Integrated strategies for management of multiple diseases and fungicide resistance in strawberry

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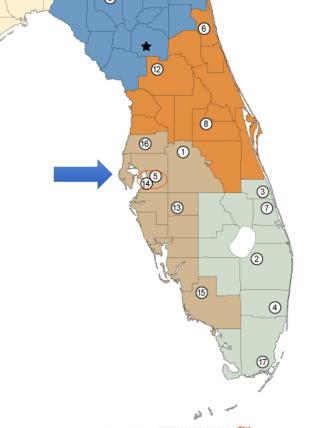
Strawberry is a high value, high risk crop

✓ FL ~10,000 acres: 95% commercial production in Hillsborough Co.

√ High value: > US\$300 million/year

√ High investment: \$30,000/ac!!

(average size farm ~20-30 acres)





Annual crop (investment) in FL





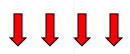
Overhead during plant establishment and freeze protection
Drip irrigation otherwise





Peak harvest periods



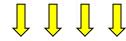


Peak bloom periods



Land prep / planting







RESET

Sept

Oct

Nov

Dec

Jan

Feb

Mar

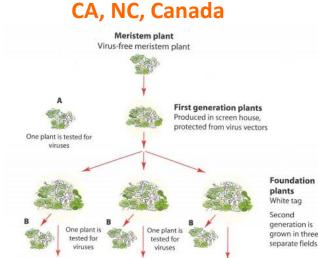
Apr

Transplants are produced far from fruit production fields (little control)











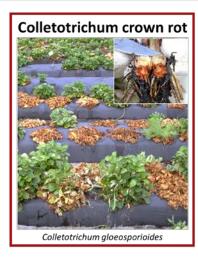
Registered plants Purple tag

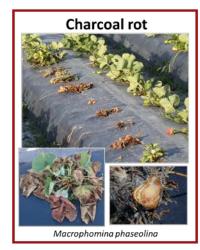
Certified plants

Many diseases introduced with transplants (High risk)

Fungal

Phytophthora crown rot Phytophthora cactorum





Bacterial











Same single-site fungicides available for production fields and nurseries



		Fungicide
	Active ingredient	group
	thiophanate-methyl	1
	iprodione	2
	Propiconazole, tetraconazole, myclobutanil	3
	mefenoxam	4
	penthiopyrad	7
	isofetamid	7
	pyrimethanil	9
	azoxystrobin, pyraclostrobin, trifloxystrobin	11
	quinoxyfen	13
Š	fenhexamid	17
外域	fenhexamid + captan	17 + M4
	fosetyl-Al, phosphites	33
	azoxystrobin + propiconazole	3 + 11
	fluoypram + pyrimethanil	7 + 9
U	fluxapyroxad + pyraclostrobin	7 + 11
	cyprodinil + fludioxonil	9 + 12
	cyflufenamid	U6
	copper	M1
	sulfur	M2
-	thiram	M3
	captan	M4



Emergence of fungicide resistance to multiple fungicides and pathogens – *C. acutatum*

Resistance in Strawberry Isolates of *Colletotrichum acutatum* from Florida to Quinone-Outside Inhibitor Fungicides

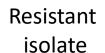
Bruna B. Forcelini and Teresa E. Seijo, Gulf Coast Research and Education Center, University of Florida, Wimauma 33598; Achour Amiri, Tree Fruit Research and Extension Center, Washington State University, Wenatchee 98801; and Natalia A. Peres, Gulf Coast Research and Education Center, University of Florida

✓ First detected during the 2013-14 season



AMMGIG-FLGYVLPYGQMSL-GATVITNLISAIP-I 51 02-159A (S) AMMGIG-FLGYVLPYGOMSL-GATVITNLISAIP-I 51 13-73 (S) AMMGIG-FLGYVLPYGOMSL-GATVITNLISAIP-I 51 02-153 (S) AMMGIG-FLGYVLPYGQMSL-GATVITNLISAIP-I 50 14-57 (S) AMMGIG-FLGYVLPYGQMSL-GATVITNLISAIP-I 51 13-494 (R) AMMGIG LGYVLPYGOMSL GATVITNLISAIP-I 51 14-87 (R) AMMGIG FLGYVLPYGQMSL-AATVITNLISAIP-I 50 13-473 (R) AMMGIG FLGYVLPYGOMSL AATVITNLISAIP-I 51 13-479 (R) AMMGIG FLGYVLPYGQMSL-AATVITNLISAIP-I 51 13-466 (R) AMMGIG-FLGYVLPYGQMSL-AATVITNLISAIP-I 52 13-475 (R) AMMGIG-FLGYVLPYGOMSL-AATVITNLISAIP-I 53 13-471 (R) AMMGIG FLGYVLPYGQMSL-AATVITNLISAIP-I 51 13-468 (R) AMMGIG-FLGYVLPYGOMSL-AATVITNLISAIP-I 52 14-61 (R) AMMGIG-FLGYVLPYGQMSL-AATVITNLISAIP-I 52 14-141 (R) AMMGIG-LGYVLPYGQMSL-GATVITNLISAIP-I 49 13-472 (R) AMMGIG-FLGYVLPYGQMSL-AATVITNLISAIP-I 51 02-160 (S) AMMGIG-FILGYVLPYGQMSL-GATVITNLISAIP-I 51 02-179 (S) AMMGIG-FILGYVLPYGQMSL-GATVITNLISAIP-I 51

Fig. 2. Amino acid sequence alignment of the partial *cytochrome b* gene for *Colleotrichum acutatum* isolates from strawberry. A mutation at codon 129 from phenylalanine to leucine was observed for moderately resistant isolates 13-494 and 14-141. A mutation at codon 143 from glycine to alanine was observed for completely resistant isolates 13-466, 13-468, 13-471, 13-472, 13-473, 13-475, 13-479, 14-61, and 14-87. Isolates 98-1, 02-153, 02-159, 02-160, 02-179, 13-73, and 14-57 were sensitive and had no point mutation.





