

**Proceedings of the
95th Annual
Cumberland-Shenandoah
Fruit Workers Conference**



December 5th-6th, 2019

Holiday Inn Winchester SE-Historic Gateway
Winchester, Virginia

(FOR ADMINISTRATIVE USE ONLY)

**Proceedings of the
Cumberland-Shenandoah
Fruit Workers Conference
95th Annual Meeting**

December 5th-6th, 2019

Holiday Inn Winchester SE-Historic Gateway
Winchester, Virginia

Edited by
Mei-Wah Choi

Cornell AgriTech – Cornell University
Geneva, New York

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Current and Past Executive Officers

2020

President: Dean Polk (Rutgers)

Secretary/Treasurer: Chris Bergh (Virginia Tech)

President-Elect: Tom Kon (NC State)

Immediate-Past President: Kerik Cox (Cornell)

2019

President: Kerik Cox (Cornell)

Secretary/Treasurer: Chris Bergh (Virginia Tech)

President-Elect: Dean Polk (Rutgers)

Immediate-Past President: Mike Dimock (Certis USA)

2018

President: Mike Dimock (Certis USA)

Secretary/Treasurer: Chris Bergh (Virginia Tech)

President-Elect: Kerik Cox (Cornell)

Immediate-Past President: Greg Krawczyk (Penn State)

2017

President: Greg Krawczyk (Penn State)

Secretary/Treasurer: Chris Bergh (Virginia Tech)

President-Elect: Mike Dimock (Certis USA)

Immediate-Past President: James Walgenbach (NC State)

2019 CSWFC Participants

Name	Affiliation	Name	Affiliation
Acimovic, Srdjan	Cornell University	Love, Kenner	Virginia Coop. Extension
Agnello, Arthur	Cornell University	Ludwick, Dalton	USDA-ARS
Allen, Carol	Virginia Tech University	Luo, Qiuchen	University of Maryland
Allen, Chester	Virginia Tech University	Mackintosh, Bill	Nutrien Ag Solutions
Ayer, Katrin	Cornell University	Madalinska, Kasia	Rutgers University
Beatty, Daniel	Nutrien Ag Solutions	Martin, Phillip	Penn State University
Bergh, Chris	Virginia Tech University	Mathew, Sudeep	Syngenta Crop Protection
Biddinger, David	Penn State University	McDougall, Robert	Rutgers University
Bielski, Jason	LAB Services	Nahiyian, Abdullah	Virginia Tech University
Bissett, Audra	University of Maryland	Nielsen, Anne	Rutgers University
Bogash, Steve	Marrone Bio Innovations	Nita, Mizuho	Virginia Tech University
Brandt, Nate	USDA-ARS	Nixon, Laura	Oakridge Assoc University
Brill, Nancy	Bayer Crop Science	O'Barr, John	BASF Corp.
Carper, Lee	USDA-ARS	Ogburn, Emily	NC State University
Carroll, Juliet	Cornell University	Peter, Kari	Penn State University
Castro, Johanny	Penn State University	Pfeiffer, Doug	Virginia Tech University
Chandler, Jeff	NC State University	Pierce, Kristen	Penn State University
Choi, Mei-Wah	Cornell University	Plasters, Kevin	Helena Agri Enterprises
Clarke, Gregory	Valent	Polk, Dean	Rutgers University
Clavet, Chris	NC State University	Pollock, Robert	Penn State University
Cosseboom, Scott	University of Maryland	Quinn, Nicole	Virginia Tech University
Cox, Kerik	Cornell University	Raines, Doug	USDA-ARS
Crassweller, Robert	Penn State University	Reed, Joseph	UPL
Cullum, John	USDA-ARS	Rice, Henry	University of Missouri
Davis, Linda	Wilbur-Ellis Co.	Rosenberger, Dave	Cornell University
Denson, Carrie	Rutgers University	Rucker, Ann	Rutgers University
Dimock, Michael	Certis USA		Alson H. Sith Jr. Ag. Res. And Ext.
Donahue, Daniel	Cornell University		Ctr.
Dyer, Jared	Alson H. Smith Jr. Ag. Res. & Ext.	Ruether, Brian	USDA-ARS
Fan, Jiangbin	Ctr.	Rugh, Tony	UPL
Fang, Emily	Rutgers University	Sasser, Cameron	Rutgers University
Farcu, Macarena	University of Maryland	Schmitt, Dave	University of Maryland
Ferelli, Angela	University of Maryland	Schoeneberg, Anita	University of Maryland
Fitzpatrick, Carrie	University of Maryland	Schoneberg, Torsten	NC State University
Ganske, Don	CSFWC, Inc.	Schoof, Steve	Penn State University
Gomyo, Mikako	Virginia Tech University	Schupp, Jim	USDA-ARS
Gresham, Sean	NC State University	Scorza, Cameron	Penn State University
Hadden, Whitney	Virginia Tech University	Seifrit, Donald	Shannon Farm Services
Harper, Jason	Penn State University	Shannon, Mark	Virginia Tech University
Hitchner, Erin	Syngenta Crop Protection	Sherif, Sherif	USDA-ARS
Holowid, John	Arysta LifeScience	Short, Brent	Michigan State University
Homer, Tyler	OmniLytics	Slack, Suzanne	Syngenta Crop Protection
Hott, Chris	USDA-ARS	Smith, Larissa	Agrofresh
Hunt, Kathy	University of Maryland	Spreen, Jacob	LAB Services
Johnson, Timothy	Marrone Bio Innovations	Steffel, Jim	Cornell University
Jones, Sharon	USDA-ARS	Strickland, David	Virginia Coop. Extension
Jurick, Wayne	USDA-ARS	Sutphin, Mark	Virginia Tech University
King, Anderson		Temkin, Mariah	BASF
Kon, Tom	NC State University	Thomas, Gar	Penn State University
Krawczyk, Greg	Penn State University	Thomas, Kate	NC State University
Ladle, Kara		Villani, Sara	NC State University
Lalancette, Norman	Rutgers University	Walgenbach, Jim	Univ. of New Hampshire
Leach, Heather	Penn State University	Wallingford, Anna	Cornell University
Leahy, Kathleen	Polaris Orchard IPM Cons	Wallis, Anna	University of Maryland
Lehman, Brian	Penn State University	Walsh, Chris	Cornell University
Leon, Chris	FMC Corp.	Wang, Tristan	Kop-Coat Protection Prod
Leskey, Tracy	USDA-ARS	Ward, John	Purdie University
Lessord, Tessa	ACDS research	Webb, Kevin	Penn State University
Liberator, Kelly	BASF	Weber, Daniel	Penn State University
Lokaj, Gail	Rutgers University	Winzeler, Edwin	Virginia Tech University
Lolic, Danijel	Rice Packing Inc.	Yoder, Keith	Michigan State University
		Zemaitis, Dan	

December 5th-6th, 2019
Holiday Inn Winchester SE-Historic Gateway
Winchester, Virginia

CONFERENCE AGENDA

Thursday, December 5:

8:00 – 9:00	Registration
9:00 – 9:10	Call to Order
9:10 – 10:10	Call of the States
10:10 – 10:30	Call of the Industry
10:30 – 10:45	BREAK
10:45 – 12:00	Plenary Session

Update on the Status of Spotted lanternfly in the Mid-Atlantic Area.
Heather Leach, Department of Entomology, Pennsylvania State University.

Before You See the Spots: Using eDNA as a Biosurveillance Tool for Spotted Lanternfly in NJ Vineyards.
Anne Nielsen, Department of Entomology, Rutgers University.

Automation and technological innovations used in apple packing.
Danijel Lolic, Engineer, Rice Fruit Company, Gardners, PA.

12:15 – 1:00	LUNCH
1:15 – 5:15	Concurrent Sessions Entomology Horticulture Plant Pathology
5:30	MIXER

Friday, December 6:

8:00 – 8:45	CSFWC Business Meeting (all are invited)
9:00 – 12:00	Concurrent Sessions continue

Concurrent Sessions Agenda
ENTOMOLOGY

Thursday, December 5:

- 1:30 - 1:45 **Redistributing *Trissolcus japonicus* in Virginia: 2019 Update.**
Chris Bergh (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA), Ashley Edwards, Kathleen Reed, Alyssa Elliott, Kate Lawrence (Virginia Cooperative Ext.), and Elijah Talamas (Division of Plant Industry, Florida Dept. of Ag. and Consumer Serv.).
- 1:45 – 2:00 **Habitat, Temporal, and Host Plant Effects on *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) Detections in Virginia.**
Nicole Quinn (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA), Elijah Talamas (Division of Plant Industry, Florida Dept. of Ag. and Consumer Serv.), Tracy Leskey (USDA-ARS, Appalachian Fruit Res. Station, Kearneysville, WV), and Chris Bergh (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA).
- 2:00 - 2:15 **Life on the Edge? Woods-to-orchard Pheromone Trap Transects for *Halyomorpha Halys*.**
Whitney Hadden (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA), Tracy Leskey (USDA-ARS, Appalachian Fruit Res. Station, Kearneysville, WV), and Chris Bergh (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA).
- 2:15 - 2:30 **Are Detections of *Halyomorpha halys* Egg Masses and *Trissolcus japonicus* Increased in Pheromone-Baited Trees?**
Jared Dyer (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA), Elijah Talamas (Division of Plant Industry, Florida Dept. of Ag. and Consumer Serv.), Tracy Leskey (USDA-ARS, Appalachian Fruit Res. Station, Kearneysville, WV), and Chris Bergh (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA).
- 2:30 - 2:45 **Residual Activity of Bifenthrin and Dinotefuran for Control of BMSB on Apples.**
Jim Walgenbach, Steven Schoof, and Amelia Heintz-Botz (NC State Univ.).
- 2:45 - 3:00 **Ambrosia Beetle Ecology and Management in NC Apple Systems.**
Sean Gresham, Seth Ellis, Netty Calvin, Amelia Heintz-Botz, Sara Villani, and Jim Walgenbach (NC State Univ.).
- 3:00 – 3:15 **BREAK**
- 3:15 - 3:30 **Efficacy of Plant Host Defense Compounds in Preventing Ambrosia Beetle Infestations in Apple Trees.**
Arthur Agnello and Dave Combs (Cornell Univ.).
- 3:30 - 3:45 **Oriental Beetle-Still A Hidden Issue.**
Carrie Denson and Dean Polk (Rutgers Univ.).
- 3:45 - 4:00 **Challenges with Spotted Lanternfly Research: Monitoring and Ovicidal Bioassays.**
Greg Krawczyk, Edwin Winzeler, and Henry Rice. (Penn State Univ. Fruit Res. and Ext. Ctr.).
- 4:00 - 4:15 **Effects of Standard Versus Reduced-Width Sticky Bands on Captures of Spotted Lanternfly Nymphs and Non-Target Organisms.**
Brian Ruether, Jaren Dyer, Whitney Hadden, Nicole Quinn, and Chris Bergh (Alson H. Smith Jr. Ag. Res. and Ext. Ctr., Virginia Tech, Winchester, VA).
- 4:15 - 4:30 **Development of Behaviorally Based Monitoring and Biosurveillance Tools for the Invasive Spotted Lanternfly, *Lycorma delicatula*.**
Laura Nixon (USDA-ARS, Appalachian Fruit Res. Station), Heather Leach (Penn State Univ.), Dalton Ludwick (USDA-ARS, Appalachian Fruit Res. Station), Julie Urban (Penn State Univ.), Danielle Kirkpatrick (Trece Inc.), and Tracy Leskey (USDA-ARS, Appalachian Fruit Res. Station).
- 4:30 – 4:45 **Spotted Lanternfly Host Breadth and Rearing: Quarantine and Field studies.**
Tracy Leskey, Sharon Jones, Dalton Ludwick, Laura Nixon (USDA-ARS), and Karen Felton (USDA Forest Serv.).

- 4:45 – 5:00 **Spotted Lanternfly Control Trials on Grape & Peach with Conventional and Bioinsecticides.**
David Biddinger (Penn State Univ. Fruit Res. & Ext. Ctr.), Heather Leach, Nina Jenkins, and Julie Urban (Penn State Univ.).
- 5:00 – 5:15 **Field Observations on Spotted Lantern Fly Behavior and Host Suitability.**
Jason Bielski and James Steffel (LABServices).

Concurrent Sessions Agenda ENTOMOLOGY

Friday, December 6:

- 9:00 - 9:15 **European Cherry Fruit Fly Quarantine in New York.**
Juliet Carroll (Cornell Univ.)
- 9:15 - 9:30 **Trapping for Brown Marmorated Stink Bug in Appalachian Forests.**
Steve Schoof and Jim Walgenbach (NC State Univ.).
- 9:30 - 9:45 **Is Codling Moth Becoming Less Susceptible to *Cydia pomonella* granulovirus in Apple Orchards?**
Jiangbin Fan, Katarzyna Madalinska, and Anne Nielsen (Rutgers Univ.).
- 9:45 - 10:00 **Update on Pesticide Impacts on Honey Bees Used for NJ Highbush Blueberry Pollination.**
Dean Polk, Chelsea Abegg, Cesar Rodriguez-Saona, and Gail Lokaj (Rutgers Univ.).
- 10:00 - 10:15 **Leveraging Pest Behavior for Implementation of Biological Control for Plum Curculio - Findings From Year 1.**
Robert McDougall (Rutgers Univ.), Clement Akotsen-Mensah (Lincoln Univ.), Tracy Leskey (USDA-ARS), Cesar Rodriguez-Saona (Rutgers Univ.), Brett Blaauw (Univ. of Georgia), and Anne Nielsen (Rutgers Univ.).
- 10:15 – 10:30 ***D. suzukii* Management - Using a Crop Sanitizer to Control Yeasts.**
Torsten Schoneberg and Kelly Hamby (Univ. of Maryland).
- 10:30 - 10:45 **Integrating *Trissolcus japonicus* into Apple IPM Programs.**
Dalton Ludwick, Jessica Patterson (USDA-ARS), Layne Leake (Univ. of Missouri-Columbia), Lee Carper, and Tracy Leskey (USDA-ARS).

Concurrent Sessions Agenda HORTICULTURE

Thursday, December 5:

- 1:15 - 1:30 **Breaking Buds with Bags: Minimizing Blind Wood on Apple.**
Thomas Kon, Chris Clavet (NC State Univ.), and Byron Phillips (Valent USA).
- 1:30 - 1:45 **Chemical Blossom Thinning in Apples: Applied Research Findings.**
W. Chester Allen, Sherif Sherif (Virginia Tech), Thomas Kon (NC State Univ.), Keith Yoder, Mariah Temkin, and Sara Pitcock (Virginia Tech).
- 1:45 - 2:00 **Evaluation of 1-Aminocyclopropane-1-carboxylic Acid (ACC) as a Potential Post-bloom Thinner of Apples.**
Sherif Sherif (Virginia Tech).
- 2:00 - 2:15 **'Honeycrisp' Bitter Pit Prediction in New York State.**
Daniel Donahue (Cornell Coop. Ext.)
- 2:15 - 2:30 **Trees Per Hectare or Leaders Per Hectare: Which is More Important?**
Rob Crassweller and Don Smith (Penn State Univ.).

- 2:30 - 3:00 **Russet and Fruit Cracking of Imperial 10-45 and GoldRush Apples: Effects of Canopy Position and GA4+7 Sprays.**
James Schupp, Melanie Schupp, and Edwin Winzeler (Penn State Fruit Res. and Ext. Ctr.).
- 3:00 – 3:15 **BREAK**
- 3:15 - 3:30 **Effects of Rootstock and In-row Tree Spacing on Mineral Nutrition and Productivity of Peach Trees in Pennsylvania.**
James Schupp, Melanie Schupp, and Edwin Winzeler (Penn State Fruit Res. and Ext. Ctr.).
- 3:30 - 3:45 **Managing Blackberry Growth with Prohexadione Calcium.**
Chris Clavet, Thomas Kon, Gina Fernandez, Penelope Perkins-Veazie (NC State Univ.), Karen Blaedow (NC Coop. Ext. Serv.).
- 3:45 - 4:00 **Comparing the Mineral Nutrition and Vigor of Northern and Southern Highbush Blueberries.**
Chris Walsh, Carol Allen, Audra Bissett, Claire Frank, Lukas Hallman, Amelia Loeb, and Sebastian Peters (Univ. of Maryland).
- 4:00 - 4:15 **Sugar Metabolism Reprogramming in Japanese Plums.**
Macarena Farcuh (Dept. of Plant Science and Landscape Architecture, Univ. of Maryland), Bosheng Li (Dept. of Plant Sciences, Univ. of California), Rosa Rivero (CEBAS, CSIC, Murcia, Spain), Avi Sadka (Dept. of Fruit Tree Sciences, ARO, The Volcani Center, Israel), and Eduardo Blumwald (Dept. of Plant Sciences, Univ. of California).
- 4:15 - 4:30 **Building a Food Safety Culture for Direct Market Growers.**
Carol Allen, Audara Bissett, Angela Ferelli, Kathy Hunr, and Chris Walsh (Univ. of Maryland).

Concurrent Sessions Agenda
PLANT PATHOLOGY

Thursday, December 5:

- 1:15 - 1:30 **Identification and Resistance Profiling of Colletotrichum spp. Isolates from Strawberries in the Mid-Atlantic.**
Qiuchen Luo (Univ. of Maryland).
- 1:30 - 1:45 **Investigating Sources for Postharvest Apple Rot Fungi in the Field and Packhouse: Conceptual Framework and Preliminary Results.**
Johanny Castro, Kari Peter (Penn State Univ.).
- 1:45 - 2:00 **Optimizing the Potential for Biological Controls to Manage Fungal Diseases of Apple.**
Katrin Ayer and Kerik Cox (Cornell AgriTech).
- 2:00 - 2:15 **Alternatives to QoI Fungicides for Glomerella Leaf Spot Management in NC.**
Sara Villani, Alejandro Llanos, and Rachel Kreis (NC State Univ.).
- 2:15 - 2:30 **Assessment of Alternative Chemical Management Programs for Apple Powdery Mildew Caused by *Podosphaera leucotricha*.**
David Strickland and Kerik Cox (Cornell AgriTech).
- 2:30 - 2:45 **Developing Tools to Detect and Manage Antimicrobial Resistance in Blue Mold Fungi Causing Postharvest Decay.**
Wayne Jurick II and Kerik Cox (Cornell Univ.).
- 2:45 - 3:00 **Influence of pH on the Efficacy of Captan for Summer Disease Control in Apple.**
W. Chester Allen, Keith Yoder, Allen Cochran, William Royston, Scott Kilmer, and Sherif Sherif (Virginia Tech).
- 3:00 - 3:15 ***Paecilomyces* Rot in Apples: A Newly Described Disease and a Possible Source of Food Spoilage and Patulin Contamination.**
Tristan Wang and Kathie T. Hodge (Cornell Univ.).
- 3:15 - 3:30 **BREAK**
- 3:30 - 3:45 **Update on In-Orchard Population Dynamics of *Erwinia amylovora*: Night Time Growth and Implications for Antibiotic Application Timing.**
Suzanne Slack, Kellie Walters, Emily Pochubay, Cory Outwater, and George Sundin (Michigan State Univ.).
- 3:45 - 4:00 **Managing Fire Blight with Prohexadione-calcium Applied Pre-bloom.**
Anna Wallis and Kerik Cox (Cornell AgriTech).
- 4:00 - 4:15 **Post-infection Applications of Prohexadione-calcium Prevent Initiation of Fire Blight Cankers on Perennial Apple Wood.**
Srdjan Acimovic, Christopher Meredith, Ricardo Santander, and Fatemeh Khodadadi (Cornell Univ.).
- 4:15 - 4:30 **Development of Viability Digital PCR to Elucidate *Erwinia amylovora* Biology and Management.**
Srdjan Acimovic, Ricardo Santander, and Christopher Meredith (Cornell Univ.).
- 4:30 - 4:45 **Quantifying Impact of Dormant Copper Sprays on Overwintering Cells of *Erwinia amylovora* in Cankers on Apple Wood.**
Srdjan Acimovic, Ricardo Santander, and Christopher Meredith (Cornell Univ.).
- 4:45 - 5:00 **The Intensity of Phytotoxicity on Grape Leaves by a Mixture of Copper and Phosphorus Acid Depends on the Copper Formulation and Water pH.**
Mizuho Nita, Abdullah Nahiyani (Virginia Tech), and Jungkwan Lee (Dong-A Univ.).

5:00 - 5:15 **Pathogenicity Behavior of *Aspergillus*, *Alternaria*, and *Pestalotiopsis* on Grape Bunches.**
Scott Cosseboom and Mengjun Hu (Univ. of Maryland).

Concurrent Sessions Agenda
PLANT PATHOLOGY

Friday, December 6:

- 9:00 - 9:15 **The Detection Rate of *Botryosphaeria* spp. is Significantly Lower in Certified Grafted Grapevine Materials.**
Mikako Gomyo, Gregory Klinger, and Mizuho Nita (Virginia Tech).
- 9:15 - 9:30 **Biocontrol Agent *Rhizobium vitis* ARK-1 Reduces Grapevine Crown Gall Against Higher Cell Numbers of Tumorigenic *R. vitis* in a Co-Inoculation Study.**
Abdullah Nahiyen, Akiko Mangan, and Mizuho Nita (Virginia Tech).
- 9:30 - 9:45 **Wine Grape Field Trials (BioSafe, PlantAid, Helena, and protective shield) at Winchester, VA, 2019.**
Mizuho Nita, Abdullah Nahiyen (Virginia Tech), and Jungkwan Lee (Dong-A Univ.).
- 9:45 - 10:00 **Quantification of *Colletotrichum fiorinae* in the Forest Suggest Its Main Ecological Role is that of a Leaf Endophyte.**
Phillip Martin and Kari Peter (Penn State Univ.).
- 10:00 - 10:15 **Sensitivity Distribution to 11 Fungicides in a Population of *Colletotrichum* Isolates from Apple.**
Kristen Pierce, Kate Thomas, Phillip Martin, Kari Peter (Penn State Univ.).
- 10:15 - 10:30 **Highlights of 2019 Apple Fungicide Tests.**
Keith Yoder, William Royston Jr., and Scott Kilmer (Virginia Tech AREC).
- 10:30 - 10:45 **Management of Peach Bacterial Spot: Integration of Biorational Bactericides and Cultivar Resistance.**
Norman Lalancette and Lorna Blaus (Rutgers Univ.).

BUSINESS AND FINANCIAL REPORTS

BUSINESS MEETING MINUTES

December 5th, 2019

Compiled and submitted by Chris Bergh, CSFWC, Inc. Secretary/Treasurer

Kerik Cox (President) called the meeting to order at 8:02 a.m.

Twenty-four members attended the Business Meeting, fulfilling the quorum requirement of 10% (conference attendance = 119)

The Minutes of 2018 Business meeting were reviewed. Motion to accept by Wayne Jurrick, seconded by Jim Schupp and the motion carried.

Chris Bergh gave the Treasurer's Report for 2018, noting record attendance in 2018. Mike Dimock moved to accept the Treasurer's Report, seconded by Wayne Jurrick, and the motion carried.

Old business:

Reduced registration fee for students. Noted that 13 graduate students attended in 2019. Noted that student's registration/membership fees are paid by advisors so no benefit to students to reduce fees. The issue was tabled.

How to increase meeting participation by small fruit and grape researchers? Suggestion of using personal contacts to recruit small fruit researchers. Ongoing issue of less small fruit researcher participation due to less small fruit content and vice versa. Jim Schupp suggested that the Proceedings be made available as public open access to entice more recruitment. Question of whether publication of data in Proceedings would preclude publication in a peer-reviewed journal was addressed and dispelled. Question of whether public access to reports that focused on pesticide trials could be used or viewed in a negative light. Suggestion of making program publicly available to promote wider participation. Wayne Jurrick moved to have open on-line access to program toward recruiting, with text to contact author to request full report. Seconded by Jim Walgenbach and the motion carried.

New business:

Members discussed the venue for and dates of the 2020 meeting. Dean Polk moved to have the 2020 meeting at the same venue on December 3-4, 2020. Seconded by Chris Walsh and motion carried.

During the Joint Session on December 5, Don Ganske indicated that he would not continue to serve as the Executive Director of the CSFWC, Inc. in 2020. It was determined that the appointment of a new Executive Director would be the responsibility of the CSFWC, Inc. Board

of Directors prior to the 2020 meeting. Mike Dimock volunteered to contact industry sponsors for the 2020 conference if a new Executive Director had not yet been identified.

Chris Walsh (UMD) was nominated by Dean Polk to serve as the CSFWC, Inc. President-Elect. Tom Kon (NCSU) was nominated by Jim Walgenbach to serve in this role and this was seconded by Sara Villani. Discussion of the pre-tenure benefit to Tom Kon and Chris Walsh withdrew his name. The members voted in favor of Tom Kon becoming President-Elect.

There was a discussion lead by Mike Dimock to not tie industry sponsorship exclusively to support an event at which alcohol is served (i.e. the Mixer), but rather in support of the conference. This was not voted on, but there were no dissenting comments.

There was discussion of enabling/encouraging Industry Representatives to submit formal 15-minute presentations about their products. It was agreed that these should focus on aspects of relevance to the audience (e.g. mode of action, resistance management, efficacy, etc.) and should not constitute a “sales pitch” for a particular product or products.

Chris Bergh recognized Kerik Cox and the rest of the Executive Committee, Mike Dimock (Past President) and Dean Polk (Incoming President) for an excellent meeting.

At 8:45 a.m., Kerik and Wayne requested a motion to adjourn. Jurrick moved to adjourn, seconded by Chris Walsh, and carried.

Cumberland-Shenandoah Fruit Workers Conference, Inc. Treasurer's Report for 2019

Respectfully submitted on December 6, 2019 by Chris Bergh, Secretary/Treasurer

INCOME		
Registration/memberships (3 comp) (119)		8,470.00
Sponsorships		2,840.00
Interest		NA*
	Total income	11,310.00
MEETING EXPENSES		
Meeting rooms		526.50
Lunch, coffee, soda		2,037.90
Mixer		1,930.69
Gratuities		642.75
Advance deposit (2017)		1,000.00
	Total meeting expenses	6,137.84
OTHER EXPENSES		
		1000.00
Deposit for 2019 meeting		100.00
Attorney		25.00
VA State Corporation registration		399.30
D. Epstein (mileage, room, M&IE)		346.36
PayPal		1,870.66
	Total other expenses	
SUMMARY		
Registrations/memberships (119)		8,470.00
Sponsorships		2,840.00
Meeting expenses		(6,137.84)
Other expenses		<u>(1,870.66)</u>
	Balance forward	3,301.50
Account balances as of Dec. 31, 2018		
BB&T		22,799.26
PayPal		258.90
	Total balance forward	23,058.16
CSFWC, INC. 2018 MEETING BREAKDOWN		
(6,137.84/119 attendees)		
Facility	526.50	(4.42 per attendee)
Food and non-adult beverages	3,335.83	(28.03 per attendee)
Adult beverages plus all gratuities	1,275.51	(10.72 per attendee)
Total cost per attendee	43.17	(59.21 in 2017)
Income per attendee	95.04	(69.04 in 2017)

*Non-interest bearing account

CALL OF THE STATES

CALL OF THE STATES – NEW JERSEY 2019

David Schmitt, Program Associate; Atanas Atanasov, Program Associate; Carrie Mansue Denson, Program Associate; Dean Polk, Statewide Agent; Norm Lalancette, Specialist in Fruit Pathology; Anne Nielsen, Specialist in Fruit Entomology
Rutgers Agricultural Research and Education Center, Bridgeton, NJ 08302

Tree Fruit - Tree phenology in 2019 was about normal based on historical observations, however Peach harvest was about a week to 10 days earlier than normal from midseason on. Cropping was very good in stone fruit resulting in a big thinning job. Fruit quality appeared good in the bin, however buyers complained of shattered and moldy pits in some midseason varieties. Cropping in pome fruit was good following an off year in 2019. Apple Harvest was about normal but growers let fruit hang late because warm temperatures in late summer made for poor color.

According to the [NJ State Climatologist](http://climate.rutgers.edu/stateclim/) (<http://climate.rutgers.edu/stateclim/>), monthly temperatures were above average for much of the growing season with 2 months: April, and July among the 5 warmest months on record. Overall total precipitation in 2019 was about normal with May, June and July above average. August and September were well below average perhaps creating the best wine vintage on record.

Disease control in the field was about average. In apples, fruit rots remain troublesome, however control was better than in recent years. Fire Blight was present on some farms in southern counties, however no widespread epidemics were noted. In peach, Bacterial Spot was difficult to impossible to control. Leaf infections were first observed during bloom and by late June epidemic levels were observed throughout the southern region. Higher summer copper rates appeared to lessen fruit symptom severity but did not improve pack-out. Observations made by Dr. Norm Lalancette's team at RAREC observed that environmental conditions were very favorable for Bacterial Spot and Brown Rot Blossom Blight. One orchard in the southern region observed a high incidence of *Botryosphaeria* canker in young orchards. In apples Dr. Lalancette reports environmental conditions were favorable for Apple Scab, and Sooty Blotch and Flyspeck. Conditions were not highly favorable for summer rots, rust diseases and fire blight.

Brown Marmorated Stink Bug populations and damage increased again this year compared to the past seasons. Dr. Anne Nielsen's lab recorded higher populations than in recent years. Codling Moth damage in apples was lower than past years, although some late damage was noted at harvest in September and October. CM trap captures were very low this year compared to recent years. Observations of Ambrosia Beetle damage remained about the same in 2019. Tree loss continues at known infestation sites. Incidence of San Jose Scale infestation in tree fruit remained significantly higher than past seasons. White Peach Scale was also observed at damaging levels in a number of orchards. Scale insects remain difficult to manage. Spotted Lantern Fly reports increased in 2019 and a number of counties in the state were placed under quarantine.

Grapes - Grape Phenology was about normal in 2019. Disease control was very good partly due to generally dry conditions in August and September. Growers that had good disease control early in the season experienced very little disease loss at harvest. Grape Berry Moth populations

were very low and little damage was noted even where controls were not implemented. Harvest was about two weeks early and favorable weather in late summer made for exceptionally high quality fruit with high sugar. Bird control remains difficult for most growers to manage.

Blueberry – The 2019 New Jersey Blueberry season experienced increased pressure from Anthracnose this year due to weather conditions.

The start of the season was a wet one. Rainfall during bloom in May was above the 30 year average. Anthracnose observations in the field were higher than normal, as were levels reflected in incubated berries. Loss of fungicide coverage due to weather or field conditions was likely a contributing factor. Even though growers had some disease problems, prices held for most growers throughout the season.

Blueberry maggot (BBM) was first detected the week of June 14th in an organic field and was later detected in commercial fields. Spotted Wing Drosophila (SWD) was first detected in Atlantic County earlier than average.

First generation Putnam Scale crawlers were first detected in early June and second generation Crawlers were first detected in mid-August. Sharpnosed Leafhopper populations were also detected in mid-August marking our earliest observation for this pest emergence.

