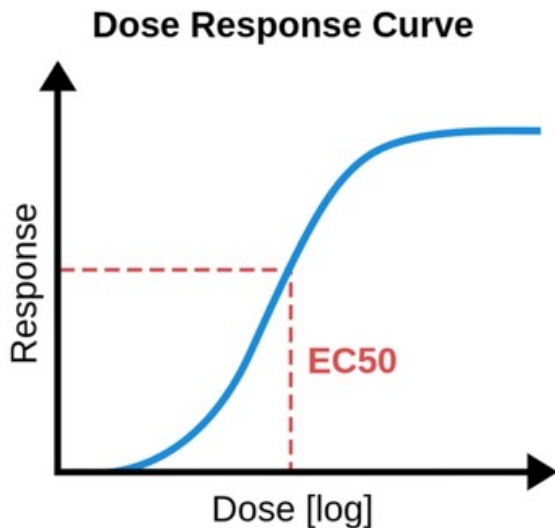
Objective 3: Fungicide poison agar test

- 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



EC₅₀ Value

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



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

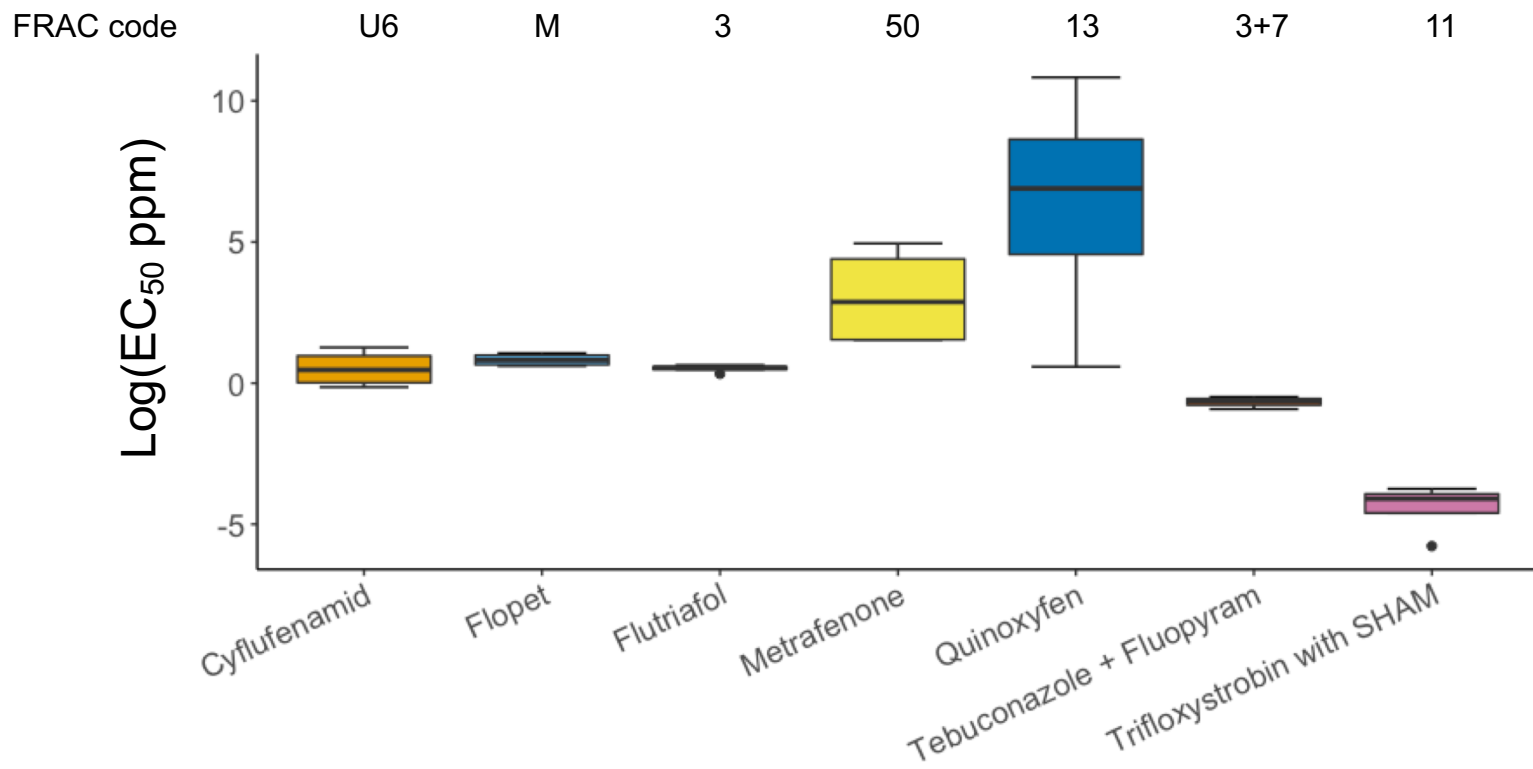