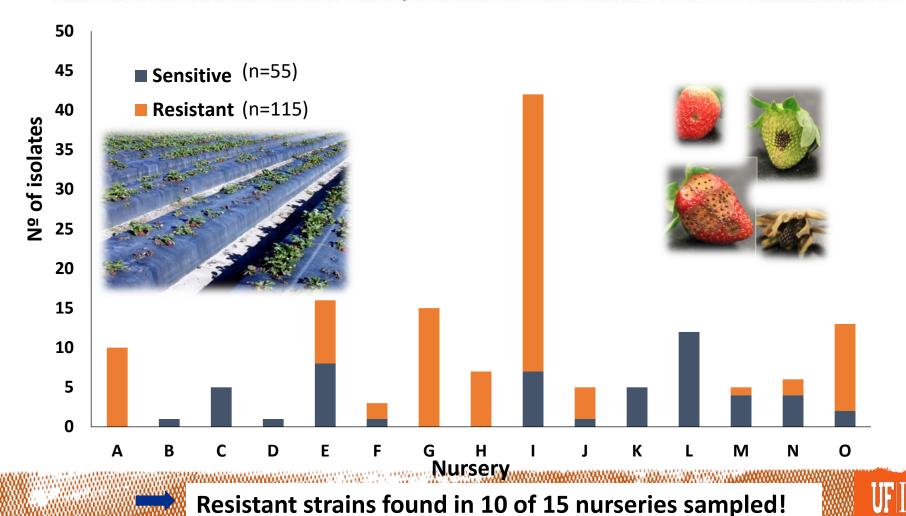


Sensitive isolate



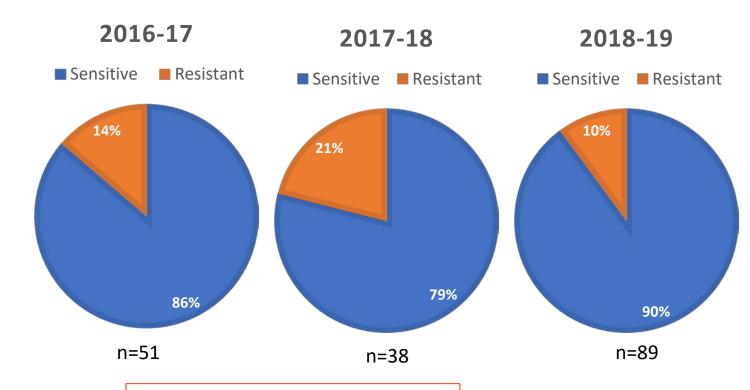
Widespread Resistance to QoI Fungicides of Colletotrichum acutatum from Strawberry Nurseries and Production Fields

Bruna B. Forcelini and Natalia A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma, 33598



Emergence of fungicide resistance to multiple fungicides and pathogens – *P. cactorum*

Phytophthora cactorum resistance to FRAC 44 (mefenoxam)

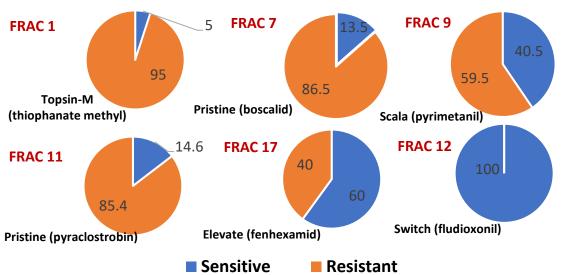


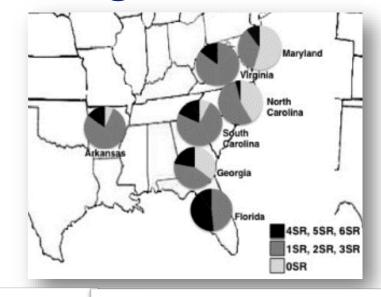


Limited to 2 nursery sources



Fungicide Resistance in Botrytis cinerea: the MRSA of Plant Pathogens





Phenotypic Characterization of Multifungicide Resistance in Botrytis cinerea Isolates from Strawberry Fields in Florida

A. Amiri, S. M. Heath, and N. A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598

Independent Emergence of Resistance to Seven Chemical Classes of Fungicides in Botrytis cinerea

Dolores Fernández-Ortuño, Anja Grabke, Xingpeng Li, and Guido Schnabel

Resistance to Pyraclostrobin and Boscalid in Botrytis cinerea Isolates from Strawberry Fields in the Carolinas

Dolores Fernández-Ortuño, Fengping Chen, and Guido Schnabel, School of Agricultural, Forestry, and Life Sciences, Clem-

Resistance to Cyprodinil and Lack of Fludioxonil Resistance in Botrytis cinerea Isolates from Strawberry in North and South Carolina

Dolores Fernández-Ortuño, Fengping Chen, and Guido Schnabel, School of Agricultural, Forestry & Life Sciences, Clemson

e-Xtra*

Diversity in the erg27 Gene of Botrytis cinerea Field Isolates from Strawberry Defines Different Levels of Resistance to the Hydroxyanilide Fenhexamid

Achour Amiri and Natalia A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma 33598

d Sciences Clemon Univer

Monitoring Resistance to SDHI Fungicides in Botrytis cinerea From Strawberry Fields

from Strawberry

Meng-Jun Hu, Department of Agricultural and Environmental Sciences, Clemson University, Clemson, SC 29634; Dolor Instituto de Hortofruticultura Subtropical y Mediterrânea "La Mayora"-Universidad de Málaga-Consejo Superior de Invi (IHSM-UMA-CSIC), Departamento de Microbiología, Campus de Teatinos, 29071 Málaga, Spain; and Guido Sch Agricultural and Environmental Sciences, Clemson University

Fungicide Resistance Profiles in Botrytis cinerea from Strawberry Fields of Seven Southern U.S. States

Dolores Fernández-Ortuño, School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC 29634 and Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora"-Universidad de Malaga-Consejo Superior de Investigaciones Científica, Departmento de Microbiología, Campus de Featinos, 20071 Málaga, Spain; Anja Grabke and Patírida Karra Bryson, School of Agricultural, Forest, and Feniromental Sciences, Clemon University, Achour Amiri and Natidia A, Peres, Gulf Coast Research and Education Center, University of Florida, Wimauma, FL 33598; and Guido Schnabel, School of Agricultural

Fenhexamid Resistance in Botrytis cinerea from Strawberry Fields in the Carolinas Is Associated with Four Target Gene Mutations

Anja Grabke, Dolores Fernández-Ortuño, and Guido Schnabel, School of Agricultural, Forest and Environ

Location-Specific Fungicide Resistance Profiles and Evidence for Stepwise Accumulation of Resistance in Botrytis cinerea