Orange-striped oakworm did heavy damage in isolated areas along wooded field borders late in the season. Growers who treated for scale and Sharpnosed Leafhoppers in August did not observe much damage from oakworms.

Weed management in blueberries remains an area of experimentation and refinement.

Tree Fruit Phenology – Southern New Jersey Counties 2019

Pest Event or Growth Stage	Approximate Date	2019 Observed Date
Bud Swell (Redhaven)	March 23 +/- 15 Days	March 25
1/4" Green Tip Red Delicious	March 31 +/- 13 Days	March 27
Pink Peach (Redhaven)	April 4 +/- 15 Days	April 4
Tight Cluster Red Delicious	April 9 +/- 13 Days	April 8
Oriental Fruit Moth Biofix	April 9 +/- 13 Days	April 8
Full Bloom Peach (Redhaven)	April 9 +/- 14 Days	April 9
Pink Apple (Red Delicious)	April 14 +/- 12 Days	April 16
Codling Moth Biofix	April 27 +/- 13 Days	April 25
Green Peach Aphid Observed	April 16 +/- 16 Days	No Observation Made

Full Bloom Apple (Red Delicious)	April 22 +/- 11 Days	April 20
Petal Fall (Redhaven)	April 22 +/- 10 Days	April 19
Petal Fall (Red Delicious)	April 27 +/- 14 Days	April 29
Shuck Split (Redhaven)	April 30 +/- 11 Days	April 24
First PC Oviposition Scars Observed	May 3 +/- 18 Days	April 23
Tufted Apple Bud Moth Biofix	May 4 +/- 10 Days	April 8
San Jose Scale Crawlers (1 st Gen.)	June 2 +/- 8 Days	May 24
Pit Hardening Peach	June 16 +/- 8 Days	June 10

CALL OF THE STATES – NEW YORK 2019

Art Agnello, Dept. of Entomology
Cornell AgriTech at NYSAES, Geneva, NY

Following in the tradition of 2018, this has been another one of those split-personality growing seasons for which New York is so well known, and which are vexing to the simplicity-seeking instincts that vainly expect a predictable or at least tolerable progression from 'when does spring arrive?' to 'is it ever going to cool off?' It's fairly apparent by now that radical swings and extreme weather events have become the new normal, so it's a good thing that most NY growers are tough enough to shake it off each year. Similar to what we saw last season, this spring was ultimately very delayed, with see-sawing temperatures and miserable rainy stretches that didn't allow much insect activity but certainly taxed most disease control efforts. By the first week of May, our degree day accumulations began to fall behind those of 2018 as well as the historical averages, and we still hadn't fully caught up by harvest time. It wasn't until we got solidly past mid-June that the rainy pattern seemed to break up and we entered the dry phase of the summer, peppered with some sporadic severe storms that nevertheless didn't do much to maintain adequate moisture. Periods of warm temperatures and low rainfall persisted through most of July and August, until finally succumbing to the late-summer pattern of pop-up thunderstorms and muggy heat that continued well into September, but which moderated with cooler afternoon and night temperatures that signalled some very favorable coloring conditions.

Again, similar to last year, insect pests appeared not to be overly troublesome this season, although there were a few oddities that we may not be certain about how everything will stand once it's all over. As in 2018, **plum curculio** seemed to be addressed adequately by most growers, despite a protracted oviposition period caused by the cool spring temperatures. Outbreaks of **European red mite** threatened briefly but then did not amount to much, again probably thanks to low temps and frequent rainfall. The main curiosity in my view was the healthy and long-lived tortricid moth flights, some of which were still very much in progress in September. Although **oriental fruit moth, codling moth and obliquebanded leafroller** all made their WNY appearances somewhat later than usual (OFM, mid-May; CM, early June; OBLR, mid-June), trap numbers were impressive at various sites around the state, and seemed to persist past the expected flight periods predicted by our 'normality-bound' developmental models. These traditional drivers of most insect management programs have stretched our concept of covering all the bases to avoid last-minute flare-ups, as the trapping and monitoring results were a challenge to translate into a reasonable protective strategy. A noteworthy trend this year was higher-than-normal levels of some foliar insect pests, including **green aphids, potato leafhopper, and Japanese beetle**, all of which seemed to materialize very quickly in July and were slow to dissipate.

First occurrence of **apple maggot** was also uniformly late around the state, and low numbers were reported from most regions outside of the Hudson Valley, so wasn't clear whether we should expect a September flush of adults that should have occurred in early August (and which ultimately didn't seem to arrive). Populations of **San Jose scale** and **woolly apple aphid**

infestations were noted in some orchards, but so far it's unknown how much damage they ended up causing by harvest time.

Our growing entourage of invasive pests also demonstrated some puzzling trends. **Spotted wing drosophila** again started showing up early (June) around the state and sustained catches eventually reached some high numbers in tart cherries, but actual fruit infestations were scarce – although to be fair, the state's crop was quite low this year owing to an April freeze event, meaning that many blocks didn't have a normal harvest. Again this season, **brown marmorated stink bug** was unaccountably difficult to find in even the favored Hudson Valley sites where it's been a frequent challenge, at least until well into September; the adventive samurai wasp parasitoid could be one of several natural enemies contributing to this trend, but a native microsporidium pathogen (*Nosema maddoxi*) should not be ruled out as another potential factor. The **European cherry fruit fly** continued its slow spread eastward, as the massive trap network maintained by USDA APHIS and the NYS Dept of Ag & Markets documented adult occurrence in actual cherry orchards this year, resulting in more of Niagara and Orleans County plantings being placed in a quarantine zone. Finally, the perennial **black stem borer** ambrosia beetle, a primary or at least secondary cause of tree decline and death in numerous plantings around the state, continued to be found in reportable numbers, primarily in sites along Lake Ontario.

CALL OF THE STATES – PENNSYLVANIA 2019

G, Krawczyk¹ and K. Peter²

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The Pennsylvania State University, Fruit Research and Extension Center, Biglerville, PA

Plant Pathology

The 2019 season kicked off with green tip around 2 April. We had a fairly wet spring; however, mid-July through harvest was quite dry. This is in stark contrast of the 2018 season.

Apple and pear diseases: **Apple scab** was an issue in many parts of the state. This was due to persistent rain events occurring when the apple scab ascospores were peaking in numbers and dispersal. There were 14/31 days in May considered an apple scab infection period; May 1 – 13 only had 2 days that were not considered an infection event. Consequently, many growers struggled with control, especially those who use alternate row middle spraying.

Bitter rot was not so much an issue during 2019 despite the wet early season. The dry latter half of the season most likely halted the progression of the disease.

Marssonina blotch, first observed in PA in 2017, is becoming more concerning with symptoms being observed as early as July in 2019. There were reports concerning premature defoliation for some cultivars, particularly Rome.

Several incidences of **Phytophthora root rot** were reported, particularly in orchard sites with high clay soils and/or poor drainage that suffered high rain fall in 2018.

Conditions for **fire blight** were not favorable during bloom in 2019. The cool, wet spring favored a protracted bloom, but ideal fire blight weather was kept at bay. Some areas saw significant shoot blight mid-season, which was most likely due to hail events during the first weekend of June.

Stone fruit diseases: Bacterial spot on peach/nectarine was especially problematic during the 2019 season. Fruit rots, particularly brown rot, was not issue most likely due to the dry conditions during the latter half of the season.

Entomology

The biofixes for most common pests occurred at dates similar to previous years. The first captures in pheromone traps during the 2019 season were observed for oriental fruit moth on April 15, codling moth on May 02, obliquebanded leafroller on June 06 and tufted apple budmoth on May 06.

Unexpectedly, the numbers of rejected loads by our only still operating fruit processor Knouse Food were significantly higher in 2019 than during the 2018 season: the total number of rejected loads reached over 200 loads (66 during the 2018 season), with **codling moth** and **Oriental fruit moth** split of 50:50

The **brown marmorated stink bug** populations survived winter in good shape however the number of adults in the spring were relatively low, mainly due to lower BMSB population going to diapause during the fall of 2018. Suitable weather conditions during the season contributed to

significant rebound in the numbers of BMSB during August and September. During the fall, many PA fruit growers reported more injuries on late apple cultivars due to the intensive feeding by BMSB adults, however the injuries caused by complex of native stink bugs can not be excluded from this observation. The parasitic wasp *Trisolcus japonicus* was detected at every location across the state where we surveyed for it, except for a single location in north-central part of the State.

Spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae) an invasive plant hopper, native to China, India and Vietnam is officially reported from 13 counties with multiple municipalities in southeast Pennsylvania. PDA imposed quarantine, however the insect appears to continuously spreading from the original areas where it was first identified during the 2014 season. The list of potential host plants includes grapes, apples and stone fruit trees.

CALL OF THE STATES – VIRGINIA 2019

Sherif Sherif¹, Keith Yoder¹, Mizuho Nita¹, and Chris Bergh¹

¹Alson H. Smith Jr. Agricultural Research and Extension Center, Virginia Tech

Horticulture (Sherif): Virginia had a full crop of peaches, apples, and sweet cherries in 2019. There were nights with below-freezing temperatures in April, but these did not coincide with the full bloom of stone or pome fruits. Although the peach crop was generally good, the market was a bit saturated, which negatively affected the farm gate price to some extent. I note that bloom and harvest dates for pome and stone fruits were about 10 days-two weeks earlier compared with 2018.

The apple crop in 2019 was generally lighter than the past two years, especially for early varieties like Gala and Honeycrisp, but fruit size and color was significantly better this year than last. One factor that contributed to good fruit size was optimal weather conditions for fruit thinning. We had several cloudy and warm days during the fruit thinning window (e.g. at 8-15 mm fruit diameter) and this was reflected in tree carbohydrate reserves and the tendency of trees to shed fruit after thinning treatments. For fruit color, the weather in the Frederick county area was also cooperative, especially with the red strains of Gala and Pink Lady. Based on our weather station, the average minimum temperatures for Winchester in August, September, and October were between 33° and 53°F, with several cold nights during the last 10 days prior to harvest. This was not the case in central Virginia, where average minimums ranged between 40° and 58°F between August and October, resulting in less fruit coloration.

We had a drier year in 2019 than 2018, with only 22 inches of rain between April and October, compared with 44 inches last year. This did not negatively impact fruit size, because of the reasons already explained. It did have an impact on the shoot length, particularly for early-season varieties.

Pathology (Yoder and Nita): From the tree fruit perspective, 2019 was not unusual for early season diseases in Virginia. Most early season scab infection periods were also cedar-apple rust infection periods, so there was considerable rust pressure, but most fruit escaped quince rust. We had 40 days favorable for apple powdery mildew through mid-June, with plenty of opportunity for secondary infection of susceptible cultivars.

For summer diseases, because many of the early spring wetting periods were relatively warm (in the 60s), this triggered some early rot activity. Wetting hour accumulation, for tracking sooty blotch and flyspeck, was the lowest we have had in 25 years of monitoring this. As of September 2, total wetting hour accumulation at the Winchester AREC was 417 hours, only half that of last year, and the 250-hour action threshold was reached on July 11, a month later than in 2018. This delay, and the fact that many early season wetting periods were quite warm, led to the appearance of bitter rot earlier than sooty blotch/flyspeck on unprotected fruit.

Many Virginia grape growers enjoyed a relatively dry season in 2019, resulting in fewer issues with common fungal diseases. Many growers experienced higher crop yield and better fruit quality than in 2018, which was a very positive change. Several growers in central Virginia

indicated the increase of Pierce's Disease, which probably took place in the previous few years. Also, most likely due to the rapid decline in temperature that happened between December 2018 and January 2019, the decline or death of vines due to winter injury was reported from growers along the Blue Ridge mountains.

Entomology (Bergh): This report also relays comments and observations from orchard consultant, Bill Mackintosh. Oriental fruit moth and codling biofixes on April 14 and May 1, respectively, were completely in line with historical averages at the Winchester AREC. There were reports of higher codling moth injury from some locations than has been seen in some time.

As yet unidentified/unconfirmed source of injury to apples was reported again from some orchards in central and northern Virginia. Pink Lady was the most commonly and most severely injured cultivar, but others such as Granny Smith and Ginger Gold also expressed the same injury. Apple curculio and/or apple greenbug have been suggested as the cause, but this has not been confirmed.

I received no reports of mite or woolly apple aphid outbreaks in 2019, likely because growers have tended to reduce their inputs for BMSB in recent seasons. However, some growers reported higher than expected levels of stink bug injury from some orchard blocks at harvest, and that they observed more brown stink bugs in their orchards than is typical. Whether the stink bug injury detected at harvest was associated with native species remains uncertain. With respect to brown marmorated stink bug (BMSB), early season captures in traps were typically low and followed by low captures through most of August. As expected, highest seasonal captures of BMSB occurred from late August – mid-October. For reasons that remain speculative but that may have been associated with the hot and dry conditions that prevailed during August and September, an unusual (for recent years) spike in BMSB invasion of buildings in late September – early October was reported from NJ to Kentucky. Growers should be cautioned that this may translate to higher populations of overwintering BMSB going into the 2020 season. Detections of *Trissolcus japonicus* continued to be common in Frederick County and new detections occurred at several of the *T. japonicus* redistribution sites in Virginia, including in Nelson (central VA) and Botetourt (near Roanoke, VA) counties.

The spotted lanternfly infestation in Winchester continued unabated, resulting in the city of Winchester and Frederick County being placed under quarantine in spring 2019. In the fall, spotted lanternfly was detected in Clarke County, VA (just east of Frederick County) and Berkeley County, WV (Bunker Hill, just north of Frederick County). As yet, there have been no reports of infestation of or damage to crops (e.g. vineyards or orchards) in this area.

ENTOMOLOGY

EFFICACY OF PLANT HOST DEFENSE AND SIGNALLING COMPOUNDS IN
PREVENTING AMBROSIA BEETLE INFESTATIONS IN APPLE TREES

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Preventive Trials for Control of Ambrosia Beetles in NY Apple Orchards

The ambrosia beetle *Xylosandrus germanus* has been documented to cause tree death and decline in dozens of NY apple orchards since 2013, mostly in young dwarf apple plantings. Preventive trunk sprays using chlorpyrifos or pyrethroids have not provided acceptable levels of control, nor have topical applications of the repellent verbenone, a component of anti-aggregation pheromone produced by various species of bark beetles that has been found to repel this and related species of scolytines from traps and attractive host trees. In our most recent trials in 2017 and 2018, we tested a mixture of verbenone and methyl salicylate, a host defense and signalling compound produced by plants under stress, as well as an SAR (Systemic Acquired Resistance) activator product. We found that all the repellent treatments had fewer infestation sites than the untreated checks, and the combined verbenone + methyl salicylate treatments had the lowest incidences of galleries containing adults or brood.

Methods

In 2019, we tested trunk applications of different repellents for *X. germanus* control in potted apple trees (2-yr old Red Delicious on Nic.29 rootstock), waterlogged to stress them to produce ethanol, and placed inside wooded areas directly adjacent to orchard sites. Additionally, individual ethanol lures were attached to each tree to increase their attractiveness to the beetles. The preventive treatments, which were applied on 15 May, included different topical formulations of methyl salicylate (a host defense and signalling compound), alone and combined with verbenone; these were in SPLAT formulations (ISCA Tech), and applied using a caulking gun. The methyl salicylate+verbenone combination product was applied at one of two timings, either 15 May (start of the 1st flight) or 12 June (start of the 2nd flight), to assess its usefulness later in the season. Additional treatments were the Systemic Acquired Resistance (SAR) activator products Actigard (acibenzolar-S-methyl, Syngenta), Regalia (*Reynoutria sachalinensis* extract, Marrone), and a formulation of Salicylic Acid (Growth Products); the insecticide Lorsban (chlorpyrifos, Dow AgroScience) was used as a grower standard comparison. The last four treatments were applied using a Solo AccuPower 416 battery-powered backpack sprayer with a TeeJet 8004 LP flat fan nozzle. Each treatment was replicated on 6 trees, which were arranged in 6-tree groupings at each of the sites, with groups of trees separated by a distance of 10 m (one group per treatment per site). The three SAR treatments were applied twice more, at 4-week intervals: 12 June and 9 July; this 3-spray regimen was used in an attempt to maximize their potential effect over the part of the season when the majority of the infestations were assumed to occur.

Trunk and tree damage was assessed among the different treatments on 9 Jul, after the end of the first adult flight, and on 3 September, as the second flight was subsiding, to determine what effect these treatments had in preventing attacks by this beetle. On each date, half the trees in each treatment group were uprooted and brought to the lab, where they were dissected to count and characterize the infestation levels.

Results

- Infestation holes: On the 3 Sept evaluation date, the treatments with the fewest infestation sites were the early application of verbenone+methyl salicylate, and verbenone. The Lorsban treatment had the highest levels on this date, while it was the Salicylic acid resulting in the most holes on the 9 July date.

- Gallery contents, adults: The fewest number of galleries containing adults was seen in the verbenone+methyl salicylate combination treatments (both timings), as well as in the Untreated Checks (for which we can propose no explanation).

- Gallery contents, brood: Brood numbers were uniformly low in all the treatments this season. The only treatment to break out statistically was the Salicylic acid, but only on the early evaluation date.

- Empty or aborted galleries: The fewest numbers were found in the combination verbenone+methyl salicylate early treatment, on both evaluation dates; Salicylic acid had the highest number on the 9 July date, and the Lorsban treatment had the highest number on the 3 Sept date.

In general, of all the treatments, the early verbenone+methyl salicylate application showed the most uniform trend of the lowest infestation characteristics on both evaluation dates. Among the SAR treatments, Actigard tended to show marginally greater effectiveness than Regalia, and the Salicylic acid had the least. SAR inducers like Actigard prime the host for stress events by inducing the expression of host defense genes; in apples, these have been used primarily for fire blight control, but our results show that a program of multiple applications could be of potential value against black stem borer (Table 1).

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This work was supported by the donation of products for testing from ISCA Technologies (Jesse Saroli, Agenor Mafra-Neto), Dow AgroSciences (Alejandro Calixto), and funds from the USDA Hatch Program and the NY Apple Research & Development Program.

Table 1. Ambrosia beetle infestations in two-year old potted flooded apple trees treated with preventive trunk applications of different materials. Data pooled across three replicated orchard sites, Wayne Co. 2019

Treatment ^a	Application		Mean #		Mean # sites with			
			Infestation Sites		Empty Galleries		Adults Brood	
	Rate	Date	9 Jul	3 Sep	9 Jul	3 Sep	9 Jul	3 Sep
	9 Jul	3 Sep						
Lorsban	1.5 qt/100 gal	5/15	1.3 b	9.7 a	0.8 b	6.1 a	0.2 b	2.9 ab
	0.2 b	0.7 a						
Verbenone	10 g/tree	5/15	1.6 b	2.2 c	1.1 ab	1.1 bc	0.4 ab	0.9 abc
	0.0 b	0.4 a						
MeSa	10 g/tree	5/15	1.2 b	3.2 bc	0.4 b	2.4 abc	0.4 ab	0.8 bc
	0.1 b	0.2 a						
Verb+MeSa	10 g/tree	5/15	0.2 b	0.8 c	0.1 b	0.1 c	0.1 b	0.6 c
	0.1 b	0.1 a						
(early)								
Verb+MeSa	10 g/tree	7/9	0.0 b	4.3 abc	0.0 b	3.9 abc	0.0 b	0.4 c
	0.0 b	0.0 a						
(late)								
Actigard 50WG	2 oz/100 gal	5/15, 6/12, 7/9	2.2 b	5.9 abc	2.0 ab	3.6 abc	0.2 b	2.1 abc
	0.0 b	0.3 a						
Regalia	30 ml/gal	5/15, 6/12, 7/9	0.6 b	8.3 ab	0.3 b	4.9 ab	0.2 b	3.0 a
	0.1 b	0.4 a						
SAR Salicylic acid	8 fl oz/100 gal	5/15, 6/12, 7/9	6.7 a	3.0 bc	4.3 a	1.7 bc	1.2 a	1.2 abc
	1.8 a	0.2 a						
Untreated Check	—	—	2.2 b	2.7 bc	1.9 ab	1.9 bc	0.0 b	0.7 c
	0.0 b	0.1 a						

Values in a column followed by the same letter not significantly different ($P < 0.05$, Student's t-test.)

^a MeSa, methyl salicylate; Verb, verbenone

FIELD OBSERVATIONS ON SPOTTED LANTERNFLY BEHAVIOR AND HOST SUITABILITY

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Introduction:

The spotted lanternfly (SLF), *Lycorma delicatula*, is an invasive planthopper native to East Asia. First discovered in Berks County, PA, in 2014 (Barringer et al., 2015), the SLF has spread to multiple states and counties. SLF are phloem-feeding insects, which results in copious amounts of liquid waste, or honeydew (Dara et al., 2015). This honeydew effectively stops photosynthesis of contaminated vegetation (Dara et al., 2015), which can ultimately result in financial losses in many different economic sectors. The USDA-APHIS implemented a trade quarantine to help limit the SLF spread. It has been shown that SLF nymphs have a wide range of hosts, while the adult host range narrows (Lee et al., 2009). SLF preferred host is commonly known as the tree-of-heaven, *Ailanthus altissima* (Lee et al., 2009). Although the preferred host for SLF is tree-of-heaven, SLF can feed on over twenty families of plants in North America (Lee et al., 2009).

The objective of this project was to review field techniques employed by LABServices over the past two years to evaluate SLF control products in the field. The objective had three goals: 1) Identify a practical method for conducting field efficacy trials on SLF. 2) Investigate adult SLF survivability on various host, tree-of-heaven (TOH), red maple (*Acer rubrum*) (RM), black walnut (BW), and native grapevine (GV). TOH, RM, BW, and GV were selected because those species are common in woodlots and the perimeter of agricultural fields within the current distribution. 3) Demonstrate techniques effective for conducting pesticide efficacy with adult SLF in field trials.

Materials and Methods:

For field trials, TOH, RM, and BW were selected to be within 6-10" diameters at breast height (DBH). GV was chosen to be within 2-3" DBH. Trees/or vines. Replicates were clustered into a group, and each replicate group was separated by at least 50' from each other. Tree cages were constructed that were durable and restrictive to maintain SLF on a treatment regimen. Tree cages were installed at breast height (4.5' from the base of the trunk). The cages took an average of fifteen minutes to install and were effective all summer unless excessive sooty mold developed and replacement with clean cages was necessary. All SLF adults were hand collected from Kaercher Creek Park, Hamburg, PA. All collections were done from Kaercher Creek exclusively to ensure limited pesticide exposure. After collection, SLF adults were held in plastic containers lined with paper towels to absorb excess honeydew and stored in coolers with refrigerated chill packs. SLF were then brought to our field site in Hamburg, PA, sexed, and five males and five females were placed directly into each cage by hand. Mortality data was collected daily or as needed.

A pyrethroid bark spray and a neonicotinoid systemic bark spray were used only on TOH test trees to demonstrate efficacy in the field. Untreated TOH was used to demonstrate the natural mortality in the cages compared to pesticide treatments. A pyrethroid bark spray (beta-cyfluthrin 2.5%) was used to represent a common homeowner product known to provide quick knockdown with limited residual activity (Table 1). A systemic neonicotinoid bark spray (dinotefuran 70%) was used to simulate an SLF control measure with extended residual properties (Table 1). Applications were made with a backpack sprayer using a TeeJet TX8003VK Full Cone nozzle held 4" from tree bark until spray run-off (60-80 DBH"/gallon solution).

Results:

A total of 5044 SLF were collected Sept 23rd – Nov 7th for use in this project. Overall the collections show that 59% of the adult SLF collected were males, while 41% were female.

To determine if collection and caging of adult SLF influenced the survivability of SLF in the cages, we compared survival at one day after infestation (1DAI) and 7DAI for average percent mortality on untreated plots. The average percent mortality was $0.40\% \pm 0.89\%$ for all adult SLF on untreated TOH 1DAI (Figure 1). Similarly, there was low percent mortality for all adult SLF on BW and GV untreated treatments 1DAI, $0.67\% \pm 1.15\%$, and $0.00\% \pm 0.00\%$, respectively (Figure 1). Adult SLF on untreated RM 1DAI resulted in a high average percent mortality of $24.33\% \pm 18.57\%$ (Figure 1). At 7 DAI, adult SLF on untreated TOH and GV had an average percent mortality not significantly different from each other, $16.11\% \pm 12.09\%$, and $21.50\% \pm 17.68\%$, respectively (Figure 1). Adult SLF on RM and BW untreated treatments 7DAI had a high average percent mortality that was not significantly different between the two species, $85.33\% \pm 16.48\%$, and $100\% \pm 0.00\%$, respectively (Figure 1). The average percent mortality for adult SLF at 14DAI follows the same trend as the 7DAI mortality. The BW average percent mortality remained at 100%, while the RM mortality increased to $96.94\% \pm 6.41\%$ (Figure 1). Adult SLF mortality on untreated TOH and GV continued to increase at 14DAI, $28.67\% \pm 21.21\%$, and $40.50\% \pm 4.95\%$ (Figure 1). As a general observation, average percent mortality tended to increase later in the season as the test trees entered abscission, and mortality increased were related to rising temperatures.

Adult SLF were capable of surviving on GV and TOH untreated treatment trees/vines in this cage design with reasonably low average percent mortality. Therefore, to quantify the duration of adult SLF survival with this caging technique, adult SLF contained in specific cages until 90% mortality occurred. Adult SLF survived 17 days and 24 days on GV and TOH, respectively, until 50% mortality (Figure 2). It took 35 and 44 days for adult SLF to then reach 90% mortality in the GV and TOH, respectively (Figure 2).

A pyrethroid bark spray and a neonicotinoid systemic bark spray were evaluated only on TOH test trees to demonstrate pesticide efficacy in the field. The pyrethroid treatment provided rapid knockdown, resulting in $100\% \pm 0.00\%$ mortality on adult SLF in less than two hours after infestation (Figure 3). Similarly, at 1DAI, the average percent mortality during the entire trial period for the neonicotinoid bark spray was $82.00\% \pm 22.98\%$, and after 4DAI average percent mortality was $100\% \pm 0.00\%$ (Figure 3). The average percent mortality for adult SLF on untreated TOH at 1DAI and 4 DAI was $0.40\% \pm 0.89\%$ and $8.12\% \pm 6.29\%$, respectively (Figure 3). The adult SLF in these evaluations were changed with newly field-collected insects every fourteen days for the duration of the trial. Evaluation of the systemic neonicotinoid bark spray continued for three months while the pyrethroid bark sprays were terminated after the second group of fresh SLF failed to differ from the untreated controls. The neonicotinoid bark spray provided $100\% \pm 0.00\%$ mortality of adult SLF at 1DAI until the 8WAT introduction of new SLF adults (Figure 4). By 8WAT, the initial mortality of adult SLF on neonicotinoid bark sprayed trees was significantly reduced from earlier introductions. However, by 4DAI, the average percent mortality was 100% (Figure 4). After three months, introduction of SLF adults on the neonicotinoid bark spray TOH treatments resulted in approximately 40% mortality at 1DAI but ultimately resulted in $100\% \pm 0.00\%$ mortality by 4DAI (Figure 4).

Discussion:

The male population, at the Kaercher Creek collection site, was significantly higher than the female SLF populations. However, about Oct 31st, when oviposition was ending, male numbers declined relative to female numbers. Lack of male SLF precluded efficacy evaluations of the neonicotinoid bark sprays after October 31. The higher mortality data for adult SLF on RM untreated treatments 1DAI suggests that the collection and caging methods influenced mortality. The high average percent mortality of adult SLF on RM and BW untreated treatments 7DAI suggest that neither species is a suitable long-term exclusive host for adult SLF. Low average percent mortality 1DAI and high average percent mortality 2DAI suggest that untreated BW may be a capable intermediate host for adult SLF. Overall, it was observed that adult SLF were not capable of surviving exclusively on RM and BW with this caged method. None the less, on TOH and GV, adult SLF were capable of surviving for extended periods of longer than one month. Adult SLF survived 20% longer on TOH compared to GV to reach 90% mortality. The neonicotinoid bark spray provided excellent control of adult SLF, up to 3 months using this caging technique.

The cage design implemented in this study could be modified to accommodate for studies on multiple host species, life stage analysis, and even biological agent related trials. Further cage modifications in the future would target smaller trees or use branches with thinner bark to create a more suitable environment for the SLF. Additionally, the cages use adult trees or vines with an established root system, mimicking what would generally be available to SLF in the field. Data collection and analysis were easily manageable because a consistent number of adult SLF used in each cage, allowing for hourly or daily data to be collected. The cages were custom made, easy to install, clean, and manage. A shortcoming observed during evaluations was the lack of available space for SLF in the cages. This presentation only represents one year of data. Therefore, additional replications should be performed.

Video evidence demonstrating a courtship behavior observed in SLF adult males in natural settings was never observed in the cages during this conduct of this research. The observed male courtship behavior involved performing a mating dance for the female SLF. The male is fluttering his hindwings to expose the brightly colored underwing in a circular motion pattern around the female.

Lastly, the presence of *Beauveria bassiana* in the field was observed. No adult SLF in our cages was observed with sporulation masses of *B. bassiana* though the confirmation of mortality due to *B. bassiana* cannot be confirmed.

Literature Cited:

- Barringer, L. E., Donovall, L. R., Spichiger, S.-E., Lynch, D., Henry, D. 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae) Entomol. News. 125, 20–23.
- Dara, S.K., Barringer, L.E., Arthurs, S.P. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): A new invasive pest in the United States. J. Integr. Pest Manag. 6, 1–6.
- Lee, J. E., Moon, S.R., Ahn, H.G., Cho, S.R., Yang, J.O., Yoon, C., Kim, J.H., 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. Korean J. Appl. Entomol. 48, 467–477.

Figures and Tables:

Table 1. Pesticide efficacy treatment list and application methods

Treatment Type	Chemical Name	% WT	Application Rate (fl. oz./gal)	Application Rate Unit	Application method
Control (no treatment)	n/a	n/a	n/a	n/a	n/a
Pyrethroid Bark Spray	Beta - Cyfluthrin	2.5	2.0	fl. oz./gal	Bark Spray 60 - 80" DBH/ gal. solution
Neonicotinoid Bark Spray	Dinotefuran	70	3.6	fl. oz./gal	Bark Spray 60 - 80" DBH/ gal. solution
	Adjuvant	95	2.5	fl. oz./gal	Bark Spray 60 - 80" DBH/ gal. solution

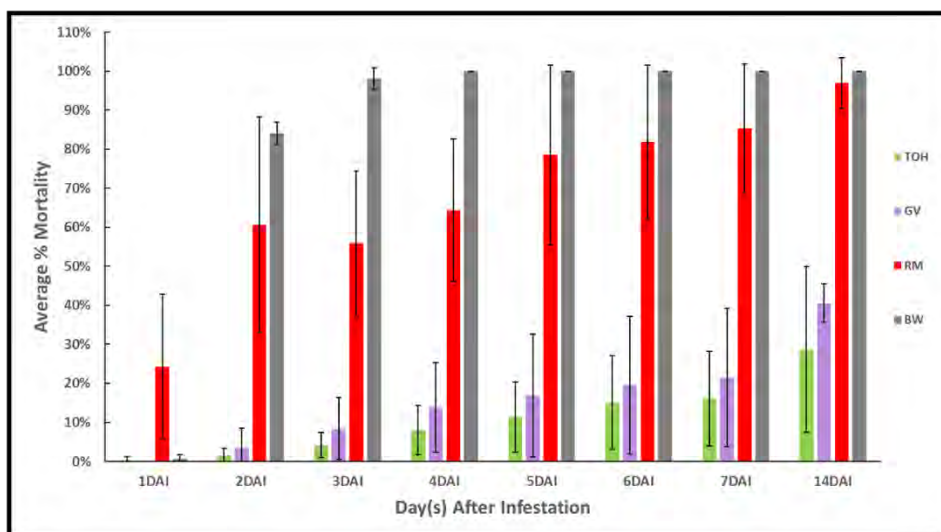


Figure 1. Adult SLF average percent mortality on TOH, GV, RM, and BW untreated treatments until 14 days after infestation.