Discriminatory Dose

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



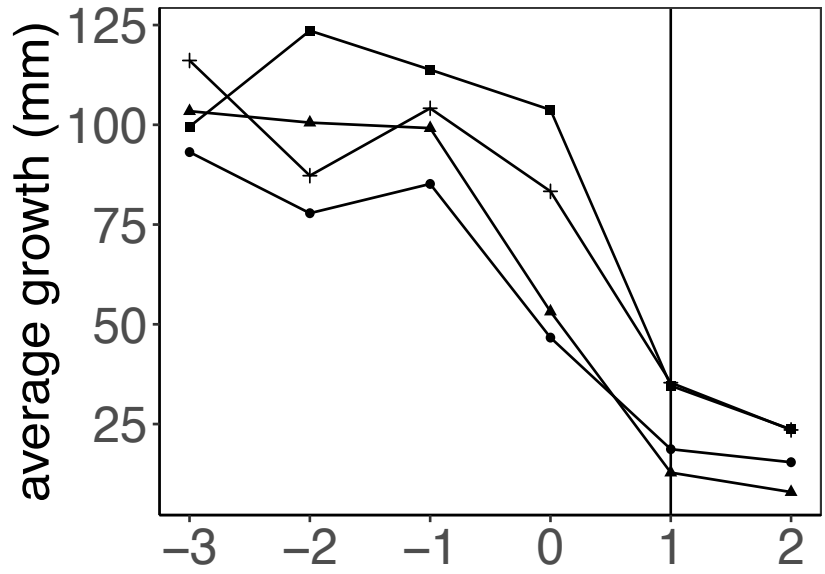
Bradley et al. 2019

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

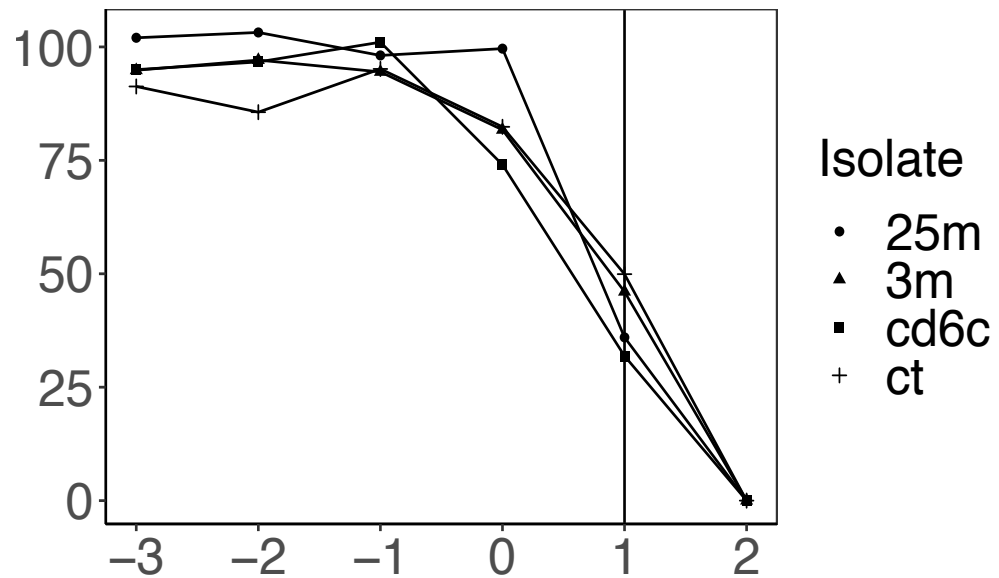


Chemicals cyflufenamid and flutriafol were tested with a discriminatory dose of 10ppm