Microbiologia, Campus de Fratinos, 2007 Millaga, Spain. Shuming Ushen, Department of Plant Pathology, Co-and Technology, and the Key Lab of Crop Disease Monistering & Safer, Control in Hobels Province, Hanzbong A, Walsan, 40070. Chian, Anja Grubke, School of Agricultural, Forest, & Environmental Sciences, Clemon Univ Department of Plant Pathology, College of Flant Science and Technology and the Key Lab of Crop Disease Control in Hobel Province, Hanzbong Agricultural University, Williams C, Bridges, Department of Mathematic Universities and Galloid-Schandet, School of Asricultural Forest & Environmental Sciences, Cermon University

Characterization of Iprodione Resistance in Botryt from Strawberry and Blackberry

Anja Grabke, Dolores Fernández-Ortuño, Achour Amiri, Xingpeng l Natália A. Peres, Powell Smith, and Guido Schnabel

Resistance to Fludioxonil in Botrytis cinerea

from Blackberry and Strawberry

Xingpeng Li, Dolores Fernández-Ortuño, Anja Grabke, and Guido S

Achour Amiri, Stacy M. Heath, and Natalia A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimaum

Resistance to Fluopyram, Fluxapyroxad, and Penthiopyrad in Botrytis cinerea

Sources of Primary Inoculum of *Botrytis cinerea* and Their Impact on Fungicide Resistance Development in Commercial Strawberry Fields

Michelle Souza Oliveira, Gulf Coast Research and Education Center, University of Florida, Wimauma 33598; Achour Amiri, Tree Fruit Research and Extension Center, Washington State University, Wenatchee 98801; and Adrian I. Zuniga and Natalia A. Peres, Gulf Coast Research and Education Center, University of Florida, Wimauma 3598

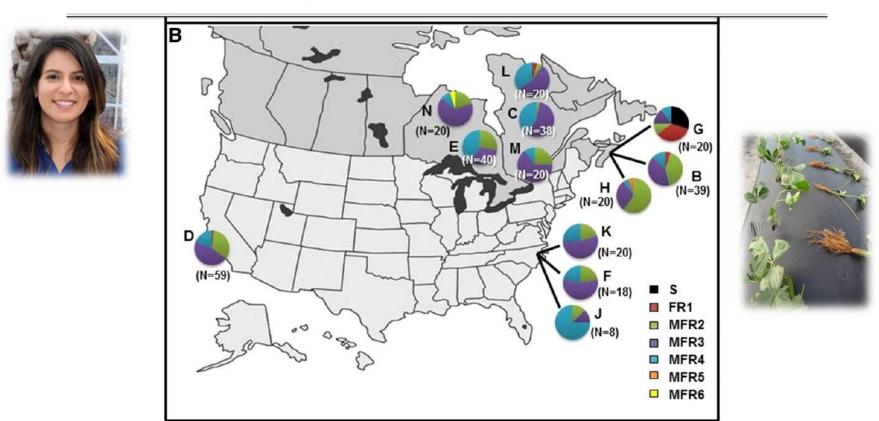


Fig. 2. Frequency of multifungicide resistance (MFR) phenotypes of *Botrytis cinerea* from strawberry transplants collected from different nurseries from California, North Carolina, Nova Scotia, Quebec, and Ontario in A, 2011-2012, and B, 2013. The fungicides tested were fenhexamid, pyrimethanil, pyraclostrobin, boscalid, fluopyram, penthiopyrad, iprodione, and fludioxonil (S = sensitive, FR1 = resistant to one fungicides, MFR2 = resistant to two fungicides, MFR3 = resistant to three fungicides, MFR4 = resistant to four fungicides, MFR5 = resistant to five fungicides, MFR6 = resistant to six fungicides). N is the number of isolates evaluated in each location. The letter next to each pie refers to the nursery code.



Many projects funded over the years with the overall goal: Integrating Disease Management Across the Nursery and Fruit Production System



Angular leaf spot (Xanthomonas fragariae)



Anthracnose (Colletotrichum acutatum)



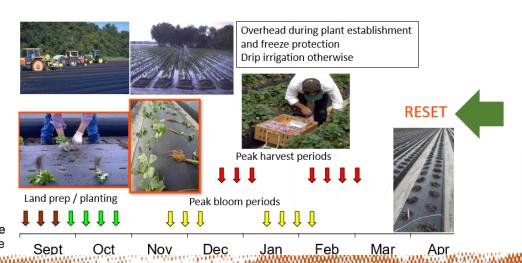
Gray mold (Botrytis cinerea)



Powdery Mildew (Podosphaera aphanis)



United States Department of Agriculture National Institute of Food and Agriculture

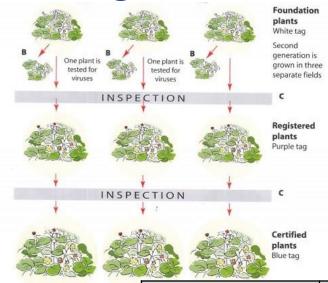






Break epidemic cycles while reducing number applications of single-site fungicides

- ✓ Identify new chemical and/or biological products for nursery usage
- ✓ Use single-sites only when needed– expand Strawberry Advisory
 - expand Strawberry Advisory System
- ✓ Development of non-chemical management alternatives

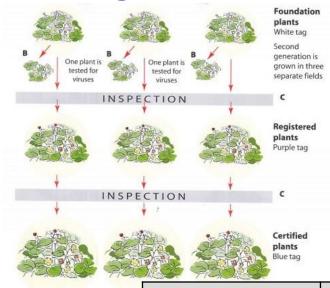




	Fungicide
Active ingredient	group
thiophanate-methyl	1
iprodione	2
propiconazole, tetraconazole,	
myclobutanil	3
mefenoxam	4
penthiopyrad	7
isofetamid	7
pyrimethanil	9
azoxystrobin, pyraclostrobin,	
trifloxystrobin	11
quinoxyfen	13
fenhexamid	17
fenhexamid + captan	17 + M4
fosetyl-Al, phosphites	33
azoxystrobin + propiconazole	3 + 11
fluoypram + pyrimethanil	7 + 9
fluxapyroxad + pyraclostrobin	7 + 11
cyprodinil + fludioxonil	9 + 12
cyflufenamid	U6
copper	M1
sulfur	M2
thiram	M3

Break epidemic cycles while reducing number applications of single-site fungicides