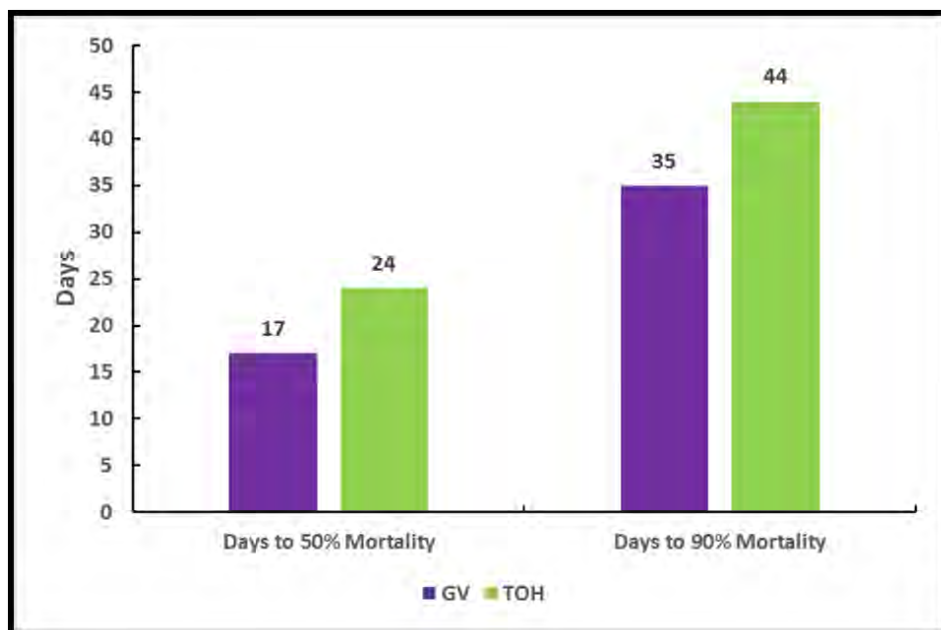


Figure 2. Days adult SLF could survive on GV and TOH exclusively in the custom cages.

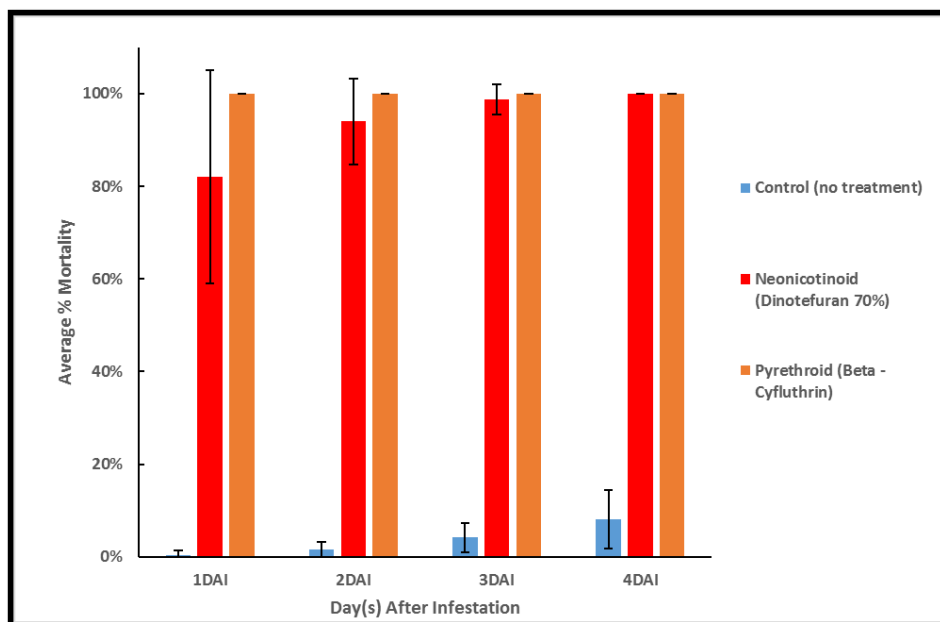


Figure 3. Average percent mortality for adult SLF on TOH treated with various pesticides.

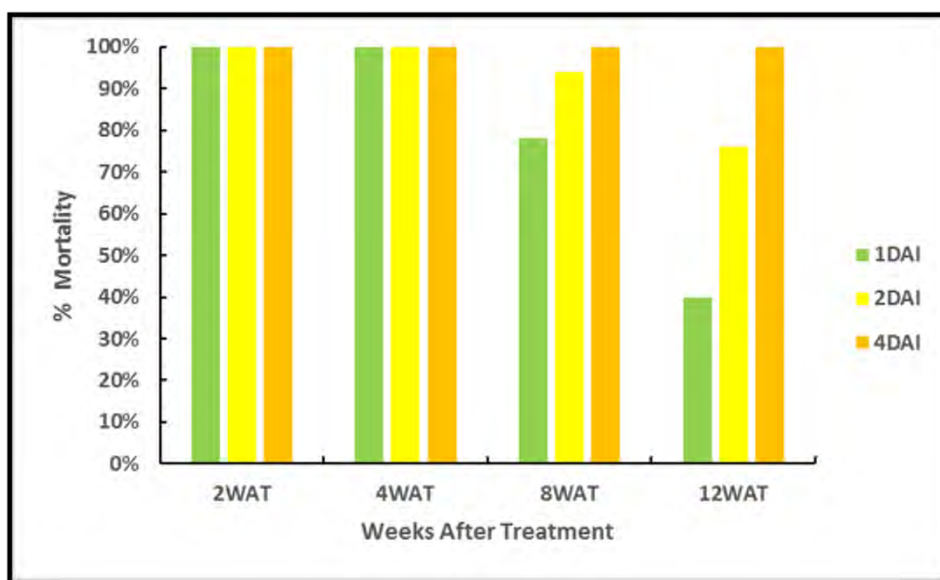


Figure 4. Percent mortality for adult SLF on TOH treated with neonicotinoid bark spray over a three-month period.

ORIENTAL BEETLE-STILL A HIDDEN ISSUE

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Oriental beetles are the most important soil feeding insect in New Jersey Blueberries. The impact is more severe on young plantings due to root feeding grubs that will reduce plant vigor and fruit yield. Imidacloprid is the only labeled chemical control option that will work on 1st and 2nd instar grubs. When spray records were analyzed from the New Jersey Blueberry IPM program from 2016 to 2018 only two farmers used Imidacloprid as a soil application for grub control. Why is this, many growers do not like to use Imidacloprid because of bee concern. As quoted from a farmer “Well let them eat, if we use Admire, we won’t have any berries next year”. Pheromone traps are highly effective in attracting adult beetles for monitoring. Over the past four years oriental beetle (OB) trap counts have been increasing in New Jersey blueberry fields. An experiment was conducted to see if trap counts would reflect on finding grubs in blueberry soils. In 2018 to 2019 a total of 201 fields were sampled, at each sample site three fields were selected; first field contained the location of the OB trap, the other two surrounded the OB field. The sampling was based on 6 bushes, undercutting 50% of the roots from one side of the bush, (roughly 27 inches in length by 16 inches wide and 9 inches deep) in a U-shape outline. Soil was then sieved, grubs found were multiplied by 2 to make up total grubs per bush. Supplies used for sampling were a shovel, bin and sieve.

In 2019, about 65% of our sampling contained grubs in the fields ranging from 2 to 32 total grubs per field. Since we know that growers prefer to not use Imidacloprid, the next step is to educating growers on other alternatives for grub control. Our future work will be supported by a SARE Grant for 2020-2021 on educated applications of Mating Disruption Tabs or Beetlegone to growers.

CAN A CROP SANITIZER CONTROL *D. SUZUKII* IN SMALL FRUITS?

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The invasive vinegar fly *D. suzukii* is a major insect pest of small fruits in the U.S. *D. suzukii* uses its' saw-like egg laying structure to penetrate the skin of ripening fruit, rendering fruit unmarketable. To achieve adequate control, weekly applications of broad spectrum insecticides are often necessary and more sustainable management practices, particularly for organic farmers are needed.

Jet-Ag[®], a peroxyacetic acid (4.9%) and hydrogen peroxide (26.5%) (PAA-HP) crop sanitizer occasionally reduced *D. suzukii* infestation in Michigan blueberries (Van Timmeren et al. 2019). Follow up laboratory experiments indicated that fruit becomes less attractive when treated (Van Timmeren et al. 2019). It is possible that this behavioral response is mediated through the fungal microbial community, in particular yeasts, which are important food resources.

To evaluate impacts on *D. suzukii* associated yeasts, the two most commonly isolated from *D. suzukii* larvae (*Hanseniaspora uvarum* and *Issatchenkia terricola*) were cultured on potato dextrose agar (PDA). Yeasts were given either one or 24 hours to establish on the potato dextrose agar (PDA) growth media. Subsequently, a central plug was removed and liquid PDA amended with increasing PAA-HP concentrations (0%, 0.5%, 1.0%, 1.5% v/v) was added. The diameter of the inhibition zone was measured after 24h. Yeasts were not able to colonize areas with higher PAA-HP concentrations when inoculated 1 hour prior to exposure. When yeasts were given 24h to establish, a zone of reduced growth occurred (Figure 1), and yeast cells transferred from this zone onto fresh PDA were unable to grow. PAA-HP prevents yeast growth and causes contact mortality under laboratory conditions.

To investigate PAA-HP under field conditions, blackberries were sampled before and 24h after the application of a 1% PAA-HP v/v applied using an airblast sprayer to two 30 ft planting rows. This application was compared to a water control application made on the same day. Fruits were collected, washed using 0.1% Tween 20, and the washing water was filtered to separate yeasts and fungi by their spore size and fungi were cultured on Rose Bengal Chloramphenicol Agar to determine fungal abundance. Two colonies of each yeast morphospecies as well as all hyphal fungi were subsequently pure cultured on PDA for identification. PAA-HP did not impact total yeast abundance (Figure 2). We are currently determining if the application impacted the yeast community. We also evaluated the impact on *D. suzukii* infestation one week after the application, using 10 fruits per treatment replicate and PAA-HP had no impact on infestation. In order to efficiently use PAA-HP for the control of *D. suzukii* in small fruits, further research about the mode of action and application frequency is needed.

Van Timmeren, S., Fanning, P.D., Schöneberg, T., Hamby, K., Lee, J., and Isaacs, R. 2019. Exploring the efficacy and mechanisms of a crop sterilant for reducing infestation by spotted-wing drosophila (Diptera: Drosophilidae). J Econ. Entomol. DOI: 10.1093/jee/toz245

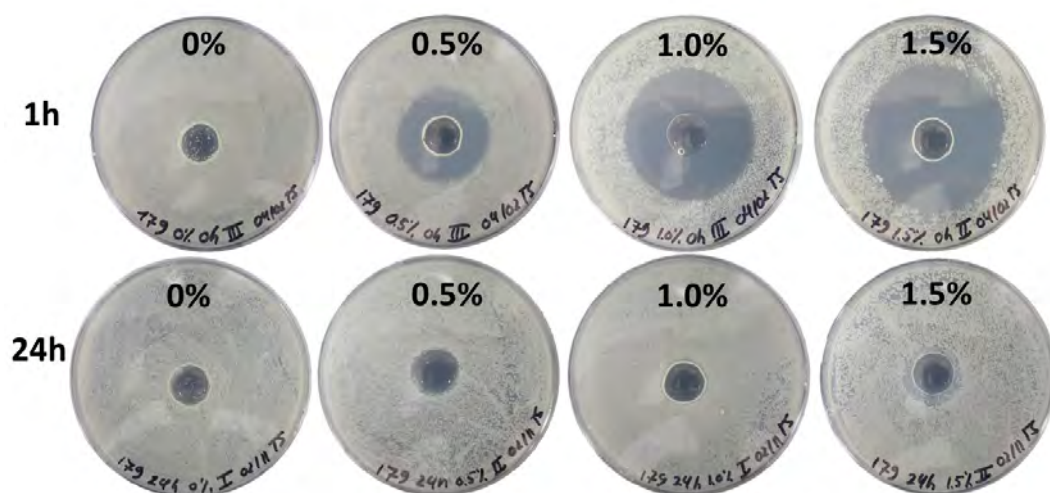


Figure 5: Growth inhibition of the yeast *Hanseniaspora uvarum* (strain 179) on artificial growth media amended with hydrogen peroxide and peroxyacetic acid (v/v) after 24h. Yeasts were given 1h or 24h to establish on the growth media.

$F_{(1,70)} = 1.28$; $P = 0.262$; $N = 2$

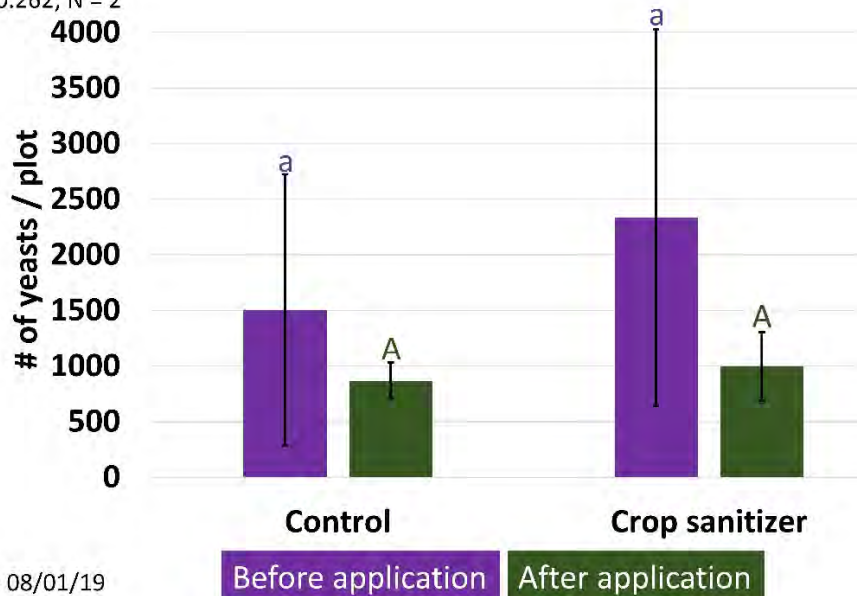


Figure 6: Number of yeasts before and 24h after application of a crop sanitizer containing hydrogen peroxide and peroxyacetic acid. Control plots were sprayed with water. Bars with the same letters do not show significant differences between treatments according to a Tukey test. Data were analyzed separately for each application time point.

HORTICULTURE

TREES/HA OR LEADERS/HA: WHICH IS MORE IMPORTANT?

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Future directions in orchard productivity strongly suggest that for commercial plantings some form of mechanization will be necessary. Work in PA with orchard platforms has shown that there is considerable savings to be realized with their use for pruning, thinning and potentially harvest. For any platform system to be successful, the tree architecture must have a thin mantle depth. The maximum depth of any canopy will be approximately 2.5 to 3 feet. An Axe/Tall Spindle type system and trellis system would seem to fit these requirements. The Axe system keeps a very narrow conical shape in the upper portions of the tree. While the trellis maintains a vertical even depth canopy the entire height of the tree. In our trellis the maximum width of the canopy at the end of the growing season is approximately 4.5 feet (2.25 ft. per side) which would provide for higher light penetration and improved fruit quality and color.

In the race to increase density to achieve instant orchards with the newest cultivar we have lost sight of the increasing costs and availability of finished trees. Robinson et al. (2013) showed that increasing tree costs restrict the profitability of an orchard system. Tustin (2014) suggested that we have overlooked canopy design in favor of increasing trees per acre when we should be thinking of *stems* per acre. The approaches of researchers to producing more “trunks per tree” in bi-axial training systems can help reduce establishment costs and increase profitability. The objective of this type of system is to determine if we can divide the vigor over more stems to reduce the establishment costs while still maintaining equal production.

One of the main reasons to begin this study was due to the high cost of orchard establishment. Well feathered trees cost more than trees that are sold as unbranched whips. In this case the TS trees cost \$9.73/tree versus \$6.59/tree for the unbranched whips. Trees with two leaders can also be planted at a lower density, but with two leaders a grower can have the same number of leaders as TS systems. The difference in the cost for the trees is shown in Table 1.

The study officially began in 2017, however, trees that were developed into the biaxial systems were planted and trained in 2016 in our nursery. In the spring of 2017, the biaxial trees having 2 leaders were transplanted to the study site. Biaxial trees were planted at two densities with their trunks spaced at either 3 ft (BiA3) or 6 ft (BiA6) in the row. New trees were purchased from the nursery and trained to a tall spindle system (TS) planted at 3 ft in the row. Rows were uniformly spaced at 13 ft. This resulted in different numbers of trees and leaders per acre as shown in Table 1. Based on the nursery price paid for the trees, the highest tree cost was for the TS followed by the BiA3 and the lowest was for the BiA6 (Table 1).

Yield per tree in 2018 was highest for the TS trees and lowest on the BiA3. However, by 2019 while the yield/tree was numerically still higher in the TS, there was no statistical difference by system (Table 2). Fruit weight in 2018 was lowest for the TS but in 2019 there was not difference between training systems.

On a land area basis, yield/A was significantly better for the TS trees in 2018 but there was no difference between either biaxial system (Table 3). In 2019 yield/A was greatest for the TS followed by the 3BiA and then 6BiA. Yield per leader in both 2018 and 2019 was significantly greater for the TS but there was no difference between the leader yield for the biaxial systems. Physiologically, yield efficiency and crop load was not influenced by any training-spacing system in either year (Table 4).

Summary: In the first 2 growing seasons the trees in the TS system have out-performed the biaxial system trees. This is most likely related to the initial size difference between the trees and because the growth of the biaxial trees was reduced due to the need to replant the trees from our nursery. The biaxial trees would have suffered from the loss of finer roots with the replanting. It does appear that the biaxial trees are overcoming their initial lower growth rate and should have similar size to the TS trees after next year. The need to train the biaxial trees a year in the nursery and to replant them is a decided disadvantage. It would appear in the first years after planting, that trees/ha is more of a factor than leaders/ha. Dormant pruning will need to be modified this coming season to restrict tree shoots extending out perpendicular to the tree row. This will be accomplished through the “click-method” of pruning the vigorous outward growing shoots.

References

Robinson, T., S. Hoying, M. Sazo. A. DeMarree & L. Dominguez. 2013. A vision for apple orchard systems of the future. NY Fruit Quart. 21(3):11-16.

Tustin, D. S. 2013. Future orchard planting systems: Do we need another revolution. Acta Horticulturae 1058:27 – 36.

Table 1. Difference in cost for trees based on tree price and spacing for Golden Delicious/M9 for three training systems					
System	Trees/ha	Stems/ha	Cost/tree	Cost/ha	% Change
Tall Spindle	2760	2760	\$9.73	\$26,855.84	
3' Biaxis	2760	5520	\$6.59	\$18,189.11	-32.3
6' Biaxis	1379	2760	\$6.59	\$9,086.41	-66.1

Table 2. Yield per tree and average fruit weight of Golden Delicious/M.9 in 2018 and 2019				
System	Average Yield/tree, kg		Average Fruit Weight, g	
	2018	2019	2018	2019
Tall Spindle	1.73 b	7.14 a	159 a	183 a
3' Biaxis	0.81 a	5.64 a	181 b	184 a
6' Biaxis	1.20 ab	5.85 a	178 b	184 a
P-Value	0.0035	0.0485	0.0030	0.9833

Letters refer to Tukey-Kramer mean separation, P = 0.05

Table 3. Yield of Golden Delicious/M.9 per hectare and per Leader in 2018 & 2019 at Rock Springs.				
System	Yield bu/ha		Yield/leader, kg	
	2018	2019	2018	2019

Tall Spindle	249 b	1,032 c	1.73 b	7.14 b
3' Biaxis	118 a	815 b	0.41 a	2.82 a
6' Biaxis	87 a	422 a	0.60 a	2.93 a
P-Value	0.0001	0.0001	0.0001	0.0001

Letters refer to Tukey-Kramer mean separation, P = 0.05

Table 4. Yield efficiency and crop load of Golden Delicious/M.9 under three training systems in 2018 and 2019

System	Efficiency, g/cm ²		Crop Load, #/cm ²	
	2018	2019	2018	2019
Tall Spindle	284 a	827 a	1.8 a	4.6 a
3' Biaxis	200 a	956 a	1.1 a	5.3 a
6' Biaxis	299 a	1013 a	1.7 a	5.5 a
P-Value	0.1361	0.3781	0.0750	0.5085

Letters refer to Tukey-Kramer mean separation, P = 0.05

Support for this project came from the State Horticultural Association of Pennsylvania Research Committee, and the USDA National Institute of Food and Federal Appropriations under Project PEN04625

Gibberellin₄₊₇ sprays to reduce skin cracking of Regal 10-45 and ‘GoldRush’ apples, 2019 PA Trials

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Objectives:

- Evaluate Novagib 5L formulation of GA₄₊₇ for managing russet and skin cracking incidence and severity of Regal 10-45 apples at 2 commercial orchards;
- Evaluate Novagib 5L formulation of GA₄₊₇ for managing russet and skin cracking incidence and severity of GoldRush apples at Penn State’s Fruit Research and Extension Center.
- Conduct a developmental study of russet and fruit cracking of Regal 10-45 and GoldRush.
 - When do symptoms, (russetting, fruit skin cracking) occur?
 - What is the effect of canopy position on these disorders?
 - Do symptoms develop more on exposed side or interior side of the fruit?

Procedures:

Novagib Trials:

We compared the early spray protocol (starting at PF), and late spray protocol (labelled ‘Stayman’ apple cracking timing) of Novagib 5L with an untreated control on Regal 10-45 at two commercial orchards and on GoldRush at FREC. The trees otherwise received fertilizers and crop protectant sprays according to local recommendations. Sprays were applied in 100 gallons per acre.

Treatments:

- 1) Early timing: Four sprays of 4 fluid oz Novagib 5L per acre, starting at PF, and at 10-day intervals. Each treatment was applied to ~1/4 acre of orchard at each commercial grower, and to buffered 5-tree plots at FREC.
- 2) Late timing: Six sprays of 12.8 fl. oz Novagib 5L per acre, starting late June, and at 3-week intervals. The trial at Boyers Orchards only received the first five sprays.

Design:

Uniform plots of ~1/4 acre in size will be selected in ‘Sweet Cheeks’ blocks with a history of fruit cracking. Five tree plots with two buffers between plots were assigned to “GoldRush / M. 9 trees at FREC. Each plot will receive treatments which will be flagged in a randomized complete block design, with three commercial orchard replications, and seven replications of GoldRush.

Sampling:

Fifty fruits per plot were non-selectively sampled at harvest. Fruit weight was measured, and mean fruit weight was calculated. Fruit length and diameter was measured and length: diameter ratio was calculated.

Fruit russet was evaluated using a visual rating scale : 1= No russet to a trace; 2= russet in stem bowl but not extending out to the shoulder and/or lenticel russet; 3= russet on ≤10% on shoulder or cheek; 4= russet on 11-20% of shoulder and cheeks; and 5= russet on ≥21% of shoulder and cheeks.

Skin cracking was evaluated using a visual rating scale of 1= none to a trace of skin cracking; 2= moderate number of concentric ring cracks on the shoulder or blossom end, or cracked lenticels on the cheek; 3= multiple cracks in exposed locations that diminish visual appeal, but no exposed tissue; 4= cracking apparent with one to three small cracks showing exposed cortical tissue (fruit flesh); and 5= multiple deep cracks with exposed tissue.

The remaining fruit from each commercial plot was harvested and segregated by treatment. All fruit from each treatment will be run separately over a commercial packing line at Hess Brothers Fruit Co. in Lancaster, PA, and the proportion of fruits downgraded for skin cracking will be documented.

Developmental Trial:

Five trees of Regal 10-45 and GoldRush were selected for study, and ribbon used to divide the canopy into inner / outer and upper / lower sections. In June and repeated in August, all persisting fruits in each sector were examined and rated for presence / absence of russet or fruit cracking (Figure 1). The Regal 10-45 trees were harvested by sector in October, and all fruits per sector were rated for fruit finish issues.

Results:

Fruit russet of Regal 10-45 apples was reduced by early sprays of Novagib 5L (Table 1). Fruit cracking scores, although not significant at a p-value of 0.05, were 20% lower at the Boyer commercial orchard site with a p-value of 0.155. Based on the 50-fruit samples, Novagib had no effect on fruit size or fruit shape.

The Boyers reported that the early protocol plots had 8 bins (176 bu.) of fruit harvested versus 7 (154 bu.) for the control plots and 7.25 for the late protocol plots. That is an increase of 419 kilos. The grower attributed this to larger, typier fruit, so it will be interesting to see the outcome of the commercial grading when it is done.

At harvest, untreated Regal 10-45 trees had no difference in cracking based on canopy position (Table 2). This suggests that the cracking is not a function of canopy microclimate.

Novagib had no effect on russet or cracking of GoldRush apples (Table 3). These results suggest that fruit cracking of these varieties is related to fruit maturity, like stem end cracking of Gala.

Table 1. Effect of Novagib 5L on fruit size, fruit shape, skin cracking and fruit russet of Regal 10-45 apples at two commercial orchards in Pennsylvania, 2019. Means are calculated from 150 fruit at commercial harvest.

SITE	TREATMENT	LENGTH	DIAM	L/D RATIO	WEIGHT	CRACKING SCORE	RUSSET SCORE	
BOYER	Control	351	378	0.93	10.11	2.31	2.95	bc
	Novagib early	366	381	0.96	10.53	1.87	2.53	c
	Novagib late	355	375	0.95	10.05	1.95	2.88	bc
RIDGE TOP	Control	360	376	0.96	10.31	1.62	3.37	a
	Novagib early	359	369	0.97	9.95	1.57	3.29	ab
	Novagib late	365	378	0.97	10.47	1.74	3.55	a
	p-value	0.740	0.667	0.305	0.910	0.155	0.006	

Table 2. Effect of canopy position on fruit size, fruit shape, skin cracking rating of Regal 10-45 apples at Penn State's Fruit Research and Extension Center, 2019.

TREATMENT	FRUIT NO.		FRUIT WT. (KG)		AVG WT (G)	CRACKING SCORE	RUSSET SCORE
LOW-INNER	32.6	ab	7.56	ab	233	2.65	3.23
LOW OUTER N	11.6	c	2.84	c	247	2.87	3.47
LOW OUTER S	15.2	bc	3.48	c	231	2.84	3.33

UPPER INNER	47.8	a	11.13	a	239	2.93	3.18
UPPER OUTER N	26.4	bc	6.25	bc	237	2.90	3.02
UPPER OUTER S	26.8	bc	5.84	bc	221	3.08	3.31
P-VALUE	0.005		0.003		0.308	0.846	0.346

Table 3. Effect of Novagib 5L on fruit size, fruit shape, skin cracking and fruit russet of GoldRush apples in Pennsylvania, 2019. Means are calculated from 350 fruit at commercial harvest.

<u>TREATMENT</u>	<u>WT</u> <u>(GRAMS)</u>	<u>LENGTH</u> <u>(CM)</u>	<u>DIAM</u> <u>(CM)</u>	<u>LENGTH/DIAM</u>	<u>RUSSET</u> <u>SCORE</u>	<u>CRACKING</u> <u>SCORE</u>
CONTROL	218	7.44	7.59	0.980	3.16	3.66
NOVAGIB EARLY	219	7.51	7.59	0.989	2.99	3.84
NOVAGIB LATE	227	7.53	7.68	0.980	3.02	3.51
P-VALUE	0.590	0.717	0.537	0.430	0.152	0.308

Effects of Rootstock and In-row Tree Spacing on Mineral Nutrition and Productivity of Peach Trees in Pennsylvania

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Penn State Fruit Research and Extension Center

In a previous study, Penn State researchers showed that peach trees trained to hex- or quad-V peach growing systems on a standard rootstock at moderate tree density produced higher yields of better-colored peaches than either low density or high-density systems. Tree spacing of 7 ft. x 18 ft., (quad V) or 10 ft. x 18 ft. (hex V) produced 65% and 53% more bushels than open vase trained trees at 14 ft. x 18 ft. These medium density V-systems also produced more large fruit per acre, with improved red fruit coloration. Although the study clearly showed the economic benefit of higher tree density, the tall height of trees in the V systems is a disincentive to many growers. Furthermore, with a standard vigor rootstock, the perpendicular V plots with the closest tree spacing, 5 ft. x 18 ft., was less productive than the medium density quad and hex V trees. One way to address both these issues is to plant trees on a dwarfing rootstock.

Development of one or more well-adapted dwarfing rootstocks is the key missing element to increasing tree density and controlling tree height in peach orchards. In addition to improving production and fruit quality, dwarfing rootstocks show great potential to increase labor efficiency of pruning, hand thinning and harvesting peaches. Reducing tree size and training the resulting smaller trees into narrow canopy sections would also ease the successful adoption of other labor-saving technology, such as blossom thinners and harvest platforms.

Maintaining optimal levels of the 16 essential mineral nutrients is necessary for crops to complete essential biological processes such as photosynthesis, and for production of large crops of quality fruits. Peach orchards are heavy consumers of nitrogen (N) and potassium (K), and require regular, usually annual, applications of N and K to be productive. Peach is sensitive to boron excess, and to zinc deficiency. While no less essential, deficiencies of other minerals such as phosphorus, calcium, and sulfur are rare in the orchard. Little is known of the extent to which increasing orchard productivity through higher tree density will affect the need for fertilizers. One can presume that as yield is increased, consumption of mineral nutrients will also increase, but no relevant data exists to make recommendations.

The primary goals of peach rootstock development are for tree size control, replant disease resistance, and tree hardiness. Apart from iron (Fe) chlorosis, caused by alkaline soils, data on rootstock effects on tree nutrition are lacking. Some of the peach-almond hybrids are reportedly better adapted to alkaline soils, showing that peach rootstock can affect nutrient uptake, at least for Fe.

The objectives of this project were to evaluate five commercially available peach rootstocks at three in-row tree spacings on leaf and soil mineral nutrient content, tree growth, and yield and fruit size of yellow-fleshed freestone peaches in Pennsylvania.