Cyflufenamid

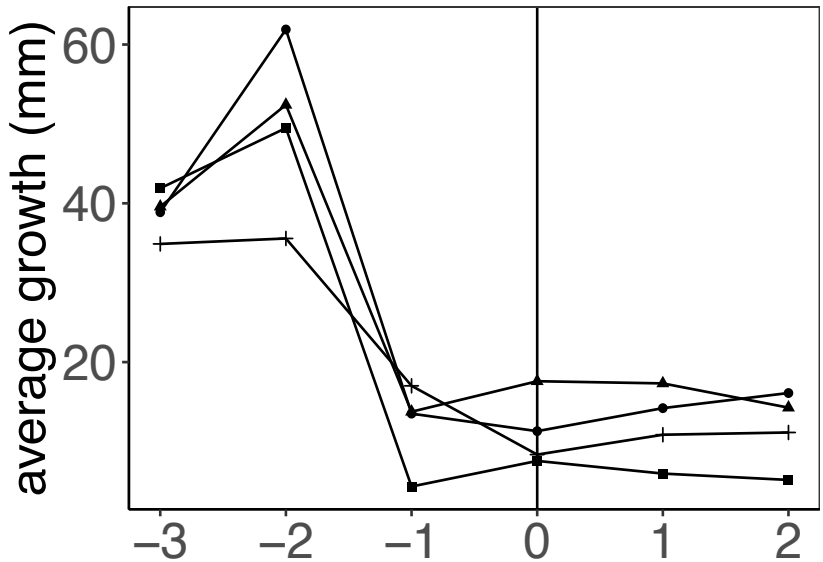


Flutriafol

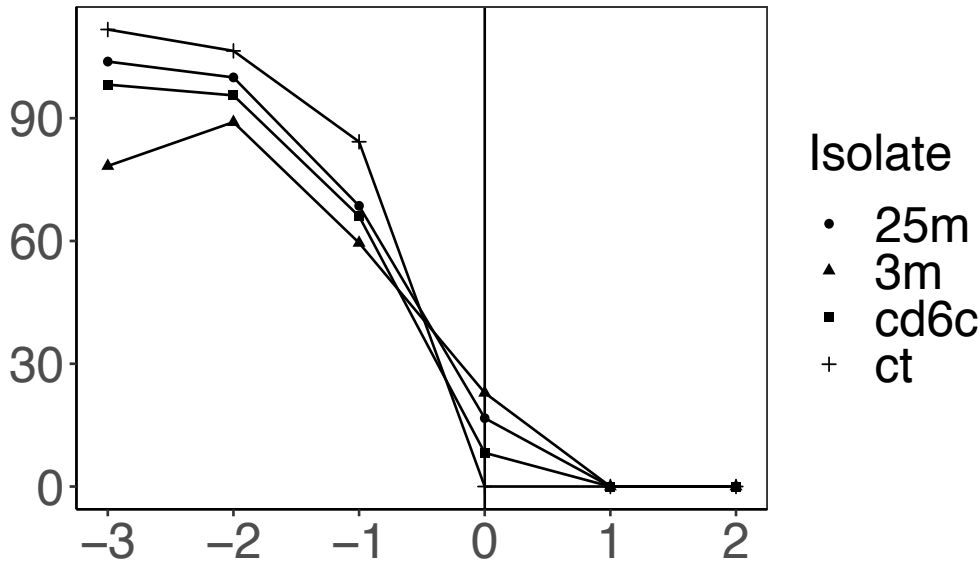


Chemicals trifloxystrobin with SHAM and tebuconazole + fluopyram were tested with a discriminatory dose of 1ppm