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mefenoxam	4
penthiopyrad	7
isofetamid	7
pyrimethanil	9
azoxystrobin, pyraclostrobin,	
trifloxystrobin	11
quinoxyfen	13
fenhexamid	17
fenhexamid + captan	17 + M4
fosetyl-Al, phosphites	33
azoxystrobin + propiconazole	3 + 11
fluoypram + pyrimethanil	7 + 9
fluxapyroxad + pyraclostrobin	7 + 11
cyprodinil + fludioxonil	9 + 12
cyflufenamid	U6
copper	M1
sulfur	M2
thiram	M3

The Strawberry Advisory System

http://sas.agroclimate.org/fl/

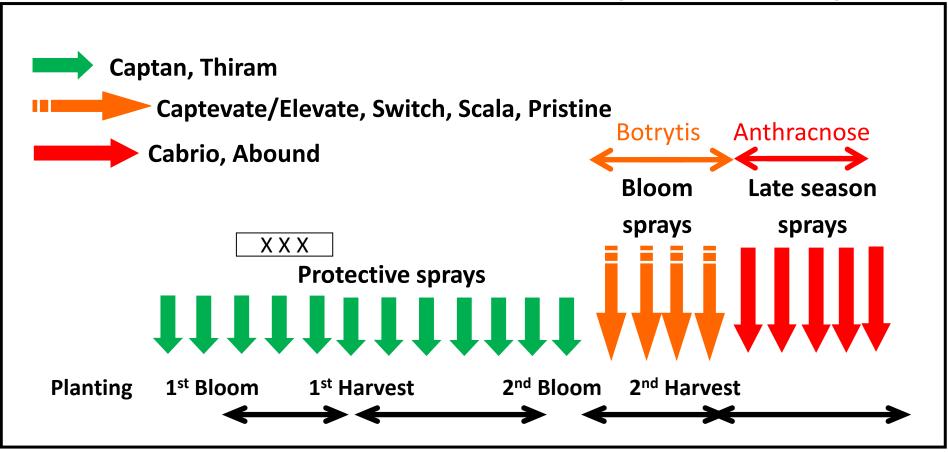








Former recommendation for management of AFR and BFR in Florida ("Low risk")



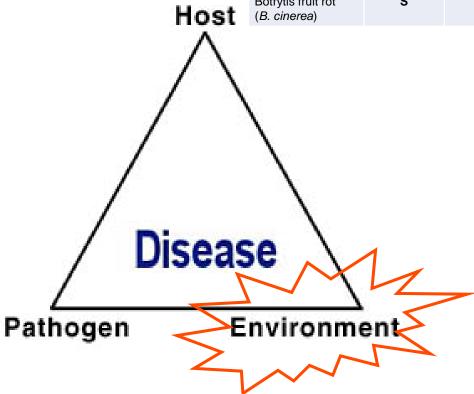
Sept/Oct Feb Nov Dec Jan Mar Apr

> Development of a Reduced Use Fungicide Program for Control of Botrytis Fruit Rot on Annual Winter Strawberry

D. E. Legard, S. J. MacKenzie, J. C. Mertely, C. K. Chandler, and N. A. Peres, University of Florida, Gulf Coast Research and Education Center, Dover, FL

Disease triangle

Disease	Radiance	FL-127	FL Beauty	Brilliance
Anthracnose (C. acutatum)	MR	R	MS	MR
Botrytis fruit rot (<i>B. cinerea</i>)	S	S	S	S







Action thresholds were determined based on disease infection models

✓ Anthracnose: Wilson-Madden *INF* > 0.15; *INF* > 0.5

Use of Leaf Wetness and Temperature to Time Fungicide Applications to Control Anthracnose Fruit Rot of Strawberry in Florida

S. J. MacKenzie and N. A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma 33598

✓ Botrytis: Bulger-Madden DI > 0.5; DI >0.7

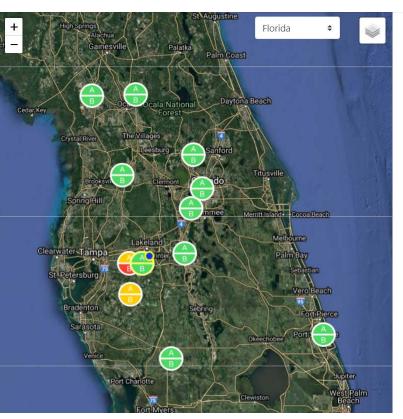
Use of Leaf Wetness and Temperature to Time Fungicide Applications to Control Botrytis Fruit Rot of Strawberry in Florida

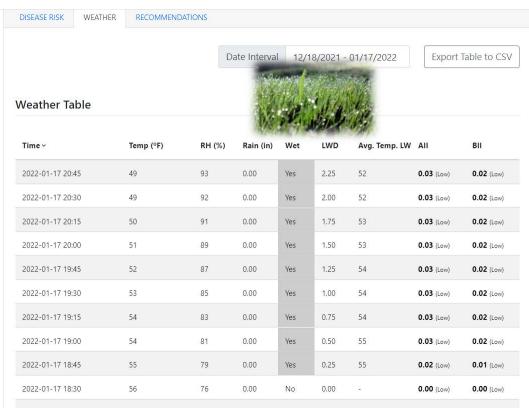
S. J. MacKenzie and N. A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma 33598

- ✓ Length of most recent wetness period
- **✓** Average temperature during wetness event
- ✓ Assumption inoculum is always present low risk



Key parameters monitored: 'Leaf Wetness Duration' and Temperature



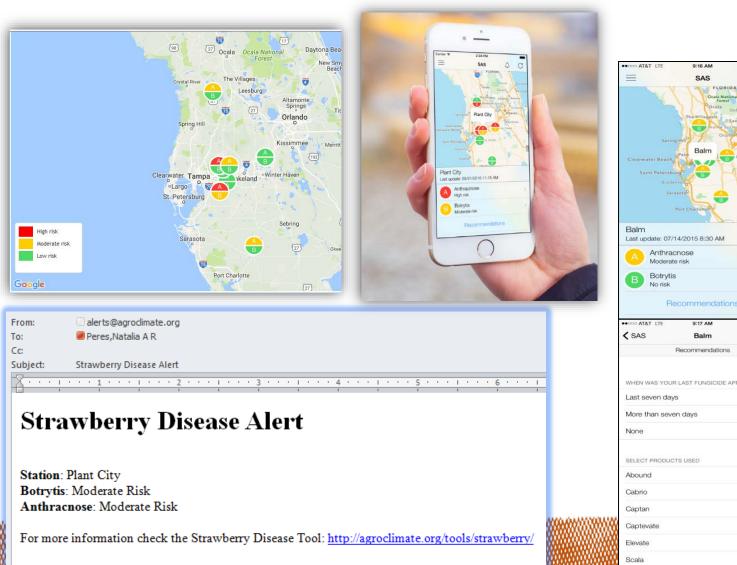


BFR High:

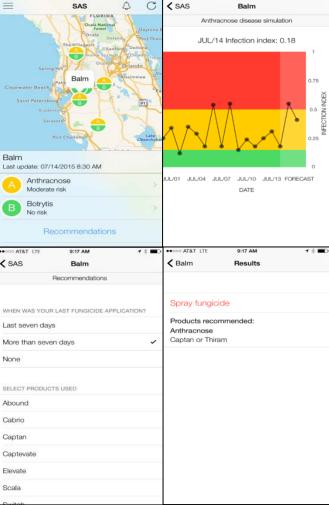
- ✓ 18 h LWD
- ✓ 17-25°C



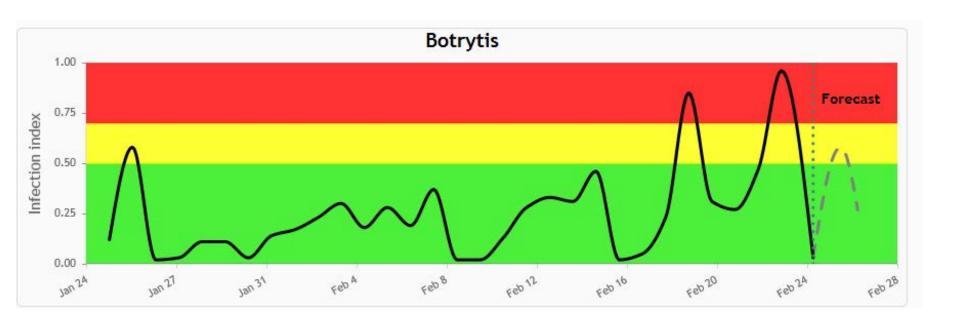
Timely alerts: Web, Email and SMS, App



SAS app



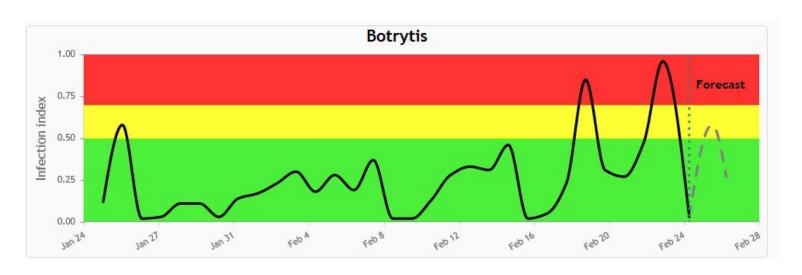
Goal to reduce number of single-site fungicide applications in periods of good weather



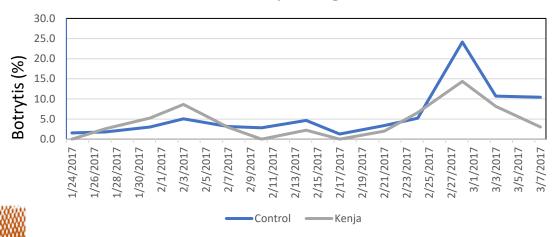
Particularly important these days since only a few fungicide products still effective



Even best fungicide not as good as good weather!!

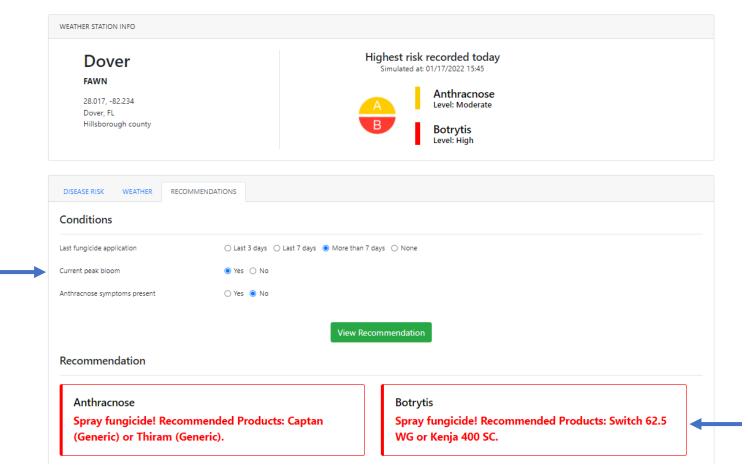


2016-17 Botrytis fungicide trial



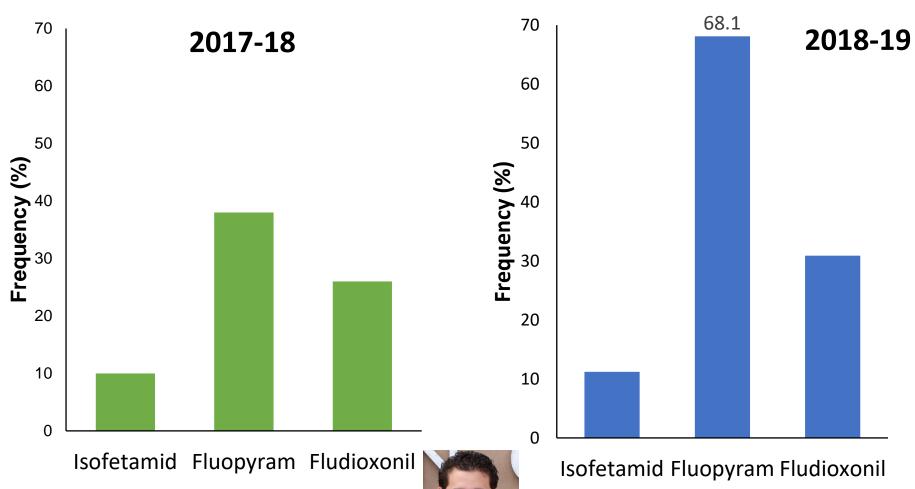


Fungicide Resistance / Wrong Fungicide Selection lead to incorporation of specific fungicide spray recommendation in the SAS