Procedures:

In 2014, a quad V peach rootstock planting was established at the Penn State Fruit Research and Extension Center to evaluate the performance of Coralstar peach on Bailey, Guardian, Krymsk 86, KV 10123, and Empyrean II rootstocks. Coralstar is mid-season, yellow fleshed freestone peach with high fruit quality. Rootstocks were selected based upon past performance (Bailey), replant tolerance (Guardian), cold hardiness (Krymsk 86), tree size control / productivity (KV 10123 and Empyrean II), and availability from commercial nurseries.

Five-tree row sections of each rootstock were planted at in-row spacing of 5, 7.5, or 10 ft. The range of in-row spacing was selected based on prior research results and allowed comparison of each rootstock at spacing ranging from close, moderate and wide. All rows were spaced 16 ft. apart to maximize light interception of trees maintained to 9-10 ft. maximum height. All trees received dormant / summer pruning, mechanized blossom thinning, hand fruit thinning, fertilizer, and pest management according to standard commercial practices.

All fruit were harvested from the two center trees of each plot in three or more pickings to collect harvest data at proper harvest maturity. Whole-tree yield and fruit size data were obtained with a Durand-Wayland electronic weight sizer. Tree size was measured when seasonal growth was complete.

Soil and leaf samples were collected 100-125 days after full bloom from each plot and sent to the Penn State Analytical lab for analysis of soil pH and leaf and soil mineral nutrient concentrations. Mineral nutrient treatment effects were compared for thresholds using established optimal ranges.

Results and Discussion:

Trees on K86 rootstock were largest and similar in size to Guardian and Penta (Table 1). KV123 trees were 23% smaller than K86. Of the rootstocks in trial, Bailey was the most dwarf; 34% smaller than trees on K86. In-row tree spacing exerted a greater effect on tree size than selected rootstocks. Trees at 7.5 foot in-row spacing were 31% smaller than those at 10 ft., while those at 5 ft between trees were 45% smaller than those at 10 ft. There were no interactions between rootstock and spacing, meaning that rootstock effects were proportional at each spacing. This outcome helps to simplify grower decision-making criteria for selection of rootstock / spacing combinations in future commercial plantings.

Yield differences in peach production systems are often the result from changes in the amount of bearing surface. Reducing in-row spacing increased the number of scaffolds per acre (Table 2). Comparing the effect of tree spacing in the crop bearing years, 2016-2018, closer tree spacing hastened development of bearing surface per acre and increased yields. Cumulative yield of trees at 7.5 ft. and 5ft. was 125% and 150% that of trees planted at 10 ft. between trees. Yields of the trees were low in 2018, due to hail, and differences between treatments were muted in 2018. Even so, trees at 5 or 7.5 ft between trees had 21-22% more yield than those at 10 ft.

Among rootstocks, Bailey was the most precocious (2016-2017), however yield of K86 trees surpassed Bailey in 2018. This is not surprising, as the bearing surface of trees on K86 was half again as big as Bailey. Trees on Penta had the least yield.

Rootstock had no effect on cumulative fruit size from 2016-2019 (Table 3). Reducing in-row spacing reduced average fruit diameter by about a tenth of an inch per 2.5-foot increment of in-row spacing. Fruit size was 6% smaller in 5 ft spacing than 10 ft. This may be attributed to the increased competition between trees at the closer spacing.

Levels of leaf mineral nutrients were all in the sufficient range throughout 2014-2019, except Zn was low in 2019. Leaf K, Ca and Mg were on high side, and this corresponds to high Mg and K saturation in soil samples. Leaf Zn levels were trending towards low, so foliar Zn was applied at the end of the growing season in 2018. K86 and Penta had higher concentrations of leaf K (Figure 1). Fruits have significant K concentration, so soil K usually declines with removal by heavy crops, but in this study, the most productive and least productive rootstocks were both higher in K (Figure 1). Penta had higher mineral nutrient levels for several elements, suggesting that the low productivity of this rootstock in the present study was not linked to mineral nutrient uptake. In-row spacing had no effect on leaf mineral nutrient levels in 2014-2019.

Conclusions:

Quad V peach trees at moderate tree densities of 272-544 trees per acre produced high yields in this study. A good grower with open vase trees at low planting density can expect to get 350 bushels per acre, while the statewide average peach yield for PA (2007-2019) was 183 bushels per acre (Jay Harper, personal communication). These yields contrast sharply with the 740 bushels per acre achieved by the quad V at 7.5 ft. x 16 ft. Our results show that quad V trees at 7.5' x 16' (363 trees per acre) with trickle irrigation during final swell, are capable of high sustained production of marketable fruit. The size reduction was only 3% at 7.5 in-row spacing, and peak sizes were still very marketable. Except for small-fruited varieties, fruit that are 3 % smaller may be a good trade-off for 25% greater yield.

For growers seeking a productive rootstock with tree size control, Bailey has been the best rootstock in this trial. K86 would be a good choice for growers seeking to maximize production on full sized trees. Penta was the least productive and is not recommended.

Mineral nutrition differences were small compared to the treatment differences in tree vigor and yield. Despite the heavy yields borne by these trees, mineral nutrients were not a limiting factor in this trial. On good orchard

sites and soils in the Mid-Atlantic, mineral nutrition requires no fine tuning when peach tree density is increased, beyond correcting any limiting nutrients based upon pre-plant soil testing, and regularly scheduled soil and leaf analyses.

Irrigation is a must at higher density, although it is required only required during final swell. Pruning of quad V is simple and quantifiable. Trellising helped with orchard mechanization. The mechanized string thinner does most of the fruit thinning at bloom. The limbs and fruit are very accessible, making the trellised quad V very compatible with labor platforms for hanging mating disruption lures, hand thinning, summer pruning and harvest. The 8 gauge black plastic wire worked, but too easy to cut / too stretchy. In new plantings we have installed 5 mm Agliner Fruitine, which is much less stretchy and is cut resistant.

Quad V production systems dramatically increase production efficiency and thus provide a means to maximize profits from peach growing enterprises.

Acknowledgements:

We thank the Pennsylvania Peach and Nectarine Program and the State Horticultural Association of Pennsylvania for support of the research.

Table 1. Effect of rootstock in-row tree spacing on tree size of ‘Coralstar’ peach trees in PA, 2019.

<u>Rootstock</u>	<u>Size (% largest)</u>	<u>In-row Spacing</u>	<u>Size (% of largest)</u>
K86	100 a	10 ft.	100 a
Guardian	90 ab	7.5 ft.	69 b
Penta	86 ab	5 ft.	55 c
KV123	77 bc		
Bailey	66 c		

Table 2. Effect of rootstock and in-row tree spacing on yield of ‘Coralstar’ peach trees in PA, 2019.

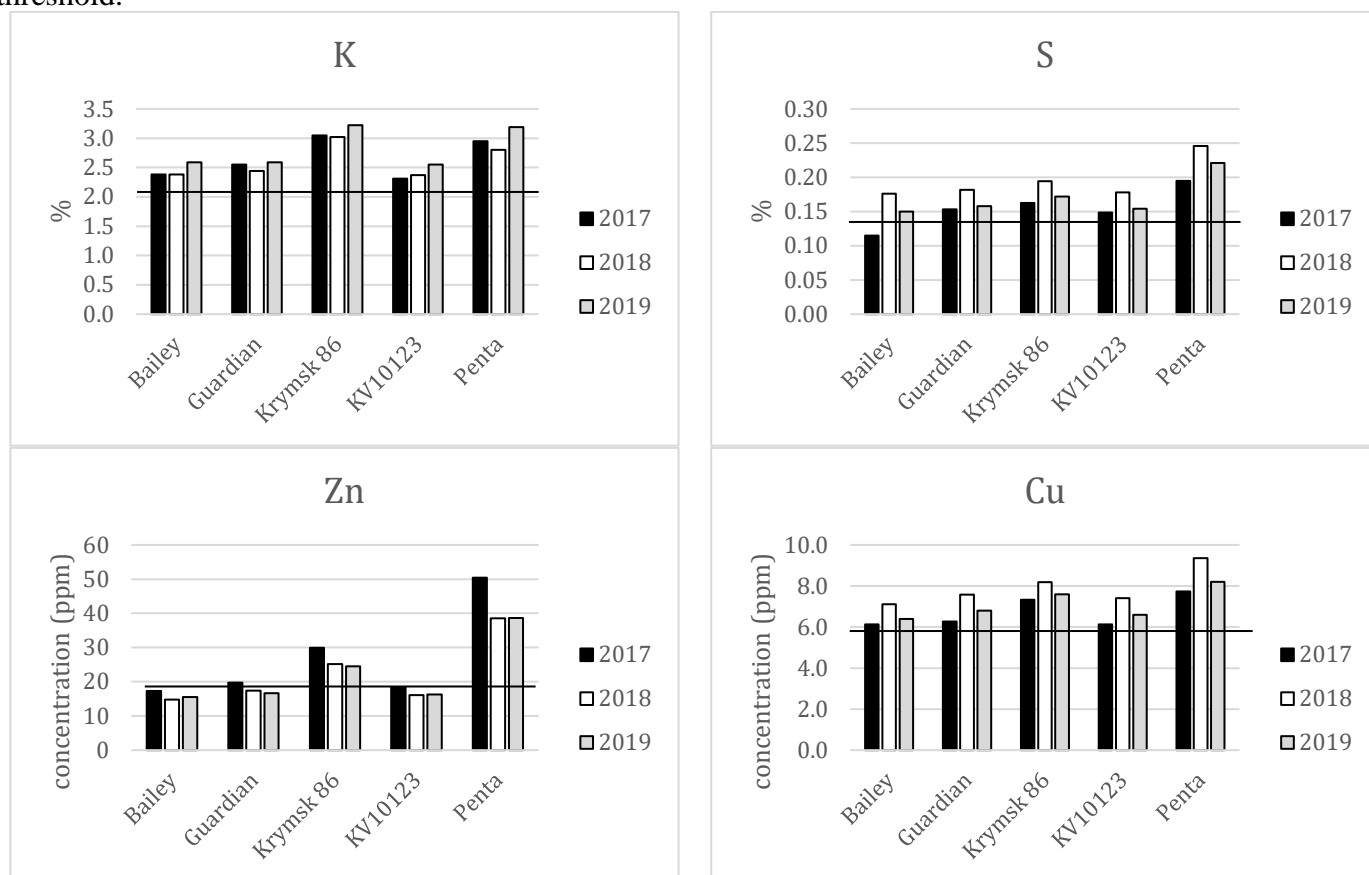
In-row space (ft.)	Trees	Scaffolds	2016	2017	2018	2019	Cum.
	----- # per acre -----		----- bushels / acre -----				
	-						
5	545	2178	196 a	539 a	108	829 a	1676 a
7.5	363	1452	134 b	408 b	110	740 a	1395 b
10	272	1088	107 b	345 c	92	572 b	1119 c
Rootstock	2016	2017	2018	2019	Cum.		
	----- bu / acre -----						
Bailey	222 a	444 a	96 b	762 a	1527 a		
Guardian	198 ab	418 ab	81 b	727 a	1427 a		
KV123	167 b	438 ab	71 b	747 a	1426 a		
K86	90 c	493 a	157 a	778 a	1522 a		
Penta	53 c	359 b	112 b	555 b	1081 b		

Table 3. Effect of In-row spacing and rootstock on fruit size of Coralstar peaches in PA (2016-2019).

<u>Rootstock</u>	<u>Avg fruit diameter (in.)</u>	<u>In-row Spacing</u>	<u>Avg fruit diameter (in.)</u>
Bailey	3.0	10 ft.	3.0 a
Guardian	2.9	7.5 ft.	2.9 b

KV123	2.9	5 ft.	2.8 c
K86	2.9		
Penta	2.9		

Figure 1. Effect of rootstock on selected leaf mineral nutrients, 2017-2018. Horizontal line shows sufficiency threshold.



PLANT PATHOLOGY

INVESTIGATING SOURCES FOR POSTHARVEST APPLE ROT FUNGI IN THE FIELD AND PACKHOUSE: CONCEPTUAL FRAMEWORK AND PRELIMINARY RESULTS

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Postharvest rots cause significant economic losses on apple production. According to Jurick and Cox (2017), between 1% and 15% of apples in the United States are lost each year as a consequence of postharvest rots, which translates into approximately 52,000-780,000 tonnes and up to \$2,500 million, (estimation based on data from FAOSTAT 2019 and the USDA National Retail Report, June 2019). Previous research in different countries has demonstrated that *Penicillium expansum*, *Botrytis cinerea*, and *Colletotrichum* spp. are the most important postharvest pathogens causing apple rots (Errampalli 2014, Rosenberger and Cox 2016, Chechi *et al.* 2019). Other species, including *Sphaeropsis pyriputrescens*, *Lambertella corni-marisi*, *Neofabrea* spp. *Alternaria alternata*, and *Mucor piriformis* are also important in specific regions of the United States, such as Washington State (Jurick and Cox 2017, Nekoduka *et al.* 2018, Bui *et al.* 2019). The management of these postharvest pathogens has been focused on using synthetic fungicides, which has the associated problem of fungicide resistance, already reported in Pennsylvania for *Penicillium expansum* and *Botrytis cinerea* (Jurick II *et al.* 2017, Yan *et al.* 2014 (II)).

Different studies since 1976 have reported multiple inoculum sources of apple postharvest pathogens. The most important are the apple fruits, bins, dump tank water, wax and fungicide drenches, and the air of packinghouse facilities and cold rooms, where some apple varieties are stored for up to eleven months (Bertrand and Sulie-Carter 1976, Sanderson and Spotts 1995, Watkins and Rosenberger 2001, Scholtz and Korsten 2016). In Pennsylvania and in most of the apple producing countries, there is limited information in regard to the identification and fungicide resistance profile of the most important apple postharvest pathogens in relation to the source of inoculum. By identifying not only the sources, but also the location of fungicide resistant isolates, targeted management strategies can be proposed to complement or eliminate the use of synthetic fungicides, which is a priority in the commercialization of apples (Ambaw *et al.* 2017). The present study was performed to determine the location of postharvest apple rot pathogens and investigate the fungicide resistance profile of *Penicillium* spp. in relation to the source of inoculum.

Materials and methods

Three orchards and packinghouses from southcentral Pennsylvania were sampled during Fall 2019. Fungal colonies were isolated from the surface of freshly harvested apples without postharvest processing (NP), processed apples (P) with less than one month in cold storage, picking bags, internal surface of field bins, and from the air of packinghouse facilities and cold rooms. Petri plates containing Potato dextrose agar (PDA) or Richards defined medium, with or without fungicide, were used to isolate the fungi from each inoculum source.

The culture medium and fungicides were used according to the following specifications, based on literature and using *Penicillium expansum* as a model (Yan *et al.* 2014 (I), Yan *et al.* 2014 (II), Amiri *et al.* 2017; Jurick II *et al.* 2019):

Culture medium	Fungicide active ingredient	FRAC code	Concentration (ppm)
PDA	Thiabendazole	1	5
PDA	Fludioxonil	12	0.5

The fungal colonies on the surface of apples were isolated by placing the fruits in sterilized water, performing serial dilutions and distributing part of the suspension on Petri plates containing the culture media, with or without fungicide. Sterile cotton swabs were used to sample the surface of picking bags and field bins. Each cotton swab was placed in sterile water, and after agitation, 100 µl of the suspension were placed and distributed on the surface of Petri plates with culture media. For the air sampling, the packinghouse facilities were divided into “wet area,” including the space between the water dump tank and waxing, and “dry area” comprising the packing line. The air of the packhouse and cold rooms was sampled by exposing the Petri plates to the air of those environments for 15 minutes. All plates were incubated at 22 °C, and after 3 days, the number of colony- forming units (CFU) of fungi was counted and the frequency of *Penicillium* spp. was determined. Pure cultures of the most frequent microorganisms were obtained for future studies and species identification.

Results

Fungal growth was observed on most of the plates with or without fungicide, and for most samples, a higher number of colony-forming units on potato dextrose agar (PDA) was observed when compared to the fungicide amended medium (Tables 1 to 3). In general, thiabendazole was the fungicide with the highest fungal load. *Cladosporium* spp. and *Penicillium* spp. were identified as the predominant genera contributing to the fungal load, independently of the inoculum source sampled. Although *Cladosporium* has been reported as a common organism on apple fruits, especially in orchards (Sholber and Haag 1996, Teixidó *et al.* 1999), it is not considered a significant pathogen causing postharvest rots. *Botrytis cinerea* and *Colletotrichum* spp., which are frequent apple postharvest pathogens (Rosenberger and Cox 2016, Chechi *et al.* 2019), were not isolated from any of the field or postharvest sources studied.

Table 1. Average colony forming units of fungi per ml of suspension of samples from the internal surface of field bins and picking bags.

Packhouse	Source	Colony forming units (CFU)/ml				
		PDA	Thiabendazole	Fludioxonil	Pyrimethanil	Difenoconazole
A	Bins	71	87	23	91	1
B		68	19	12.2	16	0.9
C		108	27	37	38	8
A	Picking bags	13	39	0	0	5
B		68	70	15	20	12.7
C		47	1	3	11	1

Table 2. Average colony forming units per plate in air samples from packinghouse facilities and cold rooms.

Colony forming units (CFU)/ plate

Packhouse	Source	PDA	Thiabendazole	Fludioxonil	Pyrimethanil	Difenoconazole
A	Wet area	14	8.2	1.2	1.4	1
B		33	7.8	2.4	7.4	3.8
C		4.6	1	0	0.8	0.2
A	Dry area	12	6	1.8	2.4	0.8
B		48.2	23.2	3.8	7.2	5.2
C		9	1.8	0.4	1.8	1.4
A	Cold room	2.1	1.9	0.3	0.3	0
B		15	7.7	1.2	9.9	0.1
C		5	2.7	0.3	2	0.5

Table 3. Average colony forming units per ml of suspension of surface samples from non-processed (NP) or processed (P) apples.

Packhouse	Source	Colony forming units (CFU)/ml				
		PDA	Thiabendazole	Fludioxonil	Pyrimethanil	Difenoconazole
A	NP fruit	20	8	0	14	4
B		556	20	0	60	20
C		484	180	118	16	190
A	P fruit	28	4	0	20	0
B		30	22	0	0	12
C		24	2	0	0	0

Tables 4 and 5 show the frequency of *Penicillium* spp. on each inoculum source sampled. The fungus was frequently isolated in the air of the packinghouse facilities and cold rooms, but not on the fruit, and at a low frequency on bins and picking bags. Fungicide resistant isolates were obtained for thiabendazole, fludioxonil, and difenoconazole, ranging between 20% and 80% frequency in the air of the wet and dry areas of the packhouse, and between 10% and 90% in the air of cold rooms. Pyrimethanil was the only fungicide with no resistant isolates. This could indicate that the packinghouse facilities and cold rooms are critical points for apple postharvest rots management. However, future research needs to determine if fungicide resistant isolates coming from the packinghouse facilities are the same organisms causing rots on stored apples.

Table 4. Frequency of *Penicillium* spp. on surface samples of bins and picking bags.

Packhouse	Source	<i>Penicillium</i> spp. frequency (%)				
		PDA	Thiabendazole	Fludioxonil	Pyrimethanil	Difenoconazole
A	Bins	0	10	0	0	0
B		20	0	0	0	0
C		10	0	0	0	0
A	Picking bags	0	0	0	0	0
B		9	0	0	0	0
C		0	0	0	0	0

Table 5. Frequency of *Penicillium* spp. on air samples of packinghouse facilities and cold rooms.

Packhouse	Source	<i>Penicillium</i> spp. frequency (%)				
		PDA	Thiabendazole	Fludioxonil	Pyrimethanil	Difenoconazole
A	Wet area	60	0	0	0	0
B		60	60	20	0	0
C		100	60	20	0	20
A	Dry area	100	80	20	0	20
B		100	60	40	0	60
C		100	0	0	0	60
A	Cold room	0	0	0	0	0
B		90	70	20	0	0
C		30	10	0	0	10

Conclusions and future work

The air of the packhouse facilities and cold rooms is the main source of *Penicillium* spp. spores, which include fungicide resistant and non-resistant isolates. This evidence supports the hypothesis that there is a difference in the fungicide resistance profile of *Penicillium* spp. in relation to the source of inoculum. However, more samples including additional packhouses during the 2020-2021 season are needed to confirm these results. Future work will be also focused on using DNA extraction, PCR amplification, and DNA sequencing to determine the most frequent *Penicillium* species obtained from different sources and study the fungicide resistance profile of *Penicillium* isolated from symptomatic apples after cold storage.

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Literature cited

- Ambaw, A., Dekeyser, D., Vanwalleghem, T., Van Hemelrijck, W., Nuyttens, D., Delele, M.A., Ramon, H., Nicolai, B., Bylemans, D., Opara, U.L., Verboven, P. 2017. Experimental and numerical analysis of the spray application on apple fruit in a bin for postharvest treatments. *Journal of Food Engineering* 202: 34-45.
- Amiri, A., Mulvaney, K.A., Pandit, L.K. 2017. First report of *Penicillium expansum* isolates with low levels of resistance to fludioxonil from commercial apple packinghouses in Washington State. *Plant Disease* 101(5): 835.
- Bertrand, P., Sulie-Carter, J. 1976. Mucor rot of pear and apples. Special report 568. Agricultural Experiment Station Oregon State University, Corvallis.
- Bui, T.T., Wright, S.A., Falk, A.B., Vanswalleghem, T., Hemelrijck, W.V., Hertog, M.L., Keulemans, J., Davey, M.W. 2019. *Botrytis cinerea* differentially induces post-harvest antioxidant responses in 'Braeburn' and 'Golden Delicious' apple fruit. *Journal of the Science of Food and Agriculture* 99: 5662-5670.
- Cechi, A., Stahlecker, J., Dowling, M.E., Schnabel, G. 2019. Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples. *Pesticide Biochemistry and Physiology* 158: 18-24.
- Errampalli, D. 2014. *Penicillium expansum* (blue mold). In: Bautista-Baños, S. Postharvest decay. Academic Press, Elsevier. CA, USA. 394 p.
- FAOSTAT. 2019. Food and agriculture data. On-line: <http://www.fao.org/faostat/en/#data>

- Jurick II, W.M., Cox, K.D. 2017. Pre- and postharvest fungal apple diseases. Pp:371-382. In: Evans, K. Achieving sustainable cultivation of apples. Burleigh Dodds Science Publishing, Philadelphia, PA. 591 p.
- Jurick II, W.M., Macarasin, O., Gaskins, V.L., Park, E., Yu, J., Janisiewicz, W., Peter, K.A. 2017. Characterization of postharvest fungicide-resistant *Botrytis cinerea* isolates from commercially stored apple fruit. *Phytopathology* 107(3): 362-368.
- Jurick II, W.M., Macarasin, O., Gaskins, V., Janisiewicz, W.J., Peter, K., Cox, K. 2019. Baseline sensitivity of *Penicillium* spp. to difenoconazole. *Plant Disease* 103(2): 331-337.
- Nekoduka, S., Tanaka, K., Sano, T. 2018. Epidemiology of Apple bitter rot caused by *Colletotrichum acutatum sensu lato*. *Journal of General Plant Pathology* 84: 262-271.
- Rosenberger, D., Cox, K. 2016. Preventing bitter rot in apples. *Scaffolds Fruit Journal* 25(22): 1-4.
- Sanderson, P.G., Spotts, R.A. 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium*. *Phytopathology* 85: 103-110.
- Scholtz, I., Korsten, L. 2016. Profile of *Penicillium* species in the pear supply chain. *Plant Pathology* 65: 1126-1132.
- Sholberg, P.L., Haag, P.D. 1996. Incidence of postharvest pathogens of stores apples in British Columbia. *Canadian Journal of Plant Pathology* 18(1): 81-85.
- Teixidó, N., Usall, J., Magan, N., Viñas, I. 1999. Microbial population dynamics on Golden Delicious apples from bud to harvest and effect of fungicide applications. *Annals of Applied Biology* 134: 109-116.
- USDA. 2019. National Retail Report- Specialty Crops, June 2019. On-line: <https://usda.library.cornell.edu/concern/publications/vq27zn46v?locale=en&page=3#release-items>
- Watkins, C.B., Rosenberger, D.A. 2000. Determining inoculum sources for postharvest decays. *Cornell Fruit Handling and Storage Newsletter*. Pp 6-8.
- Yan, H.J., Jurick II, W.M., Lou, Y.G., Gaskins, V.L. 2014 (I). First report of Pyrimethanil resistance in *Botrytis cinerea* from stored apples in Pennsylvania. *Plant Disease* 98(7): 999.
- Yan, H.J., Gaskins, V.L., Vico, I., Luo, W.J., Jurick II, W.M. 2014 (II). First report of *Penicillium expansum* isolates resistant to Pyrimethanil from stored apple fruit in Pennsylvania 98(7): 1004.

APPLE (*Malus domestica* 'Idared')
 Rots; *unspecified*
 Fruit finish

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Evaluation of pH effect on Captan fungicide mixtures for rot control on Idared apples, 2019.

The purpose of this test was to evaluate whether lowering the pH of Captan tank mixes would improve their effectiveness for summer disease suppression. The pH of Captan mixtures was adjusted to the indicated pH with citric acid and was compared to industry-standard Captan and Captan + Phostrol mixtures. Target pH of the adjusted treatments was to be pH 7, pH 6 and pH 5, but was somewhat higher in the spray tank than had been predetermined by lab testing. Final mean pH just before application was: #1, pH 7.16 (range for five applications, 7.07-7.21); #2, pH 7.00 (range, 6.96-7.05); #3, pH 6.95 (range, 6.93-6.95); #4, pH 7.09 (range, 7.07-7.17); #5, pH 6.36 (range, 6.28-6.54); #6, pH 5.64 (range, 5.16-6.65); #7, pH 5.69 (range, 5.51-5.96). The water source had a mean pH of 7.51 (range, 7.40-7.58) throughout the course of this study. The test was conducted on 33-yr-old Idared/MM.111 trees in a randomized block design with four single-tree replicates. Test treatments were applied to both sides of the tree on each application date with a Swanson Model DA-400 airblast sprayer at 100 gal/A as follows: 12 Jun, 26 Jun, 10 Jul, 30 Jul, and 30 Aug. Prior to initiation of the treatment series, early season fungicides applied to the entire test block with the same equipment included: Apr 17: Rhyme 6 fl oz/A + Manzate 3 lb/A (17 Apr and 1 May) and Merivon 5.5 fl oz + Manzate 3 lb/A (24 Apr and 9 May). Fruit counts represent means of 25-fruit samples picked from each of four single-tree replications, picked 12 Sep and 26 Sep. Each sample was rated at harvest and after one and two weeks' incubation (mean 77-78°F). Percentage data were converted by the square root arcsin transformation for statistical analysis with Tukey's HSD test, $\alpha = 0.05$.

Rainfall during the 12 Jun-26 Sep test period was 8.92 in. Rot pressure was heavy and the assessed rots were mostly bitter rot. However, sooty blotch and flyspeck development was limited by the lowest summer wetting hour accumulation in 25 years. Although the Captan + Citric Acid treatments (#4-6) showed lower mean fruit rot compared to Captan with and without Phostrol (#1 and #3), lowering the pH of Captan did not significantly reduce rot incidence ($\alpha = 0.05$). Percent infection on Citric Acid (#7) alone and Phostrol (#2) were not significantly different from non-treated fruit. Surprisingly, Captan + Phostrol (#3) had significantly more rot than Captan alone (#1) in fruit from the second sampling, 26 Sep. There was no significant difference ($\alpha = 0.05$) in fruit finish among any of the treatments compared to non-treated trees.

Rate per 100 gal per acre (mean pH prior to application)	Rot incidence (%) at harvest or after indicated incubation interval*					
	Sampled 12 Sep			Sampled 26 Sep		
	harvest	1 wk	2 wk	harves t	1 wk	2 wk
0 Non- treated control	100 c	100 c	100 c	97 c	99 c	99 c
1 Captan 4L 1 gal (pH 7.16)	29 a	43 a	43 a	36 a	40 a	45 a
2 Phostrol 4.17SL 2 qt (pH 7.00)	81 bc	87 bc	87 bc	94 c	94 c	94 bc
3 Captan 4L 1 gal + Phostrol 2 qt (pH 6.95)	45 ab	51 ab	53 ab	61 b	65 b	71 b
4 Captan 4L 1 gal + Citric acid 0.176 oz (pH 7.09)	a 19	a 19	a 24	a 22	a 31 a	a 34
5 Captan 4L 1 gal + Citric acid 2.464 oz (pH 6.36)	a 15	a 25	a 29	a 21	a 22 a	a 23

6 Captan 4L 1 gal + Citric acid 4.944 oz (pH 5.64)	a 18	a 26	a 31	a 21	a 25 a	a 29
7 Citric acid 4.944 oz (pH 5.69)	85 c	90 bc	90 bc	96 c	97 c	97 c

* Mean separation by Tukey's HSD test, $\alpha = 0.05$. Averages of four single-tree replications.

THE DETECTION RATE OF *BOTRYOSPHAERIACEAE* SPP. IS SIGNIFICANTLY LOWER IN CERTIFIED GRAFTED GRAPEVINE MATERIALS

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Grapevine trunk disease caused by fungi in the *Botryosphaeriaceae* family is a growing concern in the wine industry and major cause of grapevine decline. As of 2018, there are at least 26 species in the *Botryosphaeriaceae* known to infect the xylem tissues of grapevines, causing a disease commonly referred to as *Botryosphaeria* canker. Typical symptoms of this disease on perennial wood include brown streaking of grapevine wood underneath the bark and necrotic tissue visible in trunk cross-sections, and can considerably shorten the life of the vineyard.

Because it often takes several years after inoculation for *Botryosphaeria* canker to show symptoms in grapevines, it can be hard to determine exactly where the inoculum originated from and how it was introduced. Due to the exposure of vascular tissues to the environment, pruning is considered a major route of exposure for this disease. More importantly, nursery propagation process contains an inherent risk of trunk diseases due to the wounds inflicted on the vines and the storage of large numbers of vine cuttings in close proximity to one another. This report is an attempt to determine the level of *Botryosphaeriaceae*-infected nursery vines, and also to determine the factors influencing the incidence, i.e., cultivar, nursery, location on the vine, rootstock cultivar, and certification status of scion wood and rootstock. To do this, samples of nursery vine scion trunk wood, graft union wood, rootstock trunk wood, and rootstock root wood were obtained from 5 commercial nurseries in California and New York. DNA was extracted from each sample and a nested PCR protocol was used to selectively amplify DNA from *Botryosphaeriaceae* fungi.

Of 1,216 samples, 161 (13.24%) tested positive for grape-pathogenic *Botryosphaeriaceae*. From the selected 18 positive samples, 14 samples were grapevine-pathogenic species of the family of *Botryosphaeriaceae*. Two samples were *Botryosphaeriaceae* fungal species that are not considered to be a pathogen of *Botryosphaeria* canker. The two other samples were too poor to be precisely identified, but most likely one of *Neofusicoccum* species in *Botryosphaeriaceae*. Therefore, each of them was found to be classified with the *Botryosphaeriaceae* family. The incidence of *Botryosphaeriaceae* was significantly different in the certification status of scion wood ($P < 0.01$) and nursery ($P = 0.047$), but no significant difference in other factors (cultivar, location on the vine, and rootstock clone). Certified scion wood had significantly lower probability of positive vines. One nursery in CA resulted in significantly lower probability of positive vines than two NY vineyards.

To our knowledge, this is the first study to identify the certification status to be a significant factor, since the certification status was not investigated in previous studies. It is important to note that our certified samples have passed the previous criteria, which relied on a visual-based biological test using indicator plants, compared to the ongoing Protocol 2010 which adapts molecular tests (PCR) for pathogen detection. In other words, even fundamental tests for preventing viruses can aid in reducing the risk of infection by *Botryosphaeriaceae* species. The influence of nursery on the vine had similar numerical trends with previous studies, e.g., the incidence of *Botryosphaeria* canker differed among nurseries.

Acknowledgements

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IDENTIFICATION AND RESISTANCE PROFILING OF *COLLETOTRICHUM* SPP. ISOLATES FROM STRAWBERRY FIELDS IN THE MID-ATLANTIC

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Introduction

Strawberry anthracnose, caused by *Colletotrichum* species, is a major disease in strawberry fields. All parts of the plants, including crowns, leaves, petioles and runners, are susceptible to the pathogen. *Colletotrichum* spp. including *C. gloeosporioides* and *C. acutatum* are known to be responsible for strawberry crown rot (ACR) and fruit rot (AFR), therefore, *C. gloeosporioides* and *C. acutatum* are two species complexes. QoI fungicides (FRAC 11) are considered the primary fungicides for strawberry anthracnose control. However, resistance to FRAC 11 has been reported in the Southeastern USA and other countries. Information is lacking regarding species diversity and fungicides sensitivity in *Colletotrichum* isolates in the Mid-Atlantic strawberry fields.

Materials and Method:

1. Species Identification

A total of 200 isolates were collected from Maryland, Virginia, Pennsylvania and North Carolina. DNA was extracted from all isolates. DNA was proved to be of good quality by amplifying internal transcribed spacer (ITS) region and gel electrophoresis. Primers ITS1 and ITS4 were used to amplify ITS region and then followed by Sanger sequencing to distinguish species at the complex level. TUB2 and GAPDH gene were amplified to further identify species within each complex.

2. Fungicide Sensitivity Screening and Resistance Mechanism

QoI fungicides are commonly used to control anthracnose in strawberry fields. *C. acutatum* has been reported to be inherently resistant to MBC fungicides. Therefore, all *Colletotrichum* isolates were tested with QoI fungicides (tradename: Abound, a.i. azoxystrobin), and only *C. gloeosporioides* isolates were tested for resistance to MBC fungicides (FRAC 1, tradename: Topsin M, a.i. thiophanate-methyl). Based on previous publications, FRAC 1 or FRAC 11 fungicide at concentration of 100 µg/ml was used to distinguish resistant and sensitive phenotype. Each isolate was cultured on potato dextrose agar (PDA) plates and 4 agar plugs containing mycelia were removed from PDA with a sterile cork borer. 2 plugs were then placed on unamended PDA plate as control, and the other 2 plugs were placed on fungicide amended PDA plates. After 3 days of incubation at 25°C, colony diameter was measured to calculate the inhibition rate. Sensitive, moderately resistant, and resistant phenotypes were categorized for each isolates based on the inhibition rate of 100, 40-100, and 0-40% respectively. The experiment was conducted twice independently. Additionally, *cytb* gene was sequenced from isolates with different resistant phenotypes.

Results:

Four *Colletotrichum* species including *C. nymphaeae*, *C. fioriniae*, *C. siamense*, *C. lineola* were identified. Among them, *C. nymphaeae* and *C. fioriniae* are within *C. acutatum* complex, whereas *C. siamense* is within *C. gloeosporioides*. *C. nymphaeae* is the dominant species, which makes up 95.7% of the *C. acutatum* complex isolates (Table 1). The overall resistance frequency to QoI fungicide is 41.3% (Table 2), and 60% of the *C.*

siamense isolates were shown resistance to thiophanate-methyl (Table 3). Moreover, all *C. fioriniae* isolates were moderately resistant to QoI fungicide.

Cytb was amplified and sequenced from 3 resistant and 2 moderately resistant *nymphaeae* isolates. As a result, G143A mutation was found in the resistant isolates, but was not detected in the moderately resistant isolates. All 8 *C. fioriniae* isolates of moderately resistance were sequenced but no mutation was detected. No introns in both *C. nymphaeae* and *C. fioriniae* isolates tested (data not shown).

Table 1: Number of *Colletotrichum* spp. isolates from different states.

State	<i>C. acutatum</i>		<i>C. gloeosporiodes</i>	<i>C. lineola</i>	Total	Table 2. Number of <i>C. acutatum</i> and <i>C. gloeosporiodes</i> isolates resistant, moderately resistant or sensitive to azoxystrobin.
	<i>C. nymphaeae</i>	<i>C. fioriniae</i>	<i>C. siamense</i>			
Maryland	121	6	2	0	129	
Pennsylvania	36	2	3	1	42	
Virginia	10	0	6	0	16	
North Carolina	13	0	0	0	13	
Total	180	8	11	1	200	

gloeosporiodes isolates resistant, moderately resistant or sensitive to azoxystrobin.

Description	<i>C. acutatum</i>		<i>C. gloeosporiodes</i>	Total	Table 3. Number of <i>C. gloeosporiodes</i> isolates resistant, moderately resistant or sensitive to thiophanate-methyl.
	<i>C. nymphaeae</i>	<i>C. fioriniae</i>	<i>C. siamense</i>		
Resistant	71	0	10	81	
Moderately Resistant	5	8	0	13	
Sensitive	102	0	0	102	
Total	178	8	10	196	

thiophanate-methyl.

Description	<i>C. gloeosporiodes</i>
	<i>C. siamense</i>
Resistant	6
Moderately Resistant	0
Sensitive	4
Total	10

Discussion

In this study, we identified *Colletotrichum* isolates from strawberry fields in the Mid-Atlantic. Strawberry anthracnose was found to be at least caused by 4 *Colletotrichum* species. Anthracnose pathogen could be isolated from multiple tissues of strawberry plants, including fruit, crown, runner, stem and petiole. The majority (85%) of isolates are from fruit, which means strawberry anthracnose fruit rot (AFR) is more prevalent than strawberry anthracnose crown rot (ACR) in the fields. Of the isolates from fruit, only *C. nymphaeae* and *C. fioriniae* were isolated and *C. nymphaeae* is the dominant species, making up 95% of the isolates from fruit. 12% of our isolates were from the crown, *C. nymphaeae* and *C. siamense* makes up 49% and 49% of the isolates from crown respectively. In addition, *C. nymphaeae* was isolated from the fruit and runners as well strawberry stems. Literature suggests on strawberry, *C. acutatum* complex mainly causes black spot of fruit, but under severe epidemics, *C. acutatum* can also cause necrosis on other tissue, including crown, root and leaf. *C. siamense* can only be isolated from crown. *C. lineola* has been reported to cause leaf anthracnose on Swallow-Worts in Russia and some other herbaceous hosts. In our study, *C. lineola* was first found as a pathogen on strawberry and it was

isolated from strawberry necrotic crown as a new cause for strawberry anthracnose. As for strawberry cultivar, about 50% (40/82) of isolates are from c.v. Chandler (data not shown).

Resistance to QoI fungicides is widespread, but there is still some difference between different complexes and species. Overall, resistance frequency in *Colletotrichum* spp. against the tested QoI fungicide azoxystrobin is 41.3% (81/196). In *C. acutatum* complex, *C. nymphaeae* has a 39.9% (71/178) resistance frequency, which is closed to the overall resistance frequency, since the majority (90.8%) of the samples we collected are *C. nymphaeae*. Isolates in *C. gloeosporioides* complex, are collected from multiple locations (Table. 1), but they are all resistant to QoI fungicides (Table.2). In the fungicide sensitivity screening with thiophanate-methyl (MBC fungicides), only sensitivity of *C. siamense* was evaluated. The resistance frequency of *C. siamense* to thiophanate-methyl is 60% (6/10), which is lower compared to resistance frequency to azoxystrobin (100%).

In addition, all *C. fioriniae* isolates are of moderately resistant phenotype to QoI fungicide and are without G143A mutation nor other mutations. QoI-resistant *nymphaeae* isolates were detected with G143A mutation. The G143A mutation results in a substitution of glycine (G) by alanine at position 143. This mutation is commonly found among isolates with high level resistance. When G143A mutation is found, disease control could be extremely difficult even at high chemical concentration. There are also two other mutations, a replacement of phenylalanine (F) by leucine (L) at position 129 (F129L) and a substitution of glycine (G) by arginine (R) at position 137 (G137R), were reported to link with moderately level of resistance. However, in our study, neither of three mutations were detected in moderately resistant isolates. Therefore, a yet unknown mechanism of resistant may be evolved in these isolates. This information is not new to research, since previous publication showed there was high resistant *C. siamense* isolates without G143A mutation. Also, studies reported QoI resistance in other pathogens, including *P. fusca*, *Venturia inaequalis*, and *Puccinia horiana*, with no known mutation linked.

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QUANTIFICATION OF *COLLETOTRICHUM FIORINIAE* IN THE FOREST SUGGESTS ITS MAIN ECOLOGICAL ROLE IS THAT OF A LEAF ENDOPHYTE

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Colletotrichum fioriniae (Marcelino & Gouli), a member of the *C. acutatum* species complex (Damm et al. 2012), is an ascomycete fungus that is a pathogen on over 100 plants of temperate regions worldwide, including crops such as strawberry, blueberry, pear, peach, grapes, chestnut, and celery (Farr & Rossman 2019). A survey of over 500 apples with bitter rot collected from 38 orchards in Pennsylvania and surrounding areas found that *C. fioriniae* was the causal species in about 2/3 of them (Martin & Peter 2018). Subsequent testing of a subsample of about 200 of those isolates for sensitivity to commonly used single-mode-of-action fungicides showed wide variation among active ingredients, with pyraclostrobin, benzovindiflupyr, and fludioxonil having some of the best growth suppression (Peirce, Thomas, Martin & Peter, CSFWC proceedings, this issue). Since many of these fungicide active ingredients are limited to 4 applications per season, the timing of application becomes crucially important. *C. fioriniae* is known as a hemibiotroph, where initial penetration of plant tissue is followed by a biotrophic phase before transition to necrotrophy (Peres et al. 2005), which means that while bitter rot is usually not observed until late-season through post-harvest, the initial infection could have occurred at any point in the growing season prior to that. Knowing the timing of the initial infection is therefore crucial to knowing when to apply the most effective fungicides.

To determine the timing of initial infections, spore dispersal was quantified in the orchard throughout the growing season. Knowing that *C. fioriniae* almost exclusively reproduces via rain-splashed conidia (Peres et al. 2005) rain-splashed spore traps were constructed with Falcon® 225 mL polypropylene conical centrifuge tubes (Corning INC, Corning NY) and household grade 5 in. plastic funnels by drilling a hole in the centrifuge tube cap, inserting the funnel, and gluing them together. The centrifuge tube could then be unscrewed from the funnel and replaced as needed. A wire sink strainer was placed in the funnel to keep out large debris. The spore traps were placed in apple orchards on Penn State's Fruit Research and Extension Center (FREC) in Biglerville and Arendtsville, PA. Knowing that *C. fioriniae* has also been found in forests (Marcelino et al. 2009), spore traps were also set out in forested woodlots adjacent to the orchards. While the broad host range of *C. fioriniae* would indicate it might be abundant in diverse plant communities, its limited dispersal range and endemic nature in apple orchards led us to hypothesize that the quantity of *C. fioriniae* conidia dispersal is higher in orchards with high rates of bitter rot infections than in nearby diverse deciduous woodlots and forests.

Spores were collected within 24 hours after a rain, no more than once a week, and only after sufficient rainfall to fill the bottles at least 1/4 full. After processing of the sample, bottles were hand-washed with soap and bleach in warm water. Before reattachment of a clean bottle funnels were cleaned by spraying with 1% (0.05% NaClO) bleach solution. Samples were collected from April to August 12 times in 2018 and 9 times in 2019. The volume of water in each bottle was recorded, and the bottles centrifuged in a 2-step process in an Eppendorf 5810R centrifuge (Eppendorf North America, Hauppauge NY). The steps consisted of 8 min. at 4,000 rcf, decanting of all but 20-30 mL of water in a process that often dislodged the pellet, then a second centrifugation of 8 min. at 4,000 rcf and decanting of the remaining water.

DNA was extracted from the pellet using the NucleoSpin Soil DNA extraction kit (Macherey Nagel, Bethlehem, PA) with the following modifications. For step 1, 500 µL of buffer SL2 was pipetted into the centrifuge tube and used to dislodge the pellet, which was transferred with pipette to the bead tube. After addition of Enhancer SX in step 2, the sample was lysed using a Macherey Nagel bead tube holder attached to a Vortex Genie (Scientific Industries, Bohemia NY) at max speed for 12 min. The rest of the DNA extraction followed protocol, which was finished by eluting the purified DNA with 50 µL of buffer SE. A negative control (no initial pellet) was included in every DNA extraction to check for contaminated reagents.

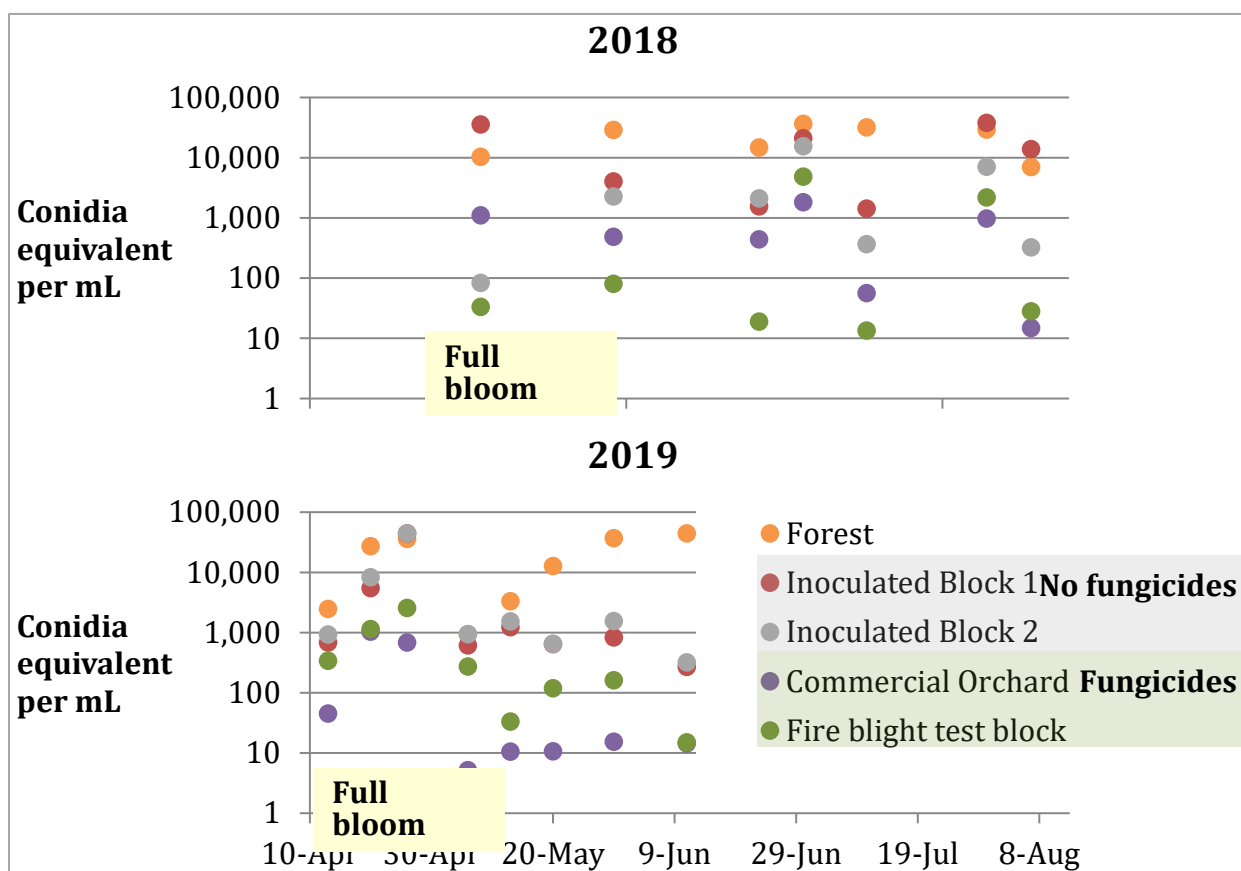


Figure 7: Graphs showing the conidia (asexual spore) equivalent per mL of rainwater captured in spore traps on the y-axis and date of spore trap collection on the x-axis. Top, collections in 2018, bottom, collections in 2019. Each dot represents the mean average conidia equivalent of the spore trap collections of the category shown in the legend.

C. acutatum-species-complex DNA was detected and quantified with q-PCR using the methods of Debode et al. (2009). Briefly, it is a TaqMan (Thermo Fisher Scientific, Waltham MA) based assay targeting the ITS1 region using primers CaITS_F701 (5'-GGATCATTACTG-AGTTACCGC-3') and CaITS_R699 (5'-GCCCCGCGAGAGGCTTC-3') and a QSY probe CaITS_P710 (5'-TACCTAAC CGTTGCTTCGGCGGG-3') that is specific to the *C. acutatum* species complex. Primers were synthesized by Integrated DNA Technologies (Skokie, IL), and the PCR plates and optically clear strip caps were from VWR (Radnor, PA). The assay was run on a Bio-Rad C1000 thermocycler with the CFX96 detection system (Bio-Rad Laboratories Inc., Hercules CA) set at 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. A standard curve was created by counting 3 separate suspensions of *C. fioriniae* spores with a hemocytometer, taking a calculated 1×10^7 spores from each suspension and extracting DNA using the method described above. The 3 samples were analyzed with the q-PCR assay for variation, then combined into a single sample and serially diluted 10-fold from 1×10^7 to 1×10^3 conidia equivalents. DNA standards of 1×10^7 , 1×10^6 , 1×10^4 , and 1×10^3 were added to each q-PCR run as an internal standard to equate cycle number to conidia number. All samples were run in duplicate and the mean average obtained.

After an initial quality control screening for contaminated samples, the remaining 308 spore trap samples across 2 growing seasons showed conidia were being dispersed throughout the season (Figure 1). A comparison of conidial quantities by location type showed a lognormal distribution, with median conidia equivalents being higher in forest than orchard samples (Figure 2). The variances of the log10 transformed spore counts by location were equal per Levene's test (p-value = 0.71) and location and date of spore trap

collection were significant ($p < 0.001$) while the interaction of location and date was not ($p = 0.36$). Tukey's HSD test showed higher conidia quantities in the forest samples than the orchard samples, and lower conidia quantities in the fungicide treated orchard samples than the untreated samples at $\alpha = 0.01$ (Figure 2).

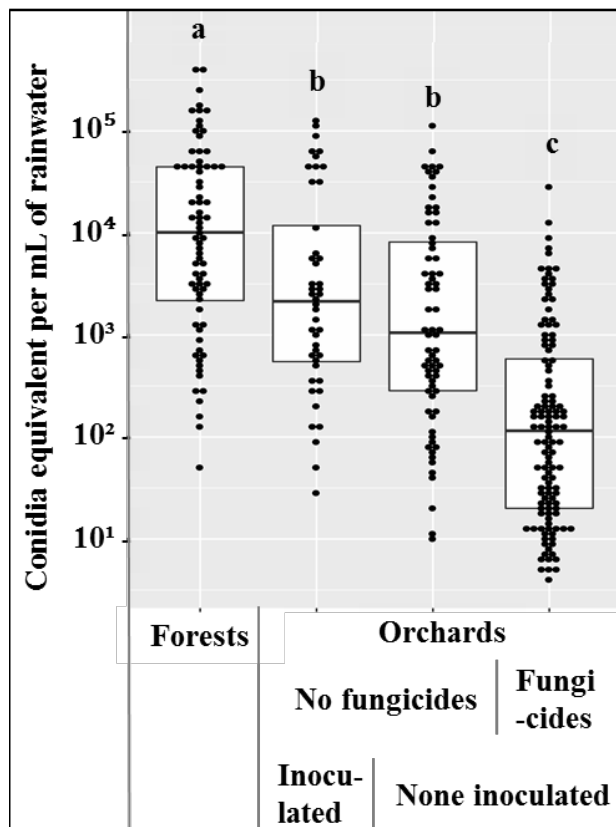


Figure 8: Dot-plot overlaid with a box-plot of the conidia concentrations (y-axis) of each rain-trap collection in forests and apple orchards. The orchards are split into 3 different management programs. Locations with a different letter are statistically different per a Tukey's HSD test with an α of 0.01.

The finding that more spores are being dispersed in the forest than in the orchard was unexpected. This led to the question of where in the forest the spores were coming from. Based on earlier findings that *C. fioriniae* can be an endophyte in various broadleaf forest plants (Marcelino et al. 2009), the leaves of several forest understory plants were collected in 2018, and a freezing method based on Børve & Stensvand (2017) and Mertely & Legard (2004) was used to detect endophytic *Colletotrichum* species infections in leaves. Leaves were surface disinfested by submersion in 70% ethanol to break the water surface tension, rinsed in deionized water, submersed in 10% (0.5% NaClO) bleach for 40s, rinsed in deionized water, submersed in 70% ethanol for 20s, and rinsed again in deionized water. They were then placed on a rack in a plastic tub (figure 3, top) with wet paper towel to maintain high humidity, frozen solid in a -20 or -80 freezer to kill the leaves, and incubated at room temperature (21-23C) for two weeks to allow endophytic fungi to sporulate. Deionized water was sprayed on the leaves as needed to maintain moist conditions. The results were abundant *Colletotrichum* conidia masses on many of the leaves (figure 3, bottom). A few pure fungal cultures were obtained from these spore masses, and sequencing of intron 1 of the GAPDH gene (using primers GDF1 5'-GCCGTCAACGACCCCTT-CATTGA-'3 and GDR1 5'-GGGTGGAGTCGTA CTTGAGCATGT-'3 (Templeton et al. 1992)) showed that the sequences were identical to those from *C. fioriniae* from apple.

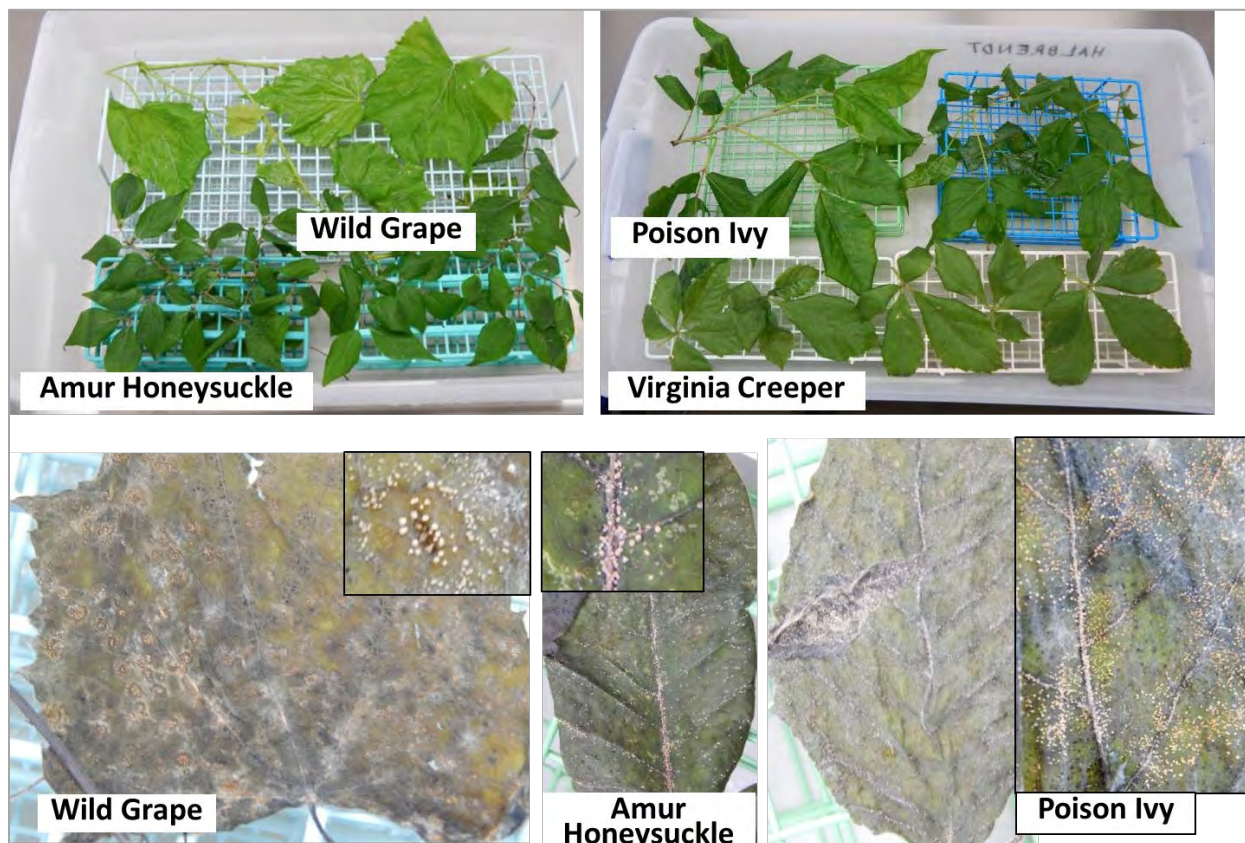


Figure 9: Photos of a sample of leaves after surface disinfestation, freezing, and incubation, showing the orange spore masses characteristic of *Colletotrichum conidia*.

To see if endophytic infections were also occurring in apple leaves, apple leaves were collected from 2 commercially fungicide treated trees and 1 untreated tree in 2018, and 3 commercially fungicide treated trees and 5 untreated trees in 2019. A few leaves of trees with fungicide treatments had endophytic infections, while endophytic infections were abundant in leaves of untreated trees (figures 4 and 5). For 4 of the untreated trees in 2019, bitter rot incidence was obtained from 25 randomly selected apples at harvest and one month post-harvest and recorded as cumulative incidence. When compared with Student's t-test, fungicide vs. non-fungicide treated leaves were different at $p = 0.01$, and a paired t-test of the 4 trees with both leaf and fruit data were different at $p = 0.07$ (Figure 5).



Figure 10: Example of orange *Colletotrichum conidia* masses on leaves of apple after surface disinfestation, freezing and incubation.

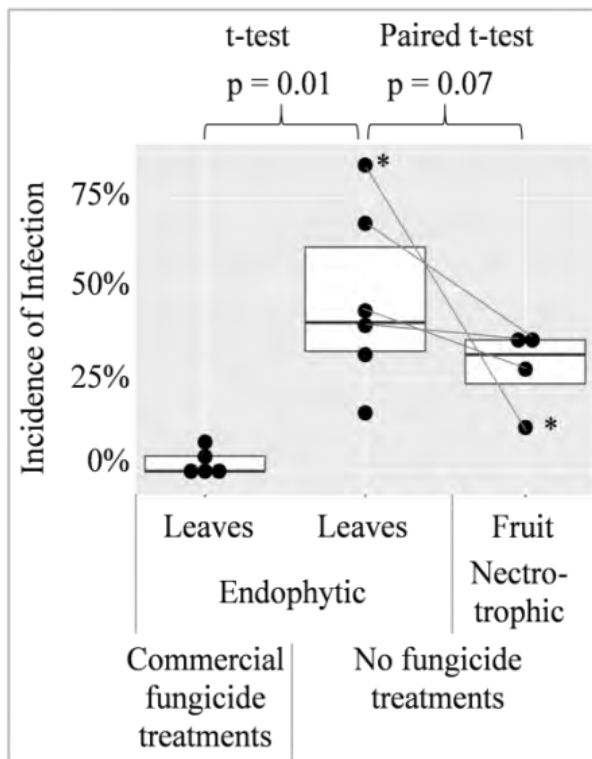


Figure 11: Dot-plot of incidence of apple leaves with endophytic *Colletotrichum* infections mid-season and fruit with necrotrophic infections (bitter rot) post-harvest, arranged by fungicide program. Each dot is a single tree and the lines are connecting leaf and fruit data from the same tree. Leaf incidence is of 24 to 28 leaves per tree and fruit incidence is of 25 fruits per tree. The tree marked with an asterisk* is cultivar 'Rome', while the rest are 'Honeycrisp'.

To make sure finding endophytic infections in leaves of forest plants wasn't just a rare, localized occurrence, in 2019 over 1,138 leaves of 24 forest plant species were collected during the months of June to September from the orchard and forested area surrounding the spore traps at FREC, and from the nearby Michaux State Forest (MSF), mostly within a kilometer of N 40.034086, W -77.342036. Permit number SFRA-1920 was obtained from the Bureau of Forestry of the Pennsylvania Department of Natural Resources for the collection of leaves from MSF. This area of MSF was heavily logged in the 1800s and saw commercial activity up to the 1970s, but has been forested since then, and is at least 4 straight-line kilometers from the nearest agricultural fields. Plant species were identified based on morphology as per Rhoads & Block (2007).

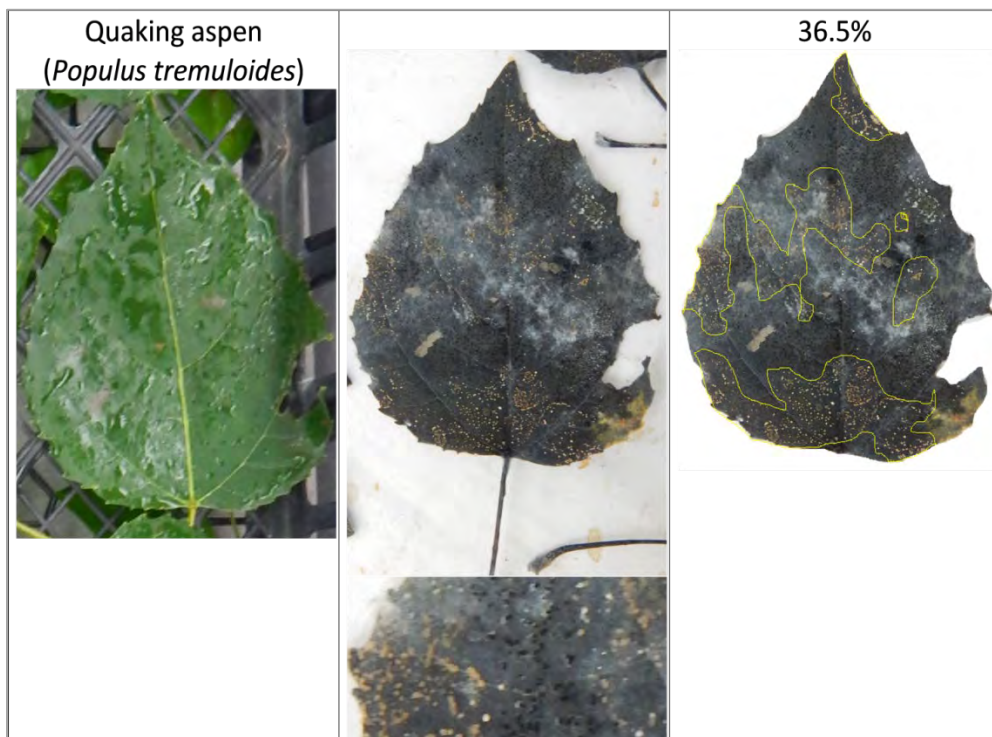


Figure 12: An example of calculation of leaf surface area with orange conidia. Left, the leaf after surface disinfestation but before freezing. Middle top, leaf after incubation, showing areas with orange conidia masses. Middle bottom, close up showing the orange conidia masses along with some black conidia masses. Right, leaf showing the area with orange conidia traced in yellow, which covered 36.5% of the total leaf surface area.