Trifloxystrobin



Tebuconazole + Fluopyram



Isolate
• 25m
▲ 3m
■ cd6c
+ ct

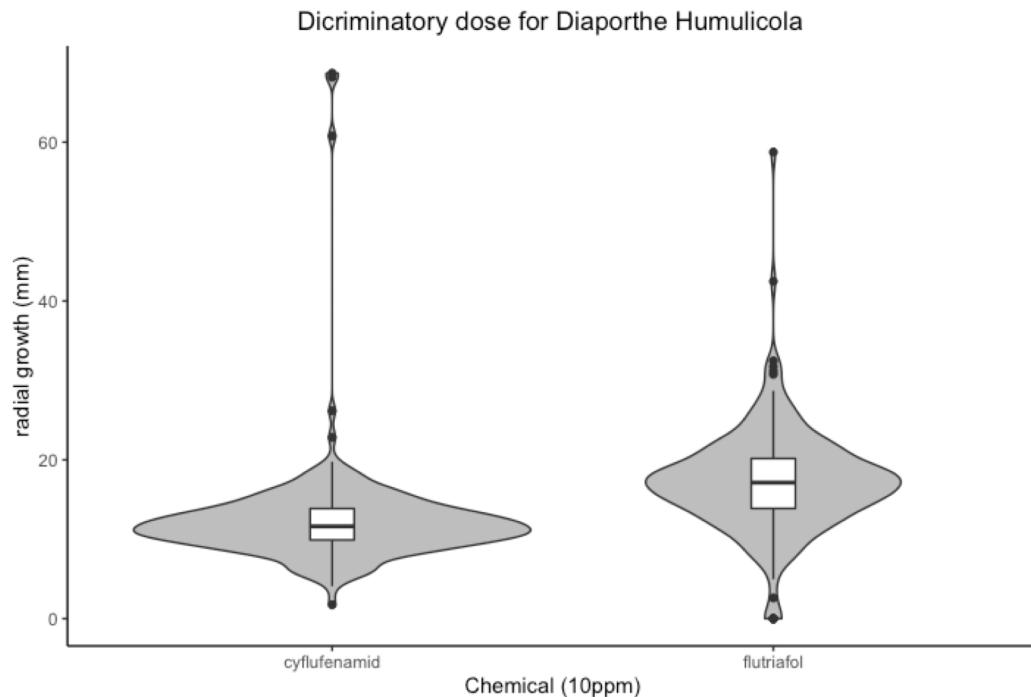
Objective 3: Discriminatory dose assay

- 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

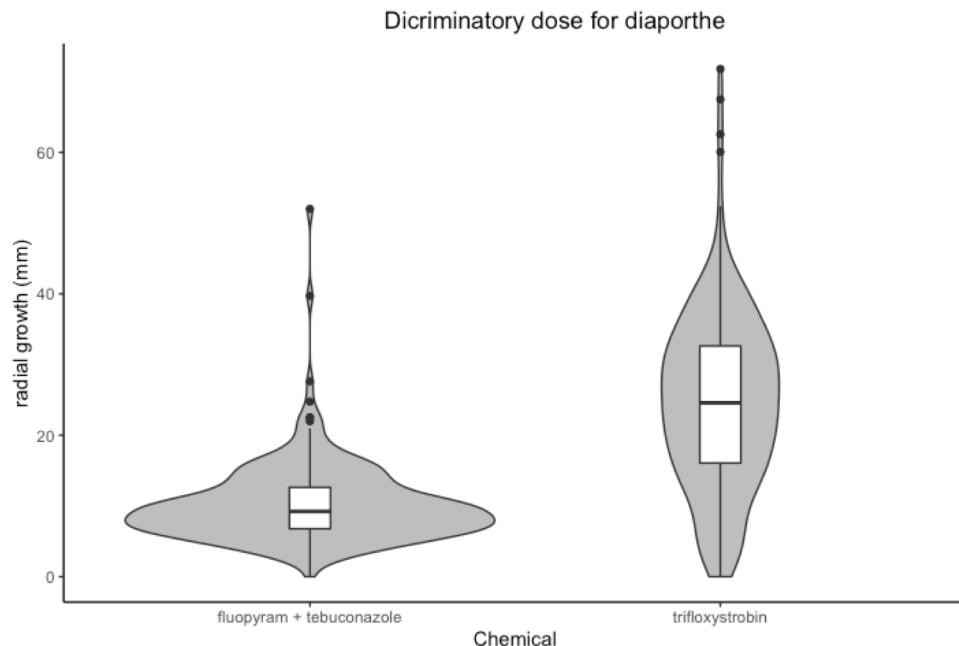
There is more variation in size when exposed to flutriafol

- Violin plots are a combination of box plots and kernel density plots and 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.