Continued monitoring for *Botrytis* fungicide resistance to adjust recommendations



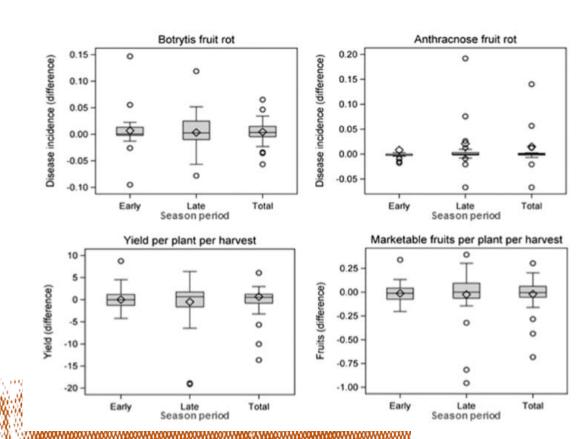


From trials in Commercial Farms to System Adoption

Meta-Analysis of a Web-Based Disease Forecast System for Control of Anthracnose and Botrytis Fruit Rots of Strawberry in Southeastern United States

Leandro G. Cordova, Gulf Coast Research and Education Center (GCREC-UF), University of Florida, Wimauma 33598; Laurence V. Madden, Department of Plant Pathology, The Ohio State University, Wooster 44691; Achour Amiri, Tree Fruit Research and Extension Center, Washington State University, Wenatchee 98801; Guido Schnabel, School of Agricultural, Forestry & Life Sciences, Clemson University, Clemson, SC 29634; and Natalia A. Peres,† GCREC-UF, University of Florida, Wimauma 33598

- √39 trials commercial farms
- ✓ Calendar vs. SAS
- ✓ AFR and BFR incidence, yield: no difference
- ✓ Number fungicide applications: 50% reduction



Strawberry Advisory System has expanded to other production areas, including nurseries

http://agroclimate.org/tools/strawberry/



FL, SC, NC, GA, VA, MD, PA, CA, OR, NY

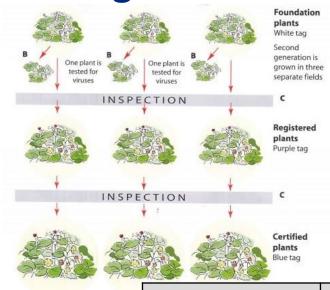
http://agroclimate.org/tools/sas/fl

http://agroclimate.org/tools/sas/va



Break epidemic cycles while reducing number applications of single-site fungicides

- ✓ Identify new chemical and/or biological products for nursery usage
- ✓ Use single-sites only when needed expand Strawberry Advisory System
- ✓ Development of non-chemical management alternatives





	Fungicide	
Active ingredient	group	
thiophanate-methyl	1	
iprodione	2	
propiconazole, tetraconazole,		
myclobutanil	3	
mefenoxam	4	
penthiopyrad	7	
isofetamid	7	
pyrimethanil	9	
azoxystrobin, pyraclostrobin,		
trifloxystrobin	11	
quinoxyfen	13	
fenhexamid	17	
fenhexamid + captan	17 + M4	
fosetyl-Al, phosphites	33	
azoxystrobin + propiconazole	3 + 11	
fluoypram + pyrimethanil	7 + 9	
fluxapyroxad + pyraclostrobin	7 + 11	
cyprodinil + fludioxonil	9 + 12	
cyflufenamid	U6	
copper	M1	
sulfur	M2	
thiram	M3	
cantan	MA	

Heat Treatment as a Non-Chemical Alternative for Management of Strawberry Diseases

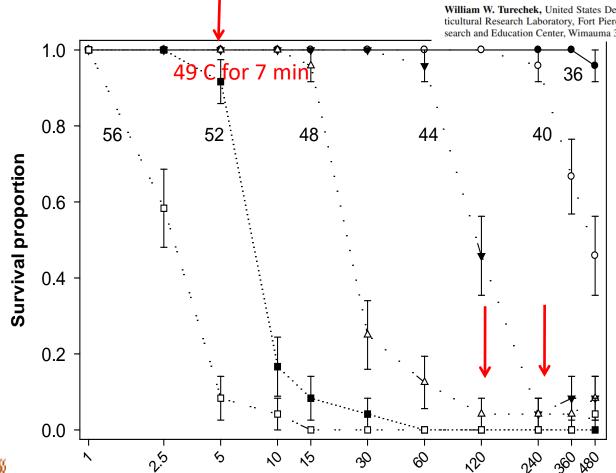
- ✓ Heat treatment had been used in nurseries in the past for management of strawberry pests
- ✓ Buchner (1991): 49 C for 7 min for reduction of cyclamen mites
- ✓ However, delayed growth, reduced flowering, and the potential spread of *Xanthomonas* with hot water treatment was observed by nurseries



Lab studies showed that much longer exposure times are needed to reduce *Xanthomonas fragariae*

Heat Treatment Effects on Strawberry Plant Survival and Angular Leaf Spot, Caused by *Xanthomonas fragariae*, in Nursery Production

William W. Turechek, United States Department of Agriculture–Agricultural Research Service, United States Horticultural Research Laboratory, Fort Pierce, FL 34945; and Natalia A. Peres, University of Florida, Gulf Coast Research and Education Center, Wimauma 33598

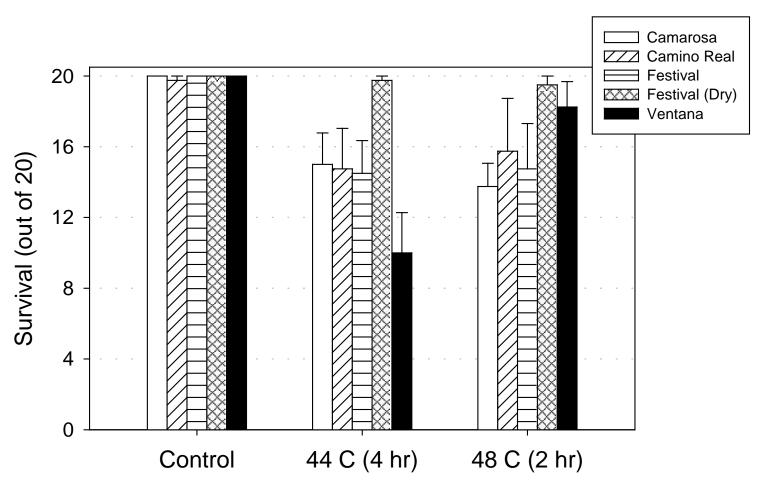


Higher temperatures or longer exposure times are needed to reduce *Xanthomonas*



Time (minutes)