The leaves were tested via the freezing method described above. After incubation, photographs were taken of the leaves for calculation of % leaf surface area covered by orange spore masses. Using ImageJ software (Schneider et al. 2012), the outline of the leaf was manually traced and the pixels of leaf area obtained. The area containing orange spore masses was then traced to obtain the pixels of leaf area with orange spores, and divided by the total leaf area to obtain a percentage of leaf area with orange spores (Figure 6). The overall results showed orange conidial masses on 43% of leaves, and quantification of spore mass area showed 30% of leaves had more than 1% leaf area with orange spores, with high variability among plant species (Figure 7). Levene's test of variances of leaf area with orange spores by plant species showed they were not equal ($p < 0.0001$) and the general linear model in SAS showed that location, plant species, and the interaction of location and plant species were all highly significant predictors of percent leaf area with orange spores, with p-values of < 0.001 . Given the in-equality of variances and the difficulties of separating the effects of location and location-plant species interactions from the effect of plant species alone, no means separation test based on plant species was performed. The results are instead shown as a dot-plot of percent leaf area with orange spores arranged by plant species, where every leaf with more than 1% leaf area with orange spores is shown as an individual dot (Figure 7).

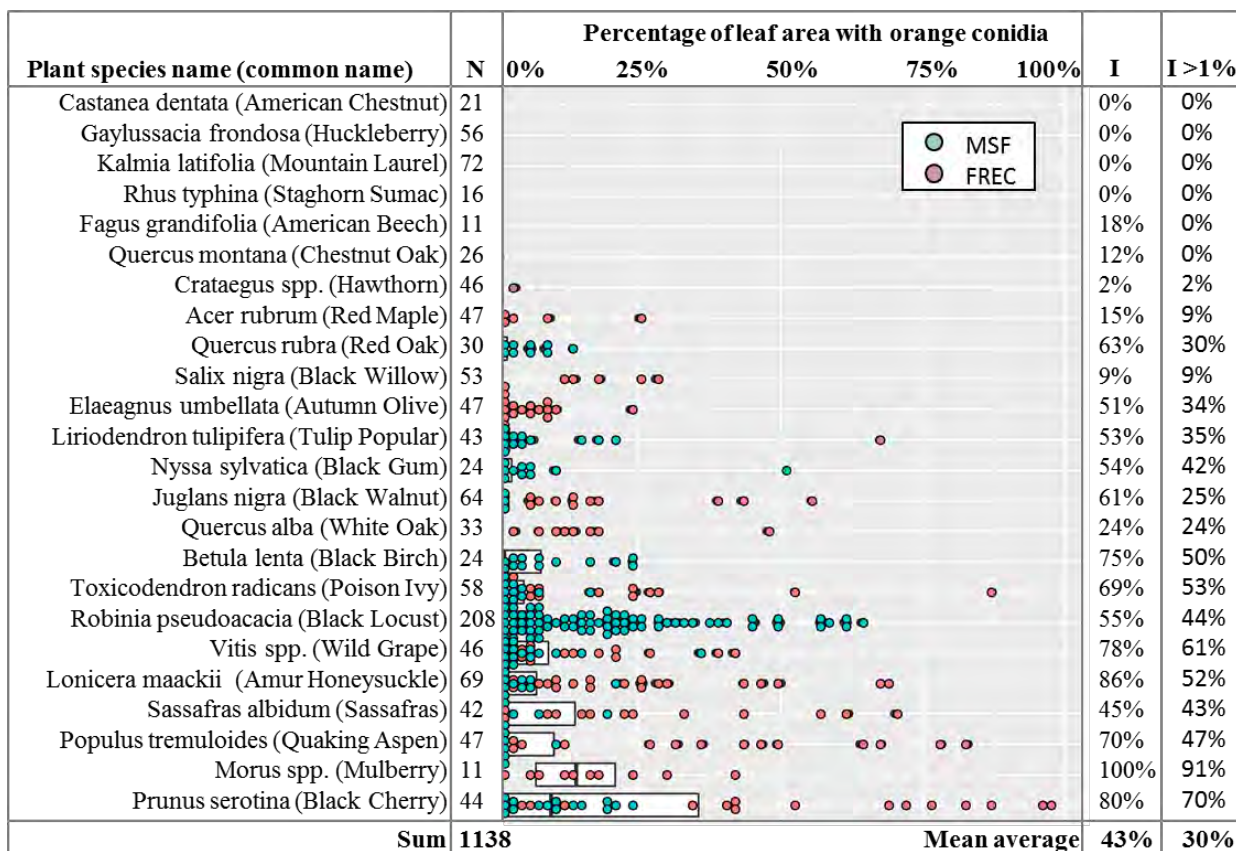


Figure 13: Quantification of endophytic *Colletotrichum* infections in leaves of forest plants. The first column is the names of plant species from which leaves were sampled. The second column is the number of leaves sampled of each plant species. The graph is a dot-plot overlaid with a box-plot of the percentage of of each leaf surface with orange conidia, with leaves from MSF in blue-green and from FREC in red. The third column is incidence of leaves of each plant species with orange conidia, and the last column is the incidence of leaves with over 1% of surface area with orange conidia.

This confirmed that *Colletotrichum* species are abundant as leaf endophytes both in farm woodlots and large forests, and raised the question of which *Colletotrichum* species these are. To answer that, 98 orange conidial masses were randomly selected from leaves of 20 plant species and the conidial masses streaked onto a Petri dish of ½ strength PDA (Potato Dextrose Agar, Difco, Franklin Lakes NJ). The resulting fungal growths were single-spore or hyphal-tip isolated to form genetically uniform fungal cultures. DNA was extracted from cultures with the NucleoSpin Microbial kit (Macherey Nagel, Bethlehem, PA) and intron 1 of the GAPDH gene was sequenced. To identify the non-*Colletotrichum* fungal cultures the ITS gene was sequenced using primers ITSF_KY02 5'-CTTGGTCATTTAGAGGAAGTAA-3 (Toju et al. 2012) and ITS4 5'-TCCTCCGCTTATTGATATGCCAG-3 (White et al. 1990).

Amplification, sequencing, and phylogenetic analysis of the GAPDH gene revealed that 88 isolates clustered with *C. fioriniae*, 2 with *C. nymphaeae*, 2 with *C. salix*, and 3 by themselves but closest to *C. aenigma* (*C. sp. indetermined C.*) (Figure 8). The ITS gene showed 3 isolates of *Gnomoniopsis paraclavulata*. The biggest takeaway though, is that the majority (~90%) of the orange-spored isolates from leaves were *C. fioriniae*.

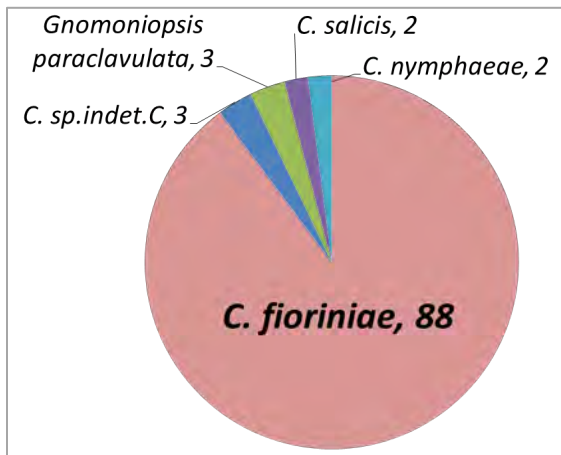


Figure 14: Pie chart showing the relative proportion of the species of orange-spored fungal isolates. The number behind the name is the number of isolates collected.

Contrary to our hypothesis, the highest quantities of conidia were in the forests, and not in heavily infected orchards. The relative quantities of conidia within orchards were as expected though, matching well with the incidence of bitter rot in those same areas (data not shown), and supported the reliability of the detection method. The finding of high conidia quantities being dispersed in the forest lead us to look for the source of those conidia, and was supported by the discovery of abundant endophytic leaf infections in many forest plants.

The source of *C. fioriniae* conidia being dispersed in the forest is likely secondary conidiation from endophytic infections, as discussed by Peres et al. (2005). This secondary conidiation would spread endophytic infections from leaf to leaf throughout the forest canopy. The production of air-dispersed ascospores from leaves is possible, but since the sexual stage of the *C. acutatum* species complex is rare and was not described until 36 years after the species was named (Guerber & Correll 2001), dispersion by ascospores seems unlikely. Endophytically infected leaves that fall to the ground could be possible sources of inoculum for the next year (Everett et al. 2018). However, given that *C. fioriniae* is predominately rain-splashed dispersed (Peres et al. 2005), and that rain splashed conidia mostly land within a meter of the source (Ntahimpera et al. 1999, 1998), it seems unlikely that leaves on the ground are a primary source of inoculum in shrub and tree canopies.

It seems most likely that *C. fioriniae* in the forest overwinters in tree and shrub canopies. Over 100 years ago infected fruit mummies and branch and twig cankers were identified as the main sources of overwintering inoculum in apple trees (Von Schrenk & Spaulding 1903), and the importance of fruit mummies and bark and twig cankers is supported by more recent work in apple (Nekoduka et al. 2018), holly (Lin & Hand 2019), and strawberry (Wilson et al. 1992). Fruit scars were identified as a key overwintering source in apple in Japan (Nekoduka et al. 2018). Buds are also sources of overwintering inoculum in plants such as blueberry (Yoshida et al. 2007; DeMarsay 2005), sweet and sour cherry (Børve & Stensvand 2006; Stensvand et al. 2017), and apple (Børve & Stensvand 2007; Everett et al. 2018). Since there generally are much higher number of buds on an average tree or shrub compared to the number of fruit mummies or cankers, even at low infection incidence, buds could play a large role as overwintering sites.

Given the evidence discussed above, we propose an infection cycle for *C. fioriniae* in the forest (Figure 9). It assumes that reproduction is dominated by asexually produced, rain-splashed conidia. Endophytic infections in leaves are hypothesized to be the main site of infections throughout the growing season. In fruit bearing plants, maturing infected fruits are also a factor. Buds, fruit mummies, fruit scars, and cankers serving as overwintering sites. This infection cycle is similar to previously published infection cycles (Everett et al. 2018) but with a greater emphasis on endophytic infections in leaves. It is also an infection cycle and not necessarily a disease cycle, only becoming a disease cycle on plants that are stressed or have susceptible fruits.

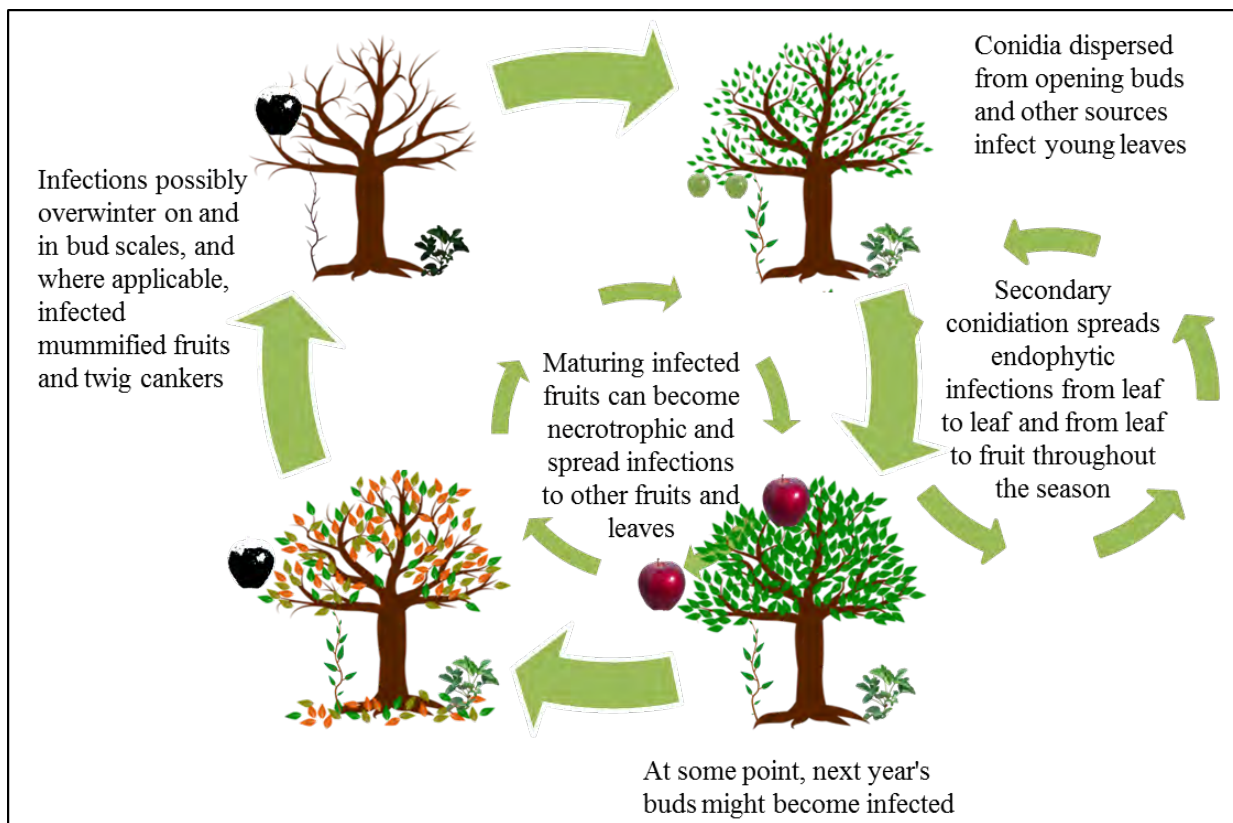


Figure 15: Hypothesized generalized infection cycle for *C. fioriniae*.

Acknowledgements

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References:

- Børve, J., and Stensvand, A. 2007. *Colletotrichum acutatum* Found on Apple Buds in Norway. Plant Manag. Netw.
- Børve, J., and Stensvand, A. 2006. *Colletotrichum acutatum* overwinters on sweet cherry buds. Plant Dis. 90:1452–1456.
- Damm, U., Cannon, P. F., Woudenberg, J. H. C., and Crous, P. W. 2012. The *Colletotrichum acutatum* species complex. Stud. Mycol. 73:37–113.
- Debode, J., Van Hemelrijck, W., Baeyen, S., Creemers, P., Heungens, K., and Maes, M. 2009. Quantitative detection and monitoring of *Colletotrichum acutatum* in strawberry leaves using real-time PCR. Plant Pathol. 58:504–514.
- DeMarsay, A. 2005. Anthracnose Fruit Rot of Highbush Blueberry: Biology and Epidemiology.
- Everett, K. R., Pushparajah, I. P. S., Timudo, A., Ah Chee, A., Scheper, R. W. A., Shaw, P. W., et al. 2018. Infection criteria, inoculum sources and splash dispersal pattern of *Colletotrichum acutatum* causing bitter rot of apple in New Zealand. Eur. J. Plant Pathol.
- Farr, D. F., and Rossman, A. Y. 2019. Fungal Databases. Accessed from [https://nt.ars-grin.gov/fungaldatabases/new_allView.cfm?whichone=FungusHost&thisName=Colletotrichum fioriniae&organismtype=Fungus&fromAllCount=yes](https://nt.ars-grin.gov/fungaldatabases/new_allView.cfm?whichone=FungusHost&thisName=Colletotrichum%20fioriniae&organismtype=Fungus&fromAllCount=yes).
- Guerber, J. C., and Correll, J. C. 2001. Characterization of *Glomerella acutata*, the teleomorph of *Colletotrichum acutatum*. Mycologia. 93:216–229.
- Lin, S., and Hand, F. P. 2019. Determining the Sources of Primary and Secondary Inoculum and Seasonal

- Inoculum Dynamics of Fungal Pathogens Causing Fruit Rot of Deciduous Holly. *Plant Dis.* 103:951–958.
- Marcelino, J. a P., Gouli, S., Parker, B. L., Skinner, M., Schwarzberg, L., and Giordano, R. 2009. Host plant associations of an entomopathogenic variety of the fungus, *Colletotrichum acutatum*, recovered from the elongate hemlock scale, *Fiorinia externa*. *J. Insect Sci.* 9:25.
- Martin, P. L., and Peter, K. A. 2018. *Colletotrichum* Species Composition and Fungicide Tolerance in Isolates Causing Bitter Rot of Apples in Pennsylvania. (Abstr.). *Phytopathology.* 108:19.
- Nekoduka, S., Tanaka, K., and Sano, T. 2018. Epidemiology of apple bitter rot caused by *Colletotrichum acutatum* sensu lato. *J. Gen. Plant Pathol.* 84:262–271.
- Ntahimpera, N., Ellis, M. A., Wilson, L. L., and Madden, L. V. 1998. Effects of a Cover Crop on Splash Dispersal of *Colletotrichum acutatum* Conidia. *Phytopathology.* 88:536–43.
- Ntahimpera, N., Wilson, L. L., Ellis, M. A., and Madden, L. V. 1999. Comparison of rain effects on splash dispersal of three *Colletotrichum* species infecting strawberry. *Phytopathology.* 89:555–63.
- Peres, N. a., Timmer, L. W., Adaskaveg, J. E., and Correll, J. C. 2005. Lifestyles of *Colletotrichum acutatum*. *Plant Dis.* 89:784–796.
- Rhoads, A. F., and Block, T. A. 2007. *The Plants of Pennsylvania: an Illustrated Manual*. University of Pennsylvania Press.
- Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. 2012. NIH Image to ImageJ : 25 years of image analysis. *Nat. Methods.* 9:671–675.
- Von Schrenk, H., and Spaulding, P. 1903. *The Bitter Rot of Apples*. ed. B. T. Galloway. Washington: U.S. Dept. of Agriculture.
- Stensvand, A., Borge, J., and Talgo, V. 2017. Overwintering Diseased Plant Parts and Newly Infected Flowers and Fruit as Sources of Inoculum for *Colletotrichum acutatum* in Sour Cherry. *Plant Dis.* 101:1207–1213.
- White, T. J., Bruns, S., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, New York: Academic Press, p. 315–322.
- Wilson, L. L., Madden, L. V., and Ellis, M. A. 1992. Overwinter survival of *Colletotrichum acutatum* in infected strawberry fruit in Ohio. *Plant Dis.* 76:948–950.
- Yoshida, S., Tsukiboshi, T., Shinohara, H., Koitabashi, M., and Tsushima, S. 2007. Occurrence and development of *Colletotrichum acutatum* on symptomless blueberry bushes. *Plant Pathol.* 56:871–877.

SENSITIVITY DISTRIBUTION TO 11 FUNGICIDES IN A POPULATION OF *COLLETOTRICHUM* ISOLATES FROM APPLE

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Apple growers in Pennsylvania and surrounding areas have been reporting increased losses to bitter rot, especially in wet years such as 2018, with losses of up to 80% in highly susceptible cultivars. Control of bitter rot starts with good horticultural practices including cultivar and rootstock selection, proper plant nutrition, and good tree training and pruning techniques. Especially important are practices that limit overwintering sites, such as removal of all diseased fruit mummies, dead twigs and limb cankers from the tree canopy, and practices that open up the canopy to allow air flow to dry off the leaves and fruits and limit wetness hours. Building on top of good cultural practices, a good bitter rot control program also includes frequent applications of fungicides. Since the fungal species that cause bitter rot are known to vary widely with regards to sensitivity to different fungicide active ingredients, building a good fungicide program requires knowing which active ingredients will provide the best control. Furthermore, bitter rot causing fungi in other locations have developed resistance to several fungicide modes of action (Chechi et al. 2019), so it is important to know whether fungicide resistance is also present in Pennsylvania.

In previous research projects over 500 apples with bitter rot have been collected from 38 orchards in Pennsylvania and surrounding areas, and over 500 fungal cultures have been isolated and identified. All of the isolates were in the genus *Colletotrichum*, with approximately 2/3 in the *C. acutatum* species complex (mostly *C. fioriniae*), and 1/3 in the *C. gloeosporioides* species complex (various species). In addition, about 100 isolates of the same species were collected from leaves of various forest plants. A total of 8 or 12 isolates representing every species isolated from apples were initially tested for sensitivity to 11 fungicide active ingredients from 6 FRAC groups (Table 1). These active ingredients were chosen because they are single-site mode of action fungicides labeled for use on apple, and were tested in their commercial formulations.

The commercial fungicide formulations were dissolved or suspended in sterile de-ionized water to active ingredient concentrations of 1,000 to 10,000 ppm and then diluted in 1/2 strength potato dextrose agar (1/2 PDA, after autoclaving and cooling to 60°C) to make a series of 10-fold dilutions with final concentrations ranging from 0.001 to 1,000 ppm, depending on the fungicide. The fungicide amended 1/2 PDA and non-amended negative control 1/2 PDA were dispensed into 100mm disposable Petri dishes and allowed to cool. Mycelial plugs (5mm dia.) from the edges of fungal colonies growing on 1/2 PDA were placed in the center of the plates, which were incubated at 26°C and ambient light. All concentrations of a single fungicide active ingredient were plated and incubated at the same time. After a 7-9 day incubation period, the diameter of the fungal colony in each plate was measured in 2 diagonal directions, and a mean average colony size obtained, minus the initial 5 mm plug. The mean average colony size of each fungicide treated plate was measured against the mean average colony size of the untreated control to obtain a percent growth relative to the untreated control. In a few cases where there was some contamination in the untreated control, growth was compared to the lowest concentration of 0.001 ppm. The growth relative to the untreated control was graphed against the log₁₀ scaled concentrations of the fungicide active ingredients (Figures 1 and 2). Based on this growth response curve, a single fungicide concentration was chosen to use as a discriminatory dose to screen the bitter rot fungal population to determine the fungicide sensitivity distributions.

Table 1: List of FRAC groups, mode action, fungicide active ingredients, and trade names of the fungicides tested. The italicized trade names were the formulations tested.
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FRAC	Fungicide target (mode of action)	Active ingredients	Trade Names
1	β -tubulin assembly	Thiophanate-methyl	<i>Topsin</i>
7	Succinate Dehydrogenase Inhibitors (SDHIs)	Fluxapyroxad Benzovindiflupyr Fluopyram	<i>Sercadis</i> (Merivon) <i>Aprovia</i> <i>Velum Prime</i> (Luna products)
9	Methionine biosynthesis inhibitors	Pyrimethanil Cyprodinil	<i>Penbotec</i> (Luna Tranquility) <i>Vanguard</i> (Inspire Super)
11	Inhibition of cytochrome-b at QoI site	Pyraclostrobin Trifloxystrobin Kresoxim-methyl	<i>Cabrio</i> (Pristine, Merivon) <i>Flint Extra</i> (Luna Sensation) <i>Sovran</i>
12	Osmotic signal transduction	Fludioxonil	<i>Scholar</i>
29	Uncoupler of oxi. Phos.	Fluazinam	<i>Omega</i>

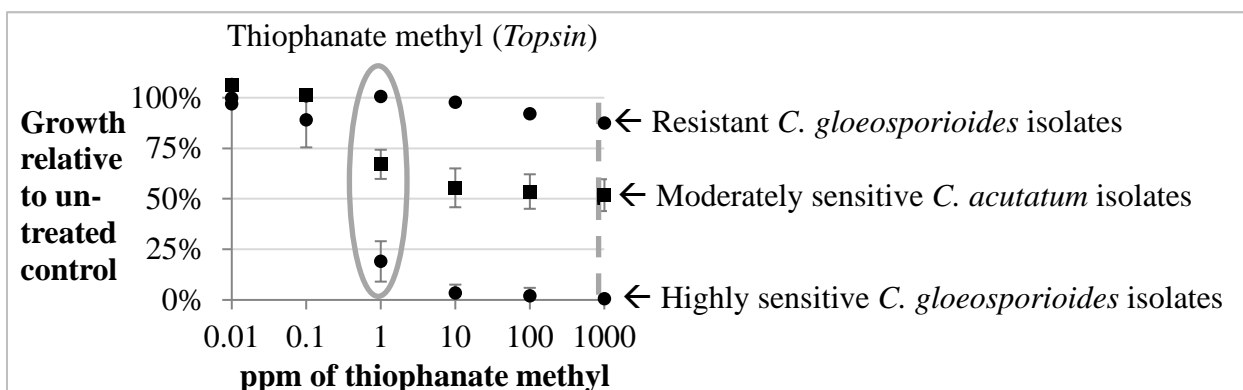


Figure 1: Graph of the dose response curves of mycelial growth of isolates in the *C. acutatum* (■) or *C. gloeosporioides* (●) species complexes to thiophanate methyl (FRAC group 1), which was tested in its commercial formulation of *Topsin*. The vertical dashed line is the field rate equivalent of thiophanate methyl when the maximum per acre labeled rate of *Topsin* is diluted in 100 gal. of water. The circled concentration was chosen as a discriminatory dose for screening of the larger fungal population. Each point is the mean of isolates in each species complex and the error bars are plus and minus the standard deviation of the mean.

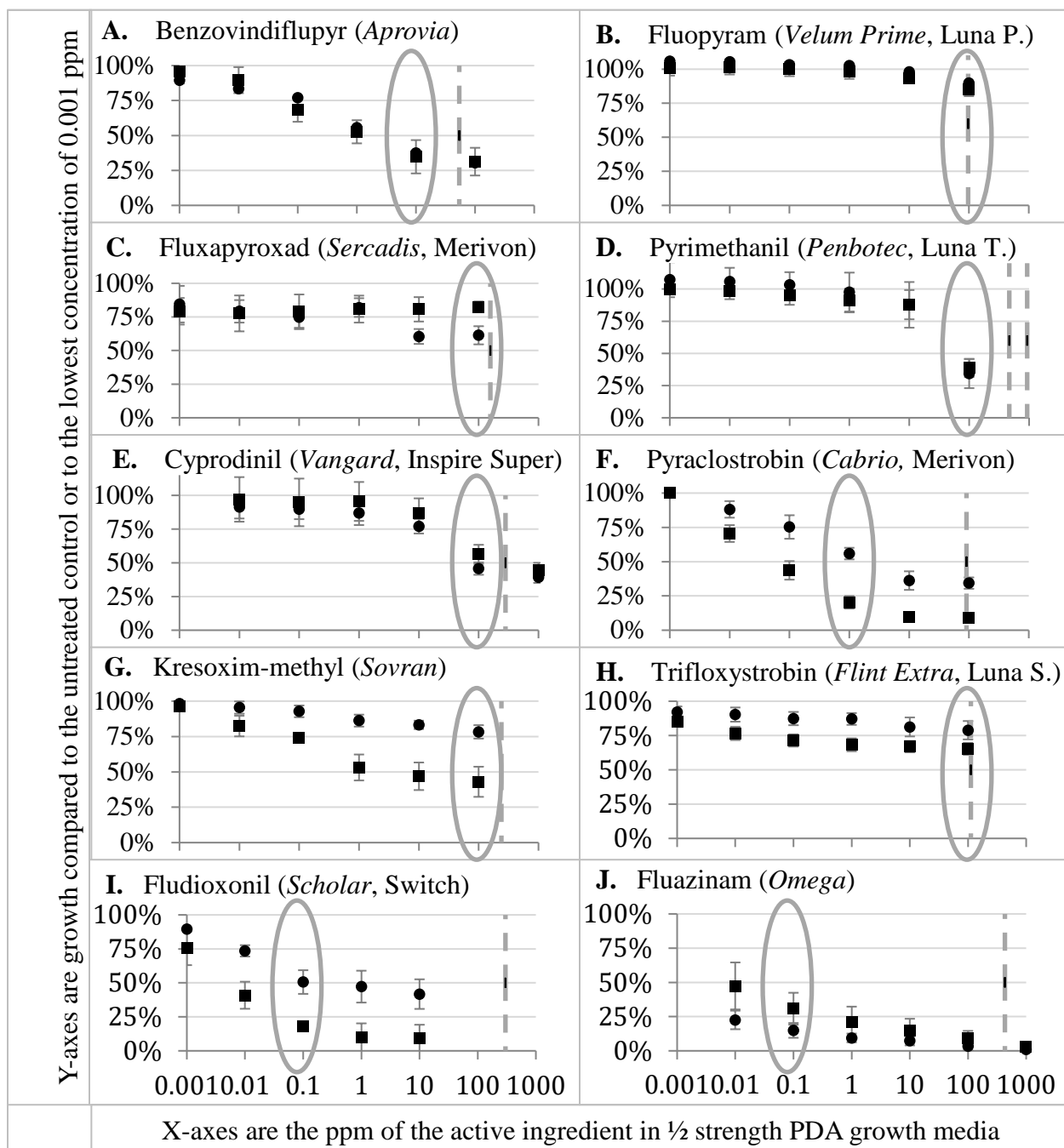
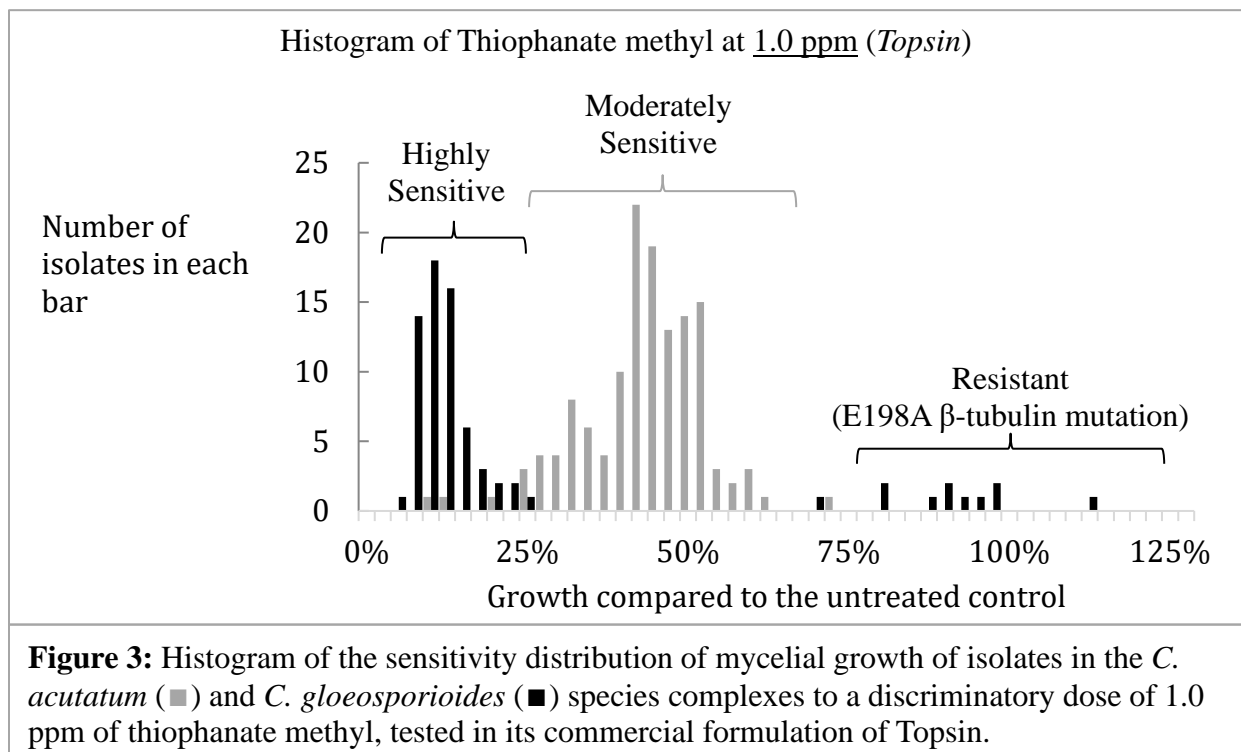


Figure 2: Graphs of dose response curves of mycelial growth of isolates in the *C. acutatum* (■) or *C. gloeosporioides* (●) species complexes to the listed fungicide active ingredients, which were tested as the italicized commercial formulations. Non-italicized names are other formulations that contain the active ingredient. Active ingredients A-C are in FRAC group 7, D and E in FRAC group 9, F-H in FRAC group 11, I in FRAC group 12, and J in FRAC group 29. Vertical dashed lines are the field rate equivalent of the fungicide active ingredient when the maximum labeled rate per acre is diluted in 100 gal. of water. Circled concentrations were chosen as discriminatory doses for screening of the larger fungal population. Each point is the mean of isolates in each species complex and the error bars are plus and minus the standard deviation of the mean.