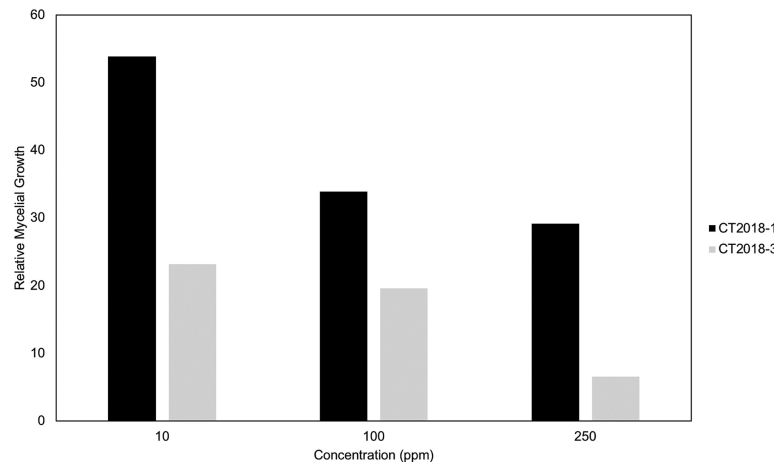


Differences in fungicide sensitivity based on regionality

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

- Quinoxifen 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_{50} 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



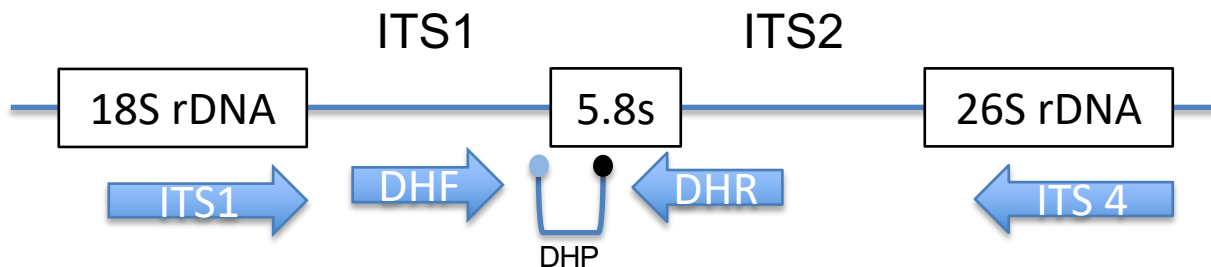
From: Allan-Perkins et al 2020

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
- Fluopyram + 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

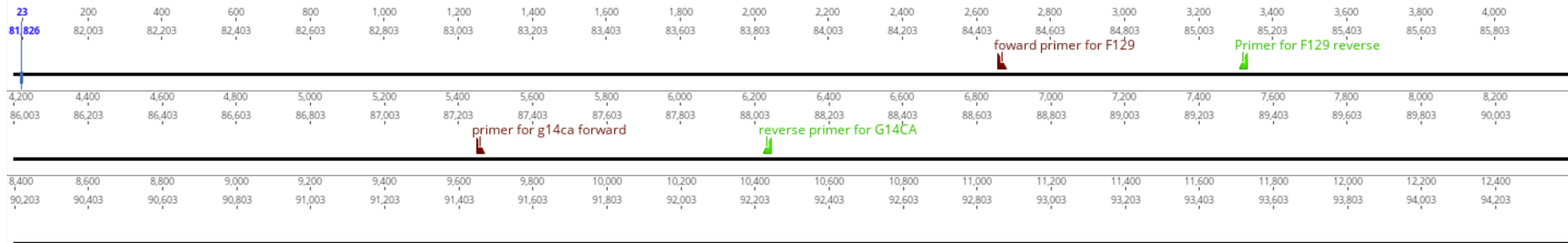
Future directions for halo blight of hops research

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

Acknowledgements

Committee members:

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

Collaborators:

Dr. Alejandro Rojas
Dr. Julien Venne
Dr. Jan Byrne
Dr. Doug Higgins
Dr. Rob Sirrine
Erin Lizotte

Miles' lab past and present:

Dr. Timothy Miles
Dr. Marivi Colle
Dr. Safa Alzohairy
Dr. Jennie Klein-Gordon
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?
hatlenro@msu.edu