Hot water treatment for prolonged time can cause some plant damage



Heat Treatment Effects on Strawberry Plant Survival and Angular Leaf Spot, Caused by *Xanthomonas fragariae*, in Nursery Production



Heat treatment units with aerated steam were built in Florida and California

Lassen Canyon nursery, CA



UF-GCREC, FL





'Aerated steam' (plant sauna) field trials

Treatment

- 1. Non-treated control
- 2. Preheat $(37^{\circ}C, 1 \text{ h}) + \text{Steam } (44^{\circ}C, 2 \text{ h})$
- 3. Preheat (37°C, 1 h) + Steam (44°C, 4 h)
- 4. Steam (44°C, 2 h)
- 5. Steam (44°C, 4 h)



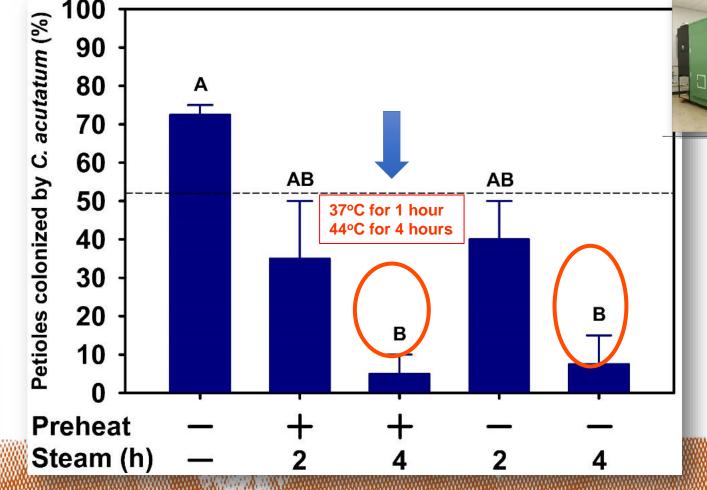


The Effects of Heat Treatment on the Gene Expression of Several Heat Shock Protein Genes in Two Cultivars of Strawberry



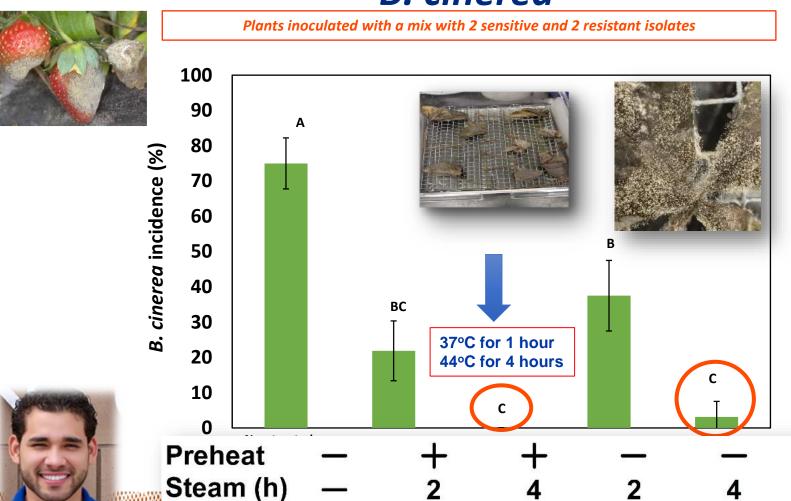
In addition to *Xanthomonas*, two-stage steam treatment (37 C for 1 hour f.b. 44 for 4 hours) reduced colonization of transplants by *C. acutatum*





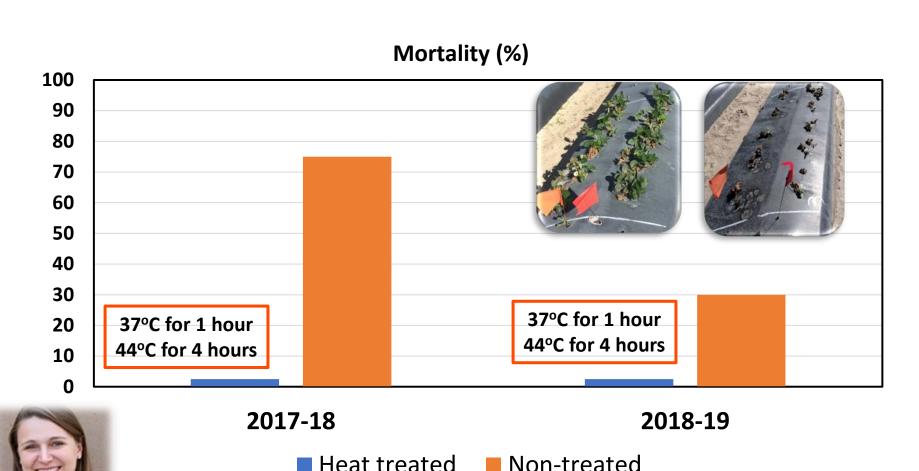


Steam treatment also reduced colonization of transplants by fungicide-resistant strains of *B. cinerea*





Heat treatment also reduce mortality of transplants inoculated with Phytophthora





Aerated steam is a better alternative than hot water

Pros

- Has less adverse effects on plants than Hot Water Treatment
- •Effective at reducing a number of pathogens, including Xanthomonas, Colletotrichum, Botrytis, Phytophthora, Podosphaera, and Neopestalotiopsis sp.