A sub-sample of 209 *Colletotrichum* isolates, which included isolates from every species at every orchard at which they were found, along with reference isolates collected from the forest and obtained from

other research labs, were screened at the previously determined discriminatory doses of the 11 fungicide active ingredients. The method was identical to the initial sensitivity test, except that only the discriminatory dose and an untreated control were plated. For each fungal isolate, a single plate of each discriminatory dose of all 11 fungicide active ingredients plus 3 untreated control plates were inoculated, incubated, and measured in a single batch. Mean average colony growth of each fungicide amended plate was divided by the mean average of the untreated control plates to obtain the percent growth compared to the untreated control. Histograms of the results were made in Excel using a bin size of 2.5% and separating the *C. acutatum* and *C. gloeosporioides* species complexes (Figures 3 and 4).



The differences in sensitivities to thiophanate methyl between the *C. acutatum* and *C. gloeosporioides* species complexes was not a novel finding, as it has long been known that these 2 species complexes vary with regards to sensitivity to FRAC group 1 fungicides (Bernstein et al. 1995). In general, there were large differences in sensitivities to the fungicide active ingredients, with the bitter rot fungal population being largely insensitive to many of them. This was true even within individual FRAC groups. In FRAC group 7, benzovindiflupyr had much greater growth suppression than fluopyram or fluxapyroxad, to which the population appeared insensitive (Figures 2A-C and 3A-C). The same was true in FRAC group 11, where pyraclostrobin had much greater growth suppression than kresoxim-methyl or trifloxystrobin (Figures 2F-H and 3F-H). In this FRAC group, these differences in sensitivity are correlated to the fungicide chemical structure class, with pyraclostrobin being a methoxy-carbamate and kresoxim-methyl and trifloxystrobin being oximino-acetates (FRAC 2018). The FRAC group 29 fungicide fluazinam looked great, although 2019 field trials did not show great bitter rot control (unpublished data).

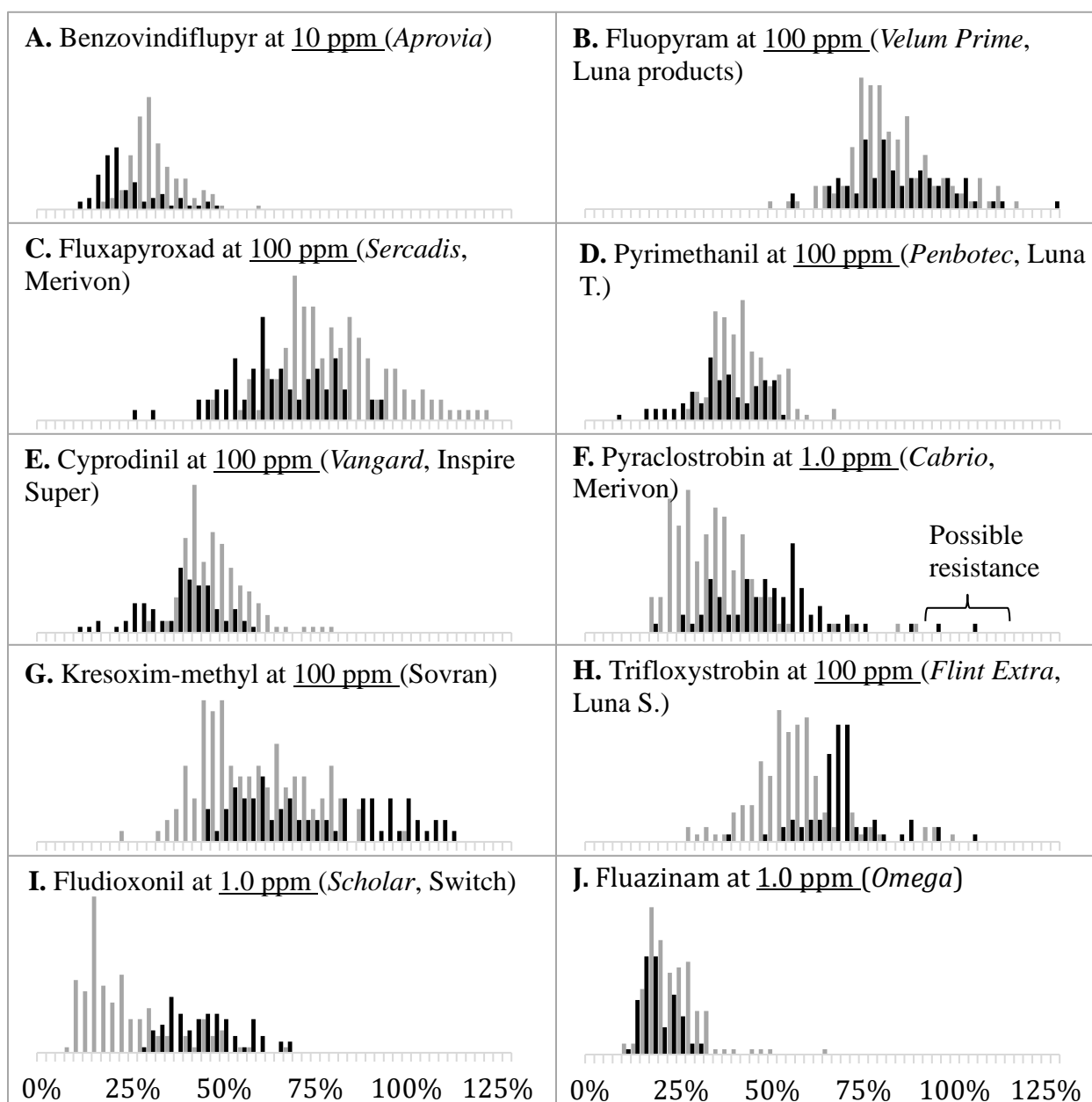


Figure 4: Histogram of the sensitivity distribution of mycelial growth of isolates in the *C. acutatum* (■) and *C. gloeosporioides* (■) species complexes to the listed discriminatory doses of active ingredients, which were tested as the italicized commercial formulations. Non-italicized names are other formulations that contain the active ingredient. Active ingredients A-C are in FRAC group 7, D and E in FRAC group 9, F-H in FRAC group 11, I in FRAC group 12, and J in FRAC group 29.

There were 2 fungicide active ingredients, thiophanate methyl (Figure 3) and pyraclostrobin (Figure 4F), in which a few isolates appeared resistant. Since thiophanate methyl targets the microtubule β -tubulin, sequences of the β -tubulin gene were obtained and checked for mutations known to confer resistance. In all of the isolates in the *C. gloeosporioides* species complex that had growth more than 70% of the untreated control had the A to C mutation in codon 198, which leads to an amino acid substitution of E198A. This mutation is well known to cause resistance to FRAC group fungicides (Mair et al. 2016). Interestingly, these isolates were all part of the *C. siamense* species, and came from 5 orchards in southeast Pennsylvania, Delaware, and

Maryland, including one organic orchard. It could be that this mutation is common in *C. siamense*, as it was also found in this species in Illinois (Chechi et al. 2019).

Pyraclostrobin targets the cytochrome-b enzyme of complex 3 in the mitochondrial electron transport chain, and several mutations in the cytochrome-b gene are well known to cause resistance (Mair et al. 2016). This research is ongoing, and sequences of this gene will be obtained and checked for the presence of known mutations.

In summary, sensitivity to FRAC group 1 was correlated with species complex, and resistance was correlated to the E198A mutation. There was large variation in sensitivity to FRAC 7, with benzovindiflupyr showing superior growth suppression. There was also large variation in sensitivity to FRAC 11 with pyraclostrobin showing superior growth suppression, which is correlated to FRAC group 11 fungicide chemical structure group. The bitter rot fungal population was less sensitive to FRAC 9, but sensitive to FRAC 12 and 29, although field trials with the FRAC group 29 fungicide did not show great bitter rot control. For future research, we will investigate the outliers, especially against pyraclostrobin, and look for correlations of sensitivity with species, location, and orchard management.

Acknowledgements

We wish to thank Brian Lehman and Teresa Krawczyk for their assistance in this work. This research was supported by the State Horticultural Association of Pennsylvania, the NSF Graduate Student Fellowship, the Northeast SARE Graduate Grant Program and the USDA National Institute of Food and Agriculture and Federal Appropriations under Project #PEN04694 and Accession #1018736.

References

- Bernstein, B., Zehr, E. I., and Dean, R. a. 1995. Characteristics of *Colletotrichum* from Peach, Apple, Pecan, and Other Hosts. Plant Dis. 79:478.
- Chechi, A., Stahlecker, J., Dowling, M. E., and Schnabel, G. 2019. Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples. Pestic. Biochem. Physiol. 158:18–24.
- FRAC. 2018. Mode of Action of Fungicides. Accessed from http://www.frac.info/docs/default-source/publications/frac-mode-of-action-poster/frac-moa-poster-2018.pdf?sfvrsn=505b4b9a_2.
- Mair, W., Lopez-ruiz, F., Stammler, G., Clark, W., Burnett, F., Hollomon, D., et al. 2016. Proposal for a unified nomenclature for target-site mutations associated with resistance to fungicides. Pest Manag. Sci. 72:1449–1459.

BIOCONTROL AGENT *R. VITIS* ARK-1 REDUCES GRAPEVINE CROWN GALL AGAINST HIGHER CELL NUMBERS OF TUMORIGENIC *R. VITIS* IN A CO-INOCULATION STUDY

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Crown gall of grapevine, caused by tumorigenic bacterium *R. vitis*, is an economically important disease in temperate as well as other grape growing regions in the world. Several cultural and preventative management practices of this disease are available, but none of them is sustainable and economically feasible. Therefore, efforts from many labs around the world have focused on finding out an effective biocontrol agent for sustainable management of this disease. Previous studies in Japan and Virginia have confirmed non-tumorigenic *R. vitis* ARK-1, isolated from a nursery in Japan, as a potential biocontrol agent according to *in planta* co-inoculation study. Detailed mechanism of ARK-1 is yet to learn, but post-co-inoculation gene expression study reveals reduced expression of several essential and non-essential virulent genes of tumorigenic strains. In this co-inoculation study in grapevine and tomato, we attempted to detect the upper limit of tumorigenic strains that can be inhibited by ARK-1 from forming galls *in planta*.

Biocontrol agent ARK-1 was obtained from Kumiai Chemicals Ltd, Japan as an unreleased commercial formulation. ARK-1 in 1X concentration ($OD_{600}=0.1$, $\sim 5 \times 10^7$ spores/ml) was mixed with different concentrations of tumorigenic strains mixture. I.e., tumorigenic strain mixtures of 1X ($OD_{600}=0.1$), 2X ($OD_{600}=0.2$), 3X ($OD_{600}=0.3$), 4X ($OD_{600}=0.4$) and 5X ($OD_{600}=0.5$) were prepared for treatments of 1:1, 1:2, 1:3, 1:4 and 1:5 ratio, respectively. The tumorigenic strains mixture (Ti-mix) consists of four tumorigenic *R. vitis* isolates originated from different parts of Virginia. Ten μ l of inoculum were inoculated in wounds that was made vertically either by drilling with 1 mm size drill bit in grapevine woody stem or by sterilized needle in tomato stem. There were five wounds per plant, each were separated ~ 1 cm and placed vertically along the stem. The control groups were received either 5 μ l of ARK-1 suspension or tumorigenic strains mixture. There was a total of three independent experimental repetitions with two internal plant replications per repetition. Following inoculation, the plants were grown on the greenhouse bench, and gall size was measured six and twelve weeks after inoculation in tomato and grapevine, respectively, by a Vernier calipers (Instant Readout Digital Calipers, Electron Microscopy Sciences). The gall size data was fitted into a linear mixed model and gall incidence data was fitted into a logistic regression model in statistical software SAS JMP Pro v. 14 (SAS Institute, Cary, NC). The means were separated by student's t test.

In tomato, ARK-1 significantly ($P < 0.05$) reduced gall incidence when it was co-inoculated with the Ti-mix up to 1:4 ratio (i.e., four times higher cell number). ARK-1 significantly reduced the gall size in all the ratio treatments up to 1:5 ratio. In grapevine, ARK-1 was able to reduce the gall incidence up to 1:3 ratio except 1:1 ratio. The gall size in grapevine was reduced by ARK-1 treatment significantly up to 1:4 ratio, but not with 1:1 ratio. Moreover, the rate of reduction by ARK-1 co-inoculation was not as high as in tomato. The inconsistency with 1:1 ratio and reduction in ARK-1's efficacy was probably due to a lower volume of cell suspension used in this study compared with the previous ones, which resulted in 90% reduction in the cell number. We will address this issue in the future experiments. In both tomato and grapevine, ARK-1 treatment applied by itself did not produce any gall, which confirmed the non-tumorigenic nature of ARK-1. In grapevine control group 0:5, where tumorigenic strain was inoculated in 5X concentration, both gall incidence and size were significantly lower than 1:5 as well as other only tumorigenic controls (0:1 - 0:4). In summary this study

demonstrated that ARK-1 can reduce the gall formation against higher cell numbers of tumorigenic strains *in planta*.

Acknowledgements

We would like to acknowledge Ms. Akiko Nita at AHS AREC for the assistance on this project. We would also like to thank the Virginia Wine Board and USDA/NIFA Hatch project (VA-160112) for funding this project

WINE GRAPE FIELD TRIALS (BIOSAFE, PLANTAID, HELENA, AND PROTECTIVE SHIELD) AT WINCHESTER, VA, 2019.

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PlantAid and Actigard program trial for grape powdery and downy mildew at Winchester VA, 2019

The trial was conducted in ‘Chardonnay’ plot planted in 2009, trained to a vertical shoot positioning system with bilateral cordons, with a spacing of 5 ft between vines and 10 ft between rows. Vines were treated with combinations of fungicides until two weeks prior to the start of the experiment to manage downy mildew, powdery mildew, Botrytis bunch rot, and black rot. At the beginning of the experiment, there were trace levels of powdery mildew throughout the plot. Plots were consisted of three consecutive vines and are arranged in a randomized complete block design with four blocks. Treatments were applied with a 4-gal backpack hand-pumped air sprayer, regulated to 21 psi by a Gate CFValve system through a single boom with a TeeJet 8003VS flat fan nozzle. Treatment applications were conducted on 22 Aug, 29 Aug, 5 Sep, and 12 Sep 2019. During the trial, one application of Phostrol (2 pt/A) was made to suppress downy mildew on 6 Sep. The estimated percentage of the infected area (disease severity) per leaf and presence or absence of diseased tissue per leaf (disease incidence) of downy mildew and powdery mildew were visually assessed on 17 Sep. From each cordon, four shoots were randomly chosen, but the outer most cordon of each plot was considered as the buffer and not included in disease assessment. Assessment was conducted on the upper most five leaves where new growth was developed after the initiation of the trial. A total of 60 selected leaves were assessed per treatment per block (a total of 240 leaves per treatment). The generalized linear model and linear mixed model in JMP Pro (ver. 15 SAS Institute, Cary, NC) was used to conduct the analysis of variance for disease incidence and severity, respectively. Treatment was considered a fixed effect, and block was considered a random effect in the mixed model. When treatment effect was found to be significant, ad hoc multiple comparisons were conducted using Fisher’s LSD with $\alpha = 0.05$.

Winchester area experienced a relatively dry growing season. The total amount of precipitation was 84.6 (3.3 in), 140.0 (5.5 in), 55.1 (2.2 in), and 110.5 (4.4 in) mm during the month of Apr, May, Jun, and Jul. Although the amount of precipitation in May was higher than a typical year (average precipitation in May is ~3.8 in. based on 1981-2010 data, usclimatedata.com), during the critical period of grape disease infection, which is approximately a month period from bloom (~ a month of June for this year), was relatively dry compared with a typical year (1981-2010 average precipitation is ~3.7 in.). Mean leaf downy mildew incidence and severity ranged from 59% to 66% and 3% to 7%, respectively. The treatment effect on downy mildew was not significant with disease incidence (Chi-square = 3.23, $P = 0.52$), but significant with disease severity ($F = 9.91$, $P < 0.01$). PlantAid applied every 14 days resulted in a significantly higher disease severity than the negative control and all the other treatments ($P \leq 0.05$). PlantAid applied every 7 days was not significantly different from the negative control ($P > 0.05$), and resulted in significantly higher mean leaf downy mildew severity than Actigard and negative control treatments. There was no significant difference between two Actigard treatments, and both resulted in significantly lower mean leaf downy mildew severity than the negative control. Mean leaf powdery mildew incidence and severity ranged from 80% to 90% and 9% to 12%, respectively. The treatment effect on downy mildew was significant with disease incidence (Chi-square = 79.8, $P = 0.03$), but not significant with disease severity ($F = 1.56$, $P = 0.18$). Actigard applied every 14 days resulted in significantly higher powdery mildew than the negative control and the all the other treatments. None of the other treatments did not significantly differ from the negative control ($P > 0.05$).

Treatment ^z	Downy mildew on leaf				Powdery mildew on leaf		
	Days after first application ^y	Disease incidence (%)	Disease severity (%)		Disease incidence (%)	Disease severity (%)	
Actigard (ASM), 14-d (57g)	0, 14	59.2	3.6	C	81.7	B	10.4
Actigard (ASM), 7-d (57g)	0, 7, 14, 21	62.5	3.0	C	90.4	A	12.0
PlantAid Cultivator and Cleaner 14-d (25 L)	0, 14	63.7	5.1	B	83.8	B	9.4
PlantAid Cultivator and Cleaner, 7-d (25 L)	0, 7, 14, 21	65.8	7.0	A	80.4	B	11.7
Negative control	n/a	60.0	5.2	B	83.3	B	10.5

- ^z Fungicide used prior to the experiment, rate per acre (in parentheses), and date were: Captan 50 WP (3 lb), 2 Jul, Cueva (100 pt), 26 Jul, 10 Aug, Microthiol D (3 lb) 4 Jun, 15 Jun, Penncozeb 75DF (3 lb) 25 Apr, 1 May, 8 May, 16 May, 22 May, 29 May, 4 Jun, 15 Jun, Phostrol (2 pt), 2 Jul, 6 Sep, Quintec (4 fl oz) 15 Jun, 2 Jul, Ranman (2.5 fl oz) 15 Jun, Revus (7 fl oz), 29 May, 4 Jun, Vivando (15 fl oz) 28 Jun.
- ^y First treatment application was 22 May.

BioSafe program trial for grape powdery and downy mildew at Winchester VA, 2019

The trial was conducted in 'Chardonnay' plot planted in 2009, trained to a vertical shoot positioning system with bilateral cordons, with a spacing of 5 ft between vines and 10 ft between rows. Plots were consisted of three consecutive vines and are arranged in a randomized complete block design with four blocks. Treatments were applied with a 4-gal backpack hand-pumped air sprayer, regulated to 21 psi by a Gate CFValve system through a single boom with a TeeJet 8003VS flat fan nozzle. All vines were treated with: Penncozeb DF (3 lb/A) on 25 April, Penncozeb plus Microthiol D (3 lb/A) on 1, 8, and 16 May, to suppress black rot, downy mildew, and powdery mildew. Treatment applications were conducted on 22 May, 30 May, 6 Jun, 13 Jun, and 24 Jun 2019. The first application was applied at approximately seven days before bloom and the second application was applied at 50% bloom. The estimated percentage of the infected area (disease severity) per leaf and presence or absence of diseased tissue per leaf (disease incidence) of downy mildew and powdery mildew were visually assessed in 5 July. Then on 12 Aug, powdery mildew on the cluster was assessed. A total of 60 and 20 randomly selected leaves and clusters, respectively, were assessed per treatment per block. I.e., a total of 240 leaves and 80 clusters per treatment. The generalized linear mixed model (PROC GLIMMIX) in SAS (ver. 9.4 SAS Institute, Cary, NC) was used to conduct the analysis of variance. Treatment was considered a fixed effect, and the block was considered a random effect. When treatment effect was found to be significant, ad hoc multiple comparisons were conducted using Fisher's LSD with $\alpha = 0.05$.

Winchester area experienced a relatively dry growing season. The total amount of precipitation was 84.6 (3.3 in), 140.0 (5.5 in), 55.1 (2.2 in), and 110.5 (4.4 in) mm during the month of Apr, May, Jun, and Jul. The amount of precipitation in May was higher than a typical year (~3.8 in. based on 1981-2010 average, usclimatedata.com). However, during the critical period of grape disease infection, which is approximately a month period from bloom (= a month of Jun for this year), was relatively dry compared with a typical year (~3.7 in. based on 1981-2010 average, usclimatedata.com). Although we observed downy mildew during the first week of May, the environmental conditions did not favor its development. The average leaf disease incidence and severity varied from 0.4 to 9.0 and 0.01 to 0.4, respectively (Table 1). Probably due to a chance, the powdery mildew negative control treatment resulted in the highest level of downy mildew. The effect of treatment was significant for both leaf disease incidence ($F = 12.06$, $P < 0.01$) and severity ($F = 16.73$, $P < 0.01$). When BioSafe treatment was compared with the downy mildew negative control treatment, both disease incidence and severity were numerically lower, but the difference was not statistically significant ($P > 0.05$). We did not observe any downy mildew cluster infection. Powdery mildew became noticeable around the second week of June, and the effect of treatment was significant for both leaf disease incidence ($F = 12.06$, $P < 0.01$) and severity ($F = 12.06$, $P < 0.01$). The mean leaf incidence and severity of powdery mildew ranged from 0.6 to 3.2 and 0.01 to 0.07, respectively (Table 1). On the cluster, the mean powdery mildew incidence varied from 2.0 to 22.2, and severity ranged from 0.03 to 0.71 (Table 2). The treatment effect was significant for both cluster disease incidence ($F = 6.35$, $P < 0.01$), and severity ($F = 5.49$, $P < 0.01$). BioSafe treatment resulted in significantly ($P \leq 0.05$) lower mean incidence and severity on both leaf and cluster than the powdery mildew negative control treatment. BioSafe treatment was not significantly different from our standard treatment ($P > 0.05$) in both powdery mildew disease incidence and severity in both leaf and cluster.

Table 1. Treatment effect on downy mildew and powdery mildew on the grape leaf

Treatment and amount/A	Days after first applicat ion ^z	Downy mildew on leaf		Powdery mildew on leaf	
		Inciden ce	Severit y	Incidenc e	Severit y
BioSafe					
Penncozeb 75DF (3 lb) + PerCarb (3 lb) + OxiPhos (4 qt)	0				
Penncozeb 75DF (3 lb) + Rev + Q + E + Oxidate T&V (50 fl oz)	8				
Penncozeb 75DF (3 lb) + Rev + V + Oxidate T&V (50 fl oz)	15				
Penncozeb 75DF (3 lb) + Q + Oxidate T&V (50 fl oz) + OxiPhos (4 qt)	22, 33	1.29	C B	0.04	B
				0.56	B
					0.01 B
Downy mildew negative control					
Microthiol D (3 lb)	0				
Microthiol D (3 lb) + Revus (8 fl oz) + Elevate (1 lb)	8				
Microthiol D (3 lb) + Vivando (11 fl oz)	15	2.94	B	0.09	B
Microthiol D (3 lb) + Quintec (4 fl oz)	22, 33			0.85	B
					1 B
Powdery mildew check					
Penncozeb 75DF (3 lb)	0				
Penncozeb 75DF (3 lb) + Revus (8 fl oz) + Elevate (1 lb)	8				
Penncozeb 75DF (3 lb) + Revus (8 fl oz)	15	9.00	A	0.39	A
Penncozeb 75DF (3 lb) + Phostrol (3 pt)	22, 33			3.23	A
					7 A
Standard					
Penncozeb 75DF (3 lb) + Microthiol D (3 lb)	0				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Revus (8 fl oz) + Quintec (4 fl oz) + Elevate (1 lb)	8				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Revus (8 fl oz) + Vivando (11 fl oz)	15				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Phostrol (3 pt) + Quintec (4 fl oz)	22, 33				
		0.42	C	0.01	B
				1.14	A B
					0.02 B

^z First treatment application was 22 May.

Table 2. Treatment effect on downy mildew and powdery mildew on the grape cluster

Treatment and amount/A	Days after first application ^z	Powdery mildew on cluster			
		Incidence		Severity	
BioSafe					
Penncozeb 75DF (3 lb) + PerCarb (3 lb) + OxiPhos (4 qt)	0				
Penncozeb 75DF (3 lb) + Rev + Q + E + Oxidate T&V (50 fl oz)	8				
Penncozeb 75DF (3 lb) + Rev + V + Oxidate T&V (50 fl oz)	15				
Penncozeb 75DF (3 lb) + Q + Oxidate T&V (50 fl oz) + OxiPhos (4 qt)	22, 33	5.16	B	0.06	B
Downy mildew negative control					
Microthiol D (3 lb)	0				
Microthiol D (3 lb) + Revus (8 fl oz) + Elevate (1 lb)	8				
Microthiol D (3 lb) + Vivando (11 fl oz)	15				
Microthiol D (3 lb) + Quintec (4 fl oz)	22, 33	6.56	B	0.33	B
Powdery mildew check					
Penncozeb 75DF (3 lb)	0				
Penncozeb 75DF (3 lb) + Revus (8 fl oz) + Elevate (1 lb)	8				
Penncozeb 75DF (3 lb) + Revus (8 fl oz)	15				
Penncozeb 75DF (3 lb) + Phostrol (3 pt)	22, 33	22.24	A	0.71	A
Standard					
Penncozeb 75DF (3 lb) + Microthiol D (3 lb)	0				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Revus (8 fl oz) + Quintec (4 fl oz) + Elevate (1 lb)	8				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Revus (8 fl oz) + Vivando (11 fl oz)	15				
Penncozeb 75DF (3 lb) + Microthiol D (3 lb) + Phostrol (3 pt) + Quintec (4 fl oz)	22, 33	2.02	B	0.03	B

^z First treatment application was 22 May.

Helena Products

Our previous research results indicated a single mode of action (of any fungicide) was not enough to successfully manage ripe rot. Thus, with the support of VWB, we started two field trials to examine combinations of modes of action group. Unfortunately, one of the selected materials (Aprovia) was compromised during the 2018 season, probably due to development of resistance. Therefore, we investigated the alternatives. In 2019, we focused on calcium (which shows to work well on apple bitter rot, that is caused by the same pathogens), and plant defense activator(s). Elemax and Brexel are two different formulations of calcium, Vacciplant is a plant defense activator, and Kendal is a foliar nutrient (but some studies suggest it is a plant defense activator as well).

To make sure these soft materials are in effect, we applied every two weeks from May to September on Cabernet Sauvignon vines in AHS AREC. Visual assessment of diseases was made in 22 September 2019. However, we did not see the positive effect on ripe rot disease management. Kendal may suppress sour rot and Botrytis, but more data is needed.

Plant Defense Activators

There are several products that help activate plants' own defense system to fight against diseases, but very limited information is available with grapes. In the initial stage of research, I will determine the rate of a new product ASM (Actigard, Syngenta) for grape and conduct experiments to see if ASM can increase the efficacy of other fungicides.

At first, we tested the rate of Actigard to determine whether it can cause phytotoxicity or not (common issue with other crops). We tested up to 200 ppm (75 ppm is the recommended rate), but we did not observe any visible symptoms. (plus, in a greenhouse, it seems to suppress powdery mildew.)

Then we conducted a field experiment to determine its efficacy against multiple diseases. We found that Actigard can be effective against downy mildew.

Protective shield

Encouraged by the results from previous bagging experiments, our lab has been experimenting with another type of protection using a sheet of plastic (Fig. 6). We placed a sheet of 4 Mil plastic (Uline Poly Sheeting S-5853) in approximately 24 inches in length to cover the area between the first catch wire (typically placed approximately 18 inches above fruiting zone) and the fruiting zone. We used a standard size staple (6.35 mm in length) and vineyard

c-clips to secure the plastic on the wire. We did not have any frame structure to support the plastic; however, grapevines naturally produce lateral shoots. These shoots pushed up the plastic to create “umbrella” (Fig. 7). The plastic was placed in mid-May (soon after bloom). We tested the system with five-year old Chardonnay grapevines, and installed on randomly assigned four panels. We placed two temperature and relative humidity sensors to monitor environmental conditions inside and outside of the plastic (Spectrum Technologies, Inc., model WatchDog A150 Temp/RH Logger)(Fig. 6).

Diseases (Botrytis bunch rot, black rot, powdery mildew, ripe rot, and sour rot) were visually measured at harvest.

It took less than 30 min with three people to place these plastic sheets onto four panels of grapevines, including time to experiment with staples and c-clips. This was considerably faster than bagging, which typically takes 2 min per 5 clusters (~ 48 min per panel of six vines, assuming 20 clusters per vine) based on our estimate. I.e., we were able to set up the shelter in less than 1/6 of time required for bagging.

Despite of the very simple set up, these shelters lasted from May to October. We noticed small holes, most likely due to our activities, but all eight plastic sheets held together. As expected, the inside of the plastic cover tends to warmer than outside, but accumulation of humidity was less than I anticipated (Fig. 8). We also noticed that since the plastic can flap with winds, if we adjust our



Figure 6. Clear plastic shield on cultivar Chardonnay, May 2019



Figure 7. Plastic shelter at harvest. Lateral shoots push plastic up to create a natural umbrella structure, Aug 2019

sprayer nozzles upwards from the underside of the fruiting zone, we were able to spray into the fruiting zone. Since 2019 season was very dry, we did not find any fruit rots on the clusters regardless of the treatment. (i.e., mean disease severity was less than 0.01 % for all measured disease, regardless of the shelter treatment.)

**THE INTENSITY OF PHYTOTOXICITY ON GRAPE LEAVES BY A MIXTURE OF
COPPER AND PHOSPHORUS ACID DEPENDS ON THE COPPER FORMULATION
AND WATER PH.**

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One of the commonly used fungicides against downy mildew, phosphorous acid (e.g., Prophyt, Phostrol, etc.), is known to cause phytotoxicity when applied in higher than recommended rate. We conducted a series of greenhouse experiments to understand this potential issue.

In order to checking the phosphorous acid's phytotoxicity by rate, we used Phostrol. Rate tested were 2.92 L/ Ha (2.5 pints/acre), 4.09 L/ Ha (3.5 pints/acre), 5.26 L/ Ha (4.5 pints/acre), 5.85 L/ Ha (5.0 pints/acre) and 8.77 L/ Ha (7.5 pints/acre). The treatment was applied to potted Chardonnay vines using a 1-gal hand sprayer. There were three replications per run and two runs. Five random leaves were visually evaluated at three days after treatment application for incidence and severity (% area affected). The data was analyzed using generalized linear regression in JMP Pro (ver. 14, SAS Institute, Cary, NC).

Our results indicated that Phostrol can cause phytotoxicity on Chardonnay at 5 pints/acre rate, which is the highest recommended rate on its label.

Next, we investigated the effect of water pH and Copper on Prophyt phytotoxicity on grape. The number of vines, cultivar, spray method, and statistical analysis were the same as the previous experiment. We prepared water at pH 5, 7, and 9. Prophyt was added to the pH adjusted water at 2 pints/acre. Then there were three copper treatments, Cueva (2%), Basic Copper (2 lb/A), and no copper control.

When Prophyt was added to the water, it adjusted the pH of water close to pH 6.0, regardless of the original water pH. The original water pH of 5 caused significantly higher phytotoxicity in both incidence and severity regardless of the type of copper treatment. The addition of copper, whether Cueva or Basic Copper, resulted in significantly higher phytotoxicity at pH 7 and 10.

In conclusion, mixture of copper compound and Prophyt can cause phytotoxicity, regardless of water pH.

MANAGING FIRE BLIGHT WITH PROHEXADIONE-CALCIUM APPLIED PRE-BLOOM

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Fire blight is routinely managed with antibiotic applications targeting blossom and shoot blight, yet the disease continues to cause erratic and devastating outbreaks, especially problematic for young, high-density plantings in which trees can be killed with a single strike. Blossom blight, considered the primary infection stage, requires extremely precise management and is a major source of inoculum for shoot blight infections later in the season, but is often difficult to detect. The challenge to effectively manage blossom blight coupled with pressure from regulators and consumers to reduce antibiotic use make it prudent that we identify alternative management strategies. The plant growth regulator prohexadione-calcium (PhCa) has been known to reduce shoot blight when applied at petal fall since it was first introduced in the U.S. in the 1990s, but at the cost of reducing tree vigor. Although a few studies indicate potential for earlier applications and carry-over effects of PhCa from the previous season, little work has been done investigating pre-bloom programs. In this work, we evaluated the effects of PhCa applied at low rates, pre-bloom on blossom and shoot blight as well as tree growth and productivity.

Methods: In 2019, paired trials were established at two research orchards: Cornell AgriTech in Geneva, NY and the University of Vermont Horticultural Research Station in Burlington, VT. At each site a mature, bearing block (Gala/B.9 or Crimson Crisp and Topaz/G.31) and a young block (NY-1/G.935 or Macoun/G.31) was selected, and treatments were applied in a RCBD to single trees or a panel of trees respectively. In the mature blocks, treatments included untreated trees, streptomycin applied at bloom, streptomycin followed by PhCa (6oz) at petal fall and 14 days later, PhCa (3 and 6oz) applied at tight cluster, PhCa (3 and 6oz) applied at pink, PhCa (2oz) + Actigard (1oz) applied at pink and again at petal fall, PhCa (3oz) + the phosphite fungicide Rampart (64fl oz) applied at pink and again at petal fall, and Regalia (32fl oz/acre) + MagnaBon (16fl oz/acre) applied at pink and again at petal fall. In young blocks, treatments included untreated trees, streptomycin applied at bloom followed by PhCa (6oz) at pink and 14 days later, PhCa (3 and 6oz) followed by Serenade Optimum at bloom, and a 'trickle' program of PhCa (2oz) applied at pink followed by PhCa (1oz) applied at bloom, 14 days, and 28 days post-bloom. Commercial products used in treatments included Firewall 17 at 24 oz (streptomycin), Apogee (PhCa, rates applied per 100 gal), and Serenade Optimum (*Bacillus subtilis* strain QST 713, applied at 20oz), and Actigard (acibenzolar-s-methyl, rates applied per 100 gal). All applications were made using a Solo 451-B gas-powered mist blower. Mature blocks were inoculated at bloom, within 24 hours of bloom treatments with Ea273 at 106 CFU/mL using a hand-pumped Solo 475 backpack sprayer. Young blocks were not inoculated, in order to observe horticultural effects in the absence of disease pressure and associated strike pruning. Blossom and shoot blight were evaluated in mature blocks as soon as symptoms were reliably visible, and were represented as percent incidence of 20 randomly

selected blossom clusters or shoots per replication. Horticultural parameters evaluated in all blocks included shoot length, trunk circumference, fruit number, and fruit size, evaluated at harvest.

In addition, trials were established in young, high-density orchards at four commercial orchards in New York representing western and eastern parts of the state. Treatments included untreated trees, Firewall17 (24oz) applied at bloom, and Apogee (3 or 6oz/100gal) applied at pink followed by Serenade Optimum (20oz) applied at bloom. Treatments were evaluated for horticultural characteristics described above.

Results: In the mature orchard at Cornell AgriTech, all PhCa treatments provided excellent blossom and shoot blight control, with less than 20% or 10% incidence respectively. Tight cluster applications tended to provide slightly better blossom blight control, while pink applications tended to provide slightly better shoot blight control (Fig.1 A&B). This could be explained by optimal application timing and the 7-14 days required for PhCa to take effect. Horticultural parameters were minimally affected by treatments, with the exception of post-bloom PhCa causing a reduction in shoot length, as expected. In some cases, tree vigor of trees treated with streptomycin was also slightly reduced. In the mature orchard at UVM, PhCa treatments were not effective at reducing blossom blight (Fig.1 C&D). This may have been due to extremely low vigor (typically <10cm shoot growth) resulting from minimal fertilization and very sandy soil of this block. With such low shoot growth, the development shoot blight was so low that it did not allow for treatment comparison. In both of the young blocks, horticultural parameters were unaffected by treatments. Similarly, at all commercial farm locations, horticultural parameters were minimally affected or varied between sites, with the exception of a streptomycin and post-bloom PhCa program, which reduced vigor as expected. Results indicate the potential for PhCa treatment pre-bloom for managing fire blight without negative horticultural impacts on mature or new orchard blocks. These positive results are corroborated by our findings from 2016-18 at Cornell AgriTech. However, results at the UVM site indicate that PhCa may not be effective in sites with exception low tree vigor. Experiments will be replicated in 2020 to validate results.

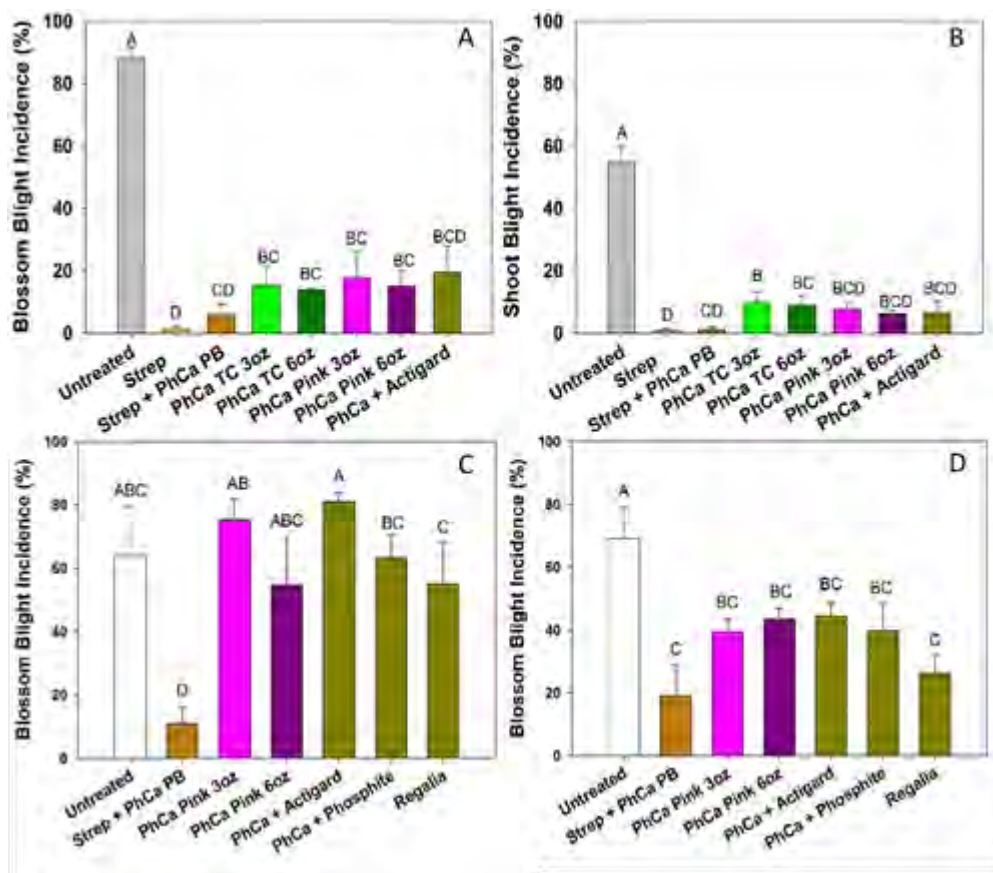


Figure 1. Mean disease incidence (± 1 SE) for semi-dwarf trees receiving various fire blight management programs at Cornell AgriTech in Geneva, NY (A&B, Gala/B.9 planted 2020) or University of Vermont in Burlington, VT (C&D, Crimson Crisp/G.31). PhCa: Prohexadione-calcium applied as Apogee at 3 or 6oz/100gal at either tight cluster (TC) or pink (Pink); Strep: streptomycin (FireWall17) applied at bloom at 24oz/acre; Strep + PhCa PB: streptomycin applied at bloom at 24oz/acre and PhCa applied at petal fall and 2 weeks after at 6oz/100gal; PhCa + Actigard: PhCa at 2oz/100gal and Actigard at 1oz/100gal applied at pink and again at petal fall; PhCa + Phosphite: PhCa at 3oz/100gal and a phosphite fungicide (Rampart) at 64fl oz/acre applied at pink and again at petal fall; Regalia: regalia at 32fl oz/acre and MagnaBon at 16fl oz/acre applied at pink and again at petal fall. Within each graph, different letters above bars indicate significant differences between means based on Tukey HSD test ($p < 0.05$).

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APPLE (*Malus domestica* 'Ramey York')
 Scab; *Venturia inaequalis*
 Cedar-apple rust; *Gymnosporangium juniperi-virginianae*
 Powdery mildew; *Podosphaera leucotricha*
 Sooty blotch; disease complex
 Flyspeck; *Zygophiala jamaicensis*
 Bitter rot; *Colletotrichum* spp.
 White rot; *Botryosphaeria dothidea*
 Fruit finish

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Evaluation of experimental and registered fungicides for disease control on York apple, 2019.

Sixteen treatments were compared for broad spectrum disease control on 20-yr-old trees. The test was conducted in a randomized block design with four single-tree replicates separated by in-row border trees. Dilute treatments were applied to runoff at 250 psi with a single nozzle handgun as first-seventh cover sprays: 10 Apr (TC, tight cluster); 17 Apr (Pk, pink); 25 Apr (Bl, bloom); 6 May (PF, petal fall); First-seventh covers (1-7C): 16 May, 30 May, 15 Jun, 28 Jun, 16 Jul, 1 Aug, and 20 Aug. Inoculum over each test tree included rust galls and bitter rot mummies placed 11 Apr and wild blackberry canes with the sooty blotch and flyspeck fungi placed 19 Apr. Other diseases developed from inoculum naturally present in the test area. Foliar data are based on all leaves on ten shoots per rep 10 Jul. Fruit ratings are based on 25-fruit samples per replication picked 4 Oct and first rated 8 Oct, then incubated in ambient warm temperatures (65-84° F.), and rated for rots 22 Oct and 1 Nov after 18 day (mean 71.7° F) and 28 days' incubation (mean 73.8° F). Maintenance materials applied to the entire test block included: Assail, Closer, Delegate, Imidan, Lannate LV, and Voliam Flexi. Percentage data were converted by the square root arcsin transformation for statistical analysis.

Under moderate early season scab pressure, all treatments gave significant control on leaves and fruit (Table 1). These involved Inspire Super + Manzate (treatment #1) or these alternated with other combinations (treatments #3 and 8). Several treatments were significantly weaker for scab control than others: Luna Sensation (#6), EcoSwing (#10) and PerCarb/OxiDate T&V (#15). Including Captan with EcoSwing and including Inspire Super with OxiDate overcame these apparent weaknesses. Mildew pressure was relatively light, and all treatments gave significant control compared to non-treated trees. Cedar rust pressure was heavy, with inoculum provided by galls placed over the test trees and from nearby cedar trees. There were eight rust infection periods ranging from 7 Apr to 13 May. Under these conditions, treatments involving Inspire Super + Manzate (treatment #1, 2, 3, 8 and 16) gave strong control, as well as Cevya (#4), Regalia + JMS Stylet-Oil (#14), the high rate of GWN-10474 (Trt #13), and BCS-AR83685 + Laguna (#9). Several other treatments gave significant suppression of rust, but were noticeably weaker than the Inspire + Manzate combination (#6, 7, 10, 11, 12, and 15). There were significantly more rust lesions per leaf where Captan was mixed with EcoSwing (#11) than with EcoSwing alone (#10). All treatments gave excellent control of cedar apple rust on fruit. The "leaf spots" on treatments with good rust control suggests that the leaf spots may have been partially inhibited rust lesions. Summer disease pressure was relatively light (Table 2), with some early accumulation of wetting hours, reaching 227 accumulated wetting hours from 12 May through 8 Jul, but then only 86 additional wetting hours occurred over the next five weeks. Under these conditions, control of sooty blotch and flyspeck (SBFS) was not difficult, and all treatments gave complete control. Fruit rots, primarily composed of bitter rot and white rot, were quite variable and increased in incidence from harvest through the 28-day incubation period. At harvest, eight treatments showed significant suppression of rots, but at the 18-day and 28-day incubation assessments, only treatment #8 (alternating schedule of Merivon or Inspire Super with Manzate TC-3C, or Captan 4C-7C) and #11 (EcoSwing + Captan) gave significant control of rots. Most treatments significantly increased russet, with treatments #12 and 13, involving GWN-10474 + Induce and Regalia + JMS Stylet-Oil (#14), resulting in the highest russet ratings. Treatments #12, 13 and 14 also significantly increased opalescence compared to non-treated trees.

Table 1. Early season disease control and fruit finish on Ramey York, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	Scab		Mildew	Cedar-apple rust			“Leaf spots”	Finish rating**	
		% lvs	% fruit	% lvs inf.	% lvs	lesions / leaf	% fruit	% leaves affected*	Russet	Opal-escence
0 No fungicide	--	39 e	41 d	9 g	69 f	18.1 d	17 b	28 d-f	0.5 a	0.9 a
1 Inspire Super 3 fl oz + Manzate 75DF 12 oz	TC-3C								1.3 b-d	0.8 a
Inspire Super 3 fl oz + Captan 80WDG 7.5 oz	4C-7C	1 a	0 a	3 f	5 a	0.2 a	0 a	17 a-c		
2 Inspire Super 3 fl oz	TC-7C	2 ab	4 a	2 d-f	7 ab	0.3 a	0 a	27 c-e	1.1 a-d	0.8 a
3 Torino 0.85SC 1.7 fl oz + Manzate 12 oz + Induce 1 pt	TC									
Inspire Super 3 fl oz + Manzate 75DF 12 oz	Pk-3C								1.6 c-e	1.2 a
Inspire Super 3 fl oz + Captan 80WDG 7.5 oz	4C-7C	1 a	3 a	4 f	4 a	0.1 a	0 a	16 ab		
4 Cevya 3.34SC (BAS 750 07F) 1 fl oz	TC-7C	6 b-d	1 a	2 c-f	6 a	0.1 a	0 a	27 c-e	1.0 a-c	1.0 a
5 Cevya 3.34SC (BAS 750 07F) 1.25 fl oz	TC-7C	4 a-c	0 a	2 ef	10 ab	0.3 a	0 a	32 ef	1.3 b-d	1.0 a
6 Luna Sensation 500SC 1.25 fl oz	TC-7C	10 cd	6 ab	0 a	22 d	1.6 ab	0 a	22 b-e	1.1 a-d	0.9 a
7 Merivon 4.18SC 1.25 fl oz	TC-7C	3 ab	0 a	<1 ab	16 b-d	0.5 a	0 a	21 a-d	1.5 b-e	0.9 a
8 Merivon 1.25 fl oz+ Manzate 75DF 12 oz	TC,BI,1C,3C								1.3 b-d	1.3 a
Inspire Super 3 fl oz + Manzate 12 oz	Pk,PF,2C									
Merivon 1.25 fl oz+ Captan 80WDG 7.5 oz	5C,7C									
Inspire Super 3 fl oz + Captan 7.5 oz	4C,6C	1 a	0 a	<1 a-c	4 a	0.1 a	0 a	14 a		
9 BCS-AR83685 1 oz + Laguna 1 oz	TC-7C	2 ab	1 a	<1 ab	8 ab	0.2 a	0 a	26 c-e	1.4 b-d	1.2 a
10 EcoSwing 8 fl oz	TC-7C	11 cd	3 a	2 c-f	38 e	2.5 b	0 a	39 fg	0.9 ab	1.0 a
11 EcoSwing 8 fl oz + Captan 80WDG 7.5 oz	TC-7C	3 ab	0 a	1 b-f	41 e	5.1 c	0 a	30 d-f	1.4 b-e	0.9 a
12 GWN-10474 7.0 oz + Induce 1 pt	TC-7C	4 a-c	0 a	0 a	18 cd	1.0 ab	0 a	38 fg	2.2 e	1.8 b
13 GWN-10474 8.75 oz+ Induce 1 pt	TC-7C	4 ab	4 a	<1 ab	9 a-c	0.3 a	0 a	32 ef	2.9 f	2.3 b
14 Regalia 1 pt + JMS Stylet-Oil 1 gal	TC-7C	7 b-d	13 bc	1 a-e	8 ab	0.2 a	0 a	47 g	2.9 f	3.5 c
15 PerCarb 3.0 lb	TC-Pk									
OxiDate T&V 50 fl oz	BI-PF								1.5 b-e	1.3 a
PerCarb 3.0 lb	1C-7C	14 d	24 c	3 ef	48 e	5.3 c	0 a	47 g		
16 OxiDate T&V 4 qt + OxiPhos 1.5 pt	TC,BI,1C,3C,5C,7C								1.7 de	1.2 a
OxiDate T&V 50 fl oz + Inspire Super 3 fl oz	Pk,PF,2C,4C,6C	2 a	1 a	1 a-d	8 a	0.3 a	0 a	26 c-e		

Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single-tree replications, ratings of all leaves on each of 10 shoots/tree, 10 Jul.

Fruit ratings were of 25-fruit samples per replication, taken 4 Oct and evaluated 8 Oct.

*Leaf spots” refers to an unidentified symptom; could be inhibited c-a rust, frog-eye leaf spot or an injury.

** Fruit finish rated on a scale of 0-5 (0 = perfect finish, 5 = severe russet or opalescence).

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi on the following dates: 10 Apr (TC, tight cluster); 17 Apr (Pk, pink); 25 Apr (BI, bloom); 6 May (PF, petal fall); First-seventh covers (1C-7C): 16 May, 30 May, 15 Jun, 28 Jun, 16 Jul, 1 Aug, 20 Aug.

Table 2. Summer disease control on Ramey York apple, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	% fruit infected at harvest			% post-storage		
		Sooty blotch	Fly speck	Fruit rot	18-day incubation		
					Any rot	Bitter rot	White rot
0 No fungicide	--	87 b	36 b	8 b-d	13 bc	5 a-c	8 c
1 Inspire Super 3 fl oz + Manzate 75DF 12 oz	TC-3C						
Inspire Super 3 fl oz + Captan 80WDG 7.5 oz	4C-7C	0 a	0 a	0 a	3 ab	0 a	3 ab
2 Inspire Super 3 fl oz	TC-7C	0 a	0 a	3 ab	7 ab	3 a	4 a-c
3 Torino 0.85SC 1.7 fl oz + Manzate 75DF 12 oz + Induce 1 pt	TC						
Inspire Super 3 fl oz + Manzate 75DF 12 oz	Pk-3C						
Inspire Super 3 fl oz + Captan 80WDG 7.5 oz	4C-7C	0 a	0 a	0 a	4 ab	0 a	4 a-c
4 Cevya 3.34SC (BAS 750 07F) 1 fl oz	TC-7C	0 a	0 a	4 ab	7 ab	5 ab	1 ab
5 Cevya 3.34SC (BAS 750 07F) 1.25 fl oz	TC-7C	0 a	0 a	14 cd	14 bc	14 bc	0 ab
6 Luna Sensation 500SC 1.25 fl oz	TC-7C	0 a	0 a	0 a	8 a-c	6 a-c	0 ab
7 Merivon 4.18SC 1.25 fl oz	TC-7C	0 a	0 a	0 a	1 ab	1 a	0 a
8 Merivon 1.25 fl oz + Manzate 75DF 12 oz	TC,BI,1C,3C						
Inspire Super 3 fl oz + Manzate 75DF 12 oz	Pk,PF,2C						
Merivon 1.25 fl oz + Captan 80WDG 7.5 oz	5C,7C						
Inspire Super 3 fl oz + Captan 80WDG 7.5 oz	4C,6C	0 a	0 a	1 a	1 a	1 a	0 ab
9 BCS-AR83685 1 oz + Laguna 1 oz	TC-7C	0 a	0 a	3 ab	5 ab	3 a	3 ab
10 EcoSwing 8 fl oz	TC-7C	0 a	0 a	15 d	22 c	14 c	7 bc
11 EcoSwing 8 fl oz + Captan 80WDG 7.5 oz	TC-7C	0 a	0 a	0 a	0 a	0 a	0 ab
12 GWN-10474 7.0 oz + Induce 1 pt	TC-7C	0 a	0 a	6 a-d	11 a-c	6 a-c	6 a-c
13 GWN-10474 8.75 oz+ Induce 1 pt	TC-7C	0 a	0 a	2 ab	5 ab	4 a	1 ab
14 Regalia 1 pt + JMS Stylet-Oil 1 gal	TC-7C	0 a	0 a	1 a	8 a-c	2 a	6 a-c
15 PerCarb 3.0 lb	TC-Pk						
OxiDate T&V 50 fl oz	BI-PF						
PerCarb 3.0 lb	1C-7C	0 a	3 a	5 a-c	7 a-c	5 a-c	1 ab
16 OxiDate T&V 4 qt + OxiPhos 1.5 pt	TC,BI,1C,3C,5C,7C						
OxiDate T&V 50 fl oz + Inspire Super 3 fl oz	Pk,PF,2C,4C,6C	0 a	0 a	0 a	8 a-c	5 a-c	3 a-c

Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single-tree replications, fruit ratings were of 25-fruit samples per replication, taken 4 Oct and first evaluated 8 Oct.

* Final rating for rots after 18 and 28 days' incubation at warm ambient temperatures 65-84° F (18-day mean 71.7° F; 28-day mean 73.8° F).

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi on the following dates: 10 Apr (TC, tight cluster); 17 Apr (Pk, pink);

25 Apr (BI, bloom); 6 May (PF, petal fall); First-seventh covers (1-7C): 16 May, 30 May, 15 Jun, 28 Jun, 16 Jul, 1 Aug, 20 Aug.

APPLE (*Malus domestica* 'Fuji')
 Scab; *Venturia inaequalis*
 Cedar-apple rust; *Gymnosporangium juniperi-virginianae*
 Powdery mildew; *Podosphaera leucotricha*
 Sooty blotch; disease complex
 Flyspeck; *Zygophiala jamaicensis*
 Bitter rot; *Colletotrichum* spp.
 White rot; *Botryosphaeria dothidea*
 Fruit finish

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Evaluation of experimental and registered fungicides for disease control on Fuji apple, 2019.

Thirteen treatments were compared for broad spectrum disease control on 24-yr-old trees. The test was conducted in a randomized block design with four single-tree replicates separated by in-row border trees. Dilute treatments were applied to runoff at 250 psi with a single nozzle handgun as first-sixth cover sprays: 18 Apr (Pk, pink); 25 Apr (Bl, bloom); 7 May (PF, petal fall); first-sixth covers (1C-6C): 16 May, 30 May, 15 Jun, 28 Jun, 19 Jul, 5 Aug. Treatments #2-7 were parallel, with the test fungicide applied only in two applications, at pink and petal fall, and these were alternated with Inspire Super at bloom and first cover. Treatment #7 did not receive the two applications at pink and petal fall, only Inspire Super at bloom and first cover. Treatments #2-7 were all covered with Captan 2nd through 6th cover. Inoculum over each test tree included rust galls, bitter rot mummies, and wild blackberry canes with the sooty blotch and flyspeck fungi, placed 19 Apr. Other diseases developed from inoculum naturally present in the test area. Foliar data are based on all leaves on ten shoots per replication 16 Jul. Fruit ratings are based on 25-fruit samples per replication picked 27 Sept and first rated 1 Oct, then incubated in ambient warm temperatures (65-89° F), and rated for rots 17 Oct (mean 72.5° F), and 28 Oct (mean 73.4° F), after 20 and 31 days' incubation. Maintenance materials applied to the entire test block included: Assail, Closer, Delegate, Imidan, Lannate LV, and Voliam Flexi. Percentage data were converted by the square root arcsin transformation for statistical analysis.

Cedar rust pressure was heavy, with inoculum provided by galls over the test trees and from nearby cedar trees, and there were eight rust infection periods from 7 Apr to 13 May. Under these conditions, Inspire Super + Manzate (treatment #1) gave the most control (Table 3). Generally, other treatments with less Inspire or mancozeb in the schedule were weaker for rust control (Trts # 8-11). Treatment #7, with just the two applications of Inspire Super through 1C, gave rust control similar to #2-6, implying that most of the rust control in those treatments came from Inspire Super. Treatments #12 and 13, involving experimental LBG-FS2 + mancozeb, showed improved rust control where Inspire was substituted for LBG-FS2 + mancozeb in just a single application at 1C (#12 vs. #13). All treatments gave excellent control of cedar apple rust on fruit. The "leaf spots" on treatments with good rust control suggests that the leaf spots may have been partially inhibited rust lesions. Under moderate early season scab pressure, all treatments gave significant control (Table 4). Treatment #7, with just the two applications of Inspire Super at pink and petal fall, shows the benefit and improved control by the other components in the schedule at pink and petal fall in treatments #2-6. Mildew pressure was light, and nearly all treatments gave significant control compared to non-treated trees (Table 4). Summer disease pressure was relatively light (Table 5), with some early accumulation of wetting hours, reaching 227 accumulated wetting hours from 12 May through 8 Jul, but then only 86 additional wetting hours occurred over the next five weeks. Under these conditions, control of sooty blotch and flyspeck was not difficult, and nearly all treatments gave complete control. Fruit rots, primarily composed of bitter rot and white rot, generally increased in incidence from harvest through the 31-day incubation period and all treatments gave significant control at all assessment intervals (Table 5 and 6). Among the best treatments were #4 (Luna Sensation/Inspire Super/ Captan) and #8 (Manzate/A19649B/Inspire Super/ Captan). Most treatments significantly increased russet compared to non-treated trees, and several of them also significantly increased opalescence compared to non-treated trees.

Table 3. Rust control and leaf spot suppression on Fuji apple, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	Cedar-apple rust			“Leaf spots”*	
		% leaves infected	lesions/leaf	% fruit	% leaves affected	lesions/leaf
0 No fungicide	--	46 f	2.6 c	4 b	58 g	3.3 d
1 Inspire Super 3 fl oz + Manzate 12 oz Captan 80WDG 20 oz	Pk-1C 2C-6C	5 a	0.2 a	0 a	10 a	0.2 ab
2 Excalia 2.84SC 0.75 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	9 bc	0.4 ab	0 a	11 a	0.2 a
3 Excalia 2.84SC 1.0 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	8 a-c	0.3 ab	0 a	21 cd	0.8 bc
4 Luna Sensation 1.25 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	10 b-d	0.4 ab	0 a	11 a	0.2 ab
5 Aprovia 0.83EC 1.39 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	7 ab	0.3 ab	0 a	14 a-c	0.3 a-c
6 Sercadis 2.47SC 0.88 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	10 b-d	0.3 ab	0 a	13 ab	0.3 a-c
7 --- Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	No Pk or PF Bl, 1C 2C-6C	9 bc	0.3 ab	0 a	30 f	0.8 a-c
8 Manzate Pro-Stick 75DF 12 oz A19649B 0.86 fl oz Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	11 b-d	0.4 ab	0 a	22 de	0.6 a-c
9 Manzate Pro-Stick 75DF 12 oz A19649H 0.86 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	14 de	0.5 ab	0 a	27 ef	0.8 c
10 Manzate Pro-Stick 75DF 12 oz Aprovia 0.83EC 1.38 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	12 c-e	0.4 ab	0 a	24 d-f	0.7 a-c
11 Manzate Pro-Stick 75DF 12 oz Luna Sensation 4.17SC 1.25 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C covers	9 bc	0.4 ab	0 a	18 b-d	0.4 a-c
12 Manzate 75DF 12 oz + LBG-FS2 14 fl oz Inspire Super