Cons

- Treatment application requires specialized units
- •Treatment times take ~6 hours



Research

The Use of Aerated Steam as a Heat Treatment for Managing Angular Leaf Spot in Strawberry Nursery Production and Its Effect on Plant Yield



Heat treatment can reduce risk of spreading diseases among nurseries



Quiescently- infected pathogens

X. fragariae

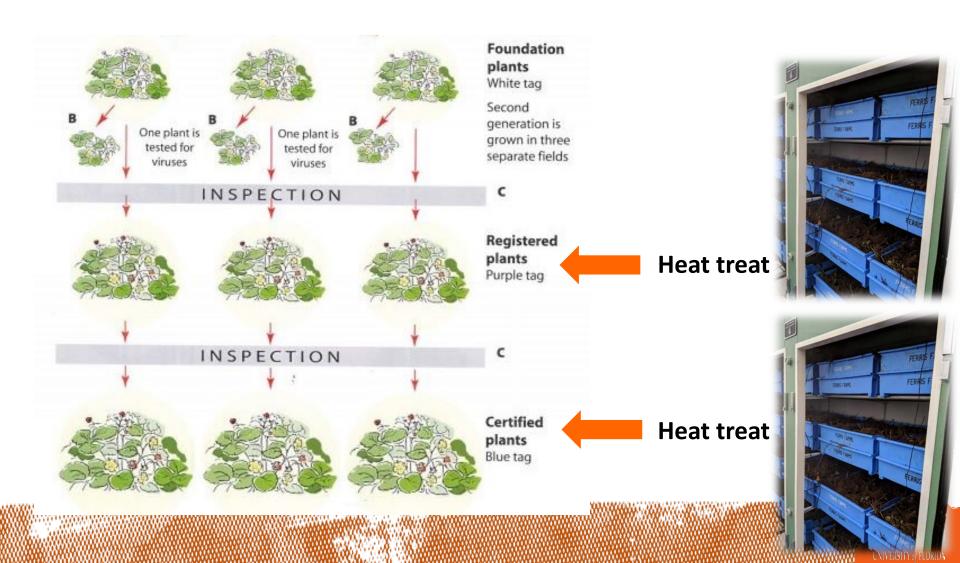
C. acutatum

B. cinerea

P. aphanis Phytophthora spp Neopestalotiopsis



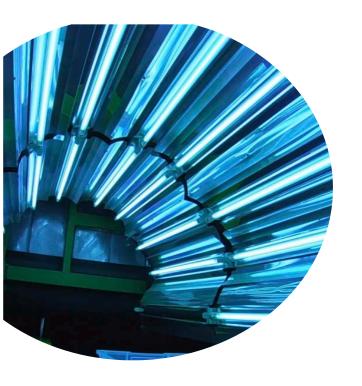
Heat treatment of transplants can reduce risks of epidemics

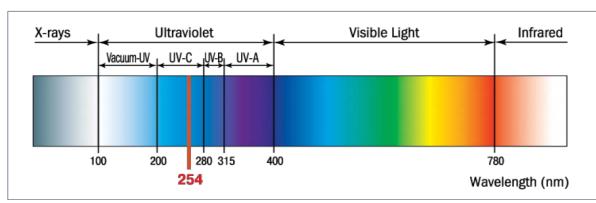


UV Light as a Non-Chemical Alternative for Management of Strawberry Diseases

✓ Lamps producing ultraviolet light have been commonly available for over 75 years

Schuch et al., 2013





✓ UV wavelength from 250 to 280 nm is most effective

UV Light as a Non-Chemical Alternative for Management of Plant Diseases

- ✓ Mode of action of UV: damage to pathogen DNA
- ✓ Many pathogens have evolved systems that repair DNA damage by UV (UVA) in daylight
- ✓ Breakthrough in Norway by Suthaparan et al. (2016) using nighttime application of UV
- √ Repair systems are [recharged by blue and UVA] reduced during darkness



What pathogens can be controlled with UV?

- ✓ Powdery mildews among the most exposed plants pathogens to UV light
- ✓ Wholly external to the host
- ✓ More severe in greenhouses/protected structures







UV Light has been used for management of Powdery Mildew in greenhouses

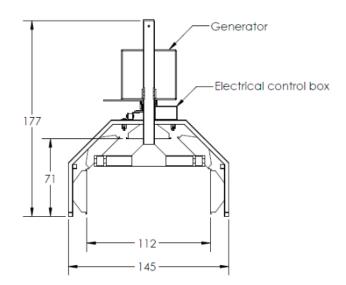


Research and some commercial usage of UV in greenhouses in Europe





UV Light Use: From Greenhouse to Field Application



- Tractor-drawn units designed;
- Internal hemicylindrical array;
- 20 UVC germicidal lamps (254nm);
- Powered by a generator



Dr David Gadoury



Dr Mark Rea Dr Andrew Bierman





UV Light Use: From Greenhouse to Field Application

Field trials in Florida

- Applied 1x or 2x per week (after sunset)
- Speed = 1.4 mph (2.3 kph) to 3.4 mph (5.6 kph)
- Doses evaluated = 68, 85, 170 J/m²

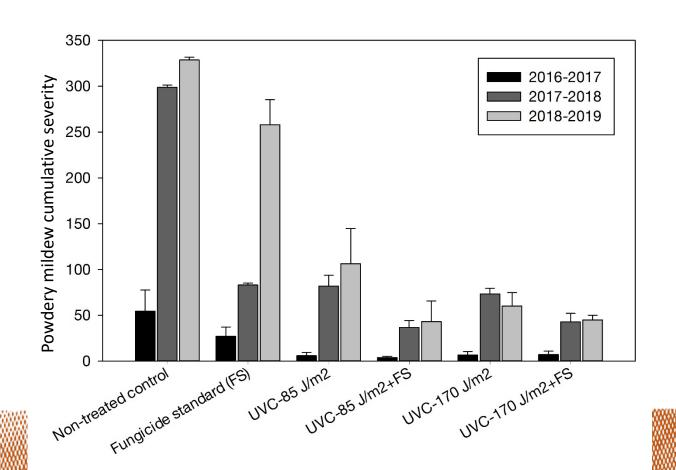








UV treatments were similar or more effective than standard fungicide sprays for PM







Many other units built and currently being tested on strawberry and other crops









Autonomous UV Robot "Thorvald"





Summary integrated strategies to manage multiple diseases and fungicide resistance in strawberry

- ✓ Create a better linkage between fruit and plant producers [improved extension efforts to nurseries]
- ✓ Recommendation for nurseries to rely more on multi-site fungicides (captan, thiram, sulfur, chlorotalonil) as the basis of disease management program [alternative groups]
- ✓ Save single-site materials more expensive and prune to resistance selection for fruit and critical periods! [SAS]
- ✓ Integrate other disease management practices instead of relying only on fungicides [resistant cultivars, non-chemical alternatives such as heat and UV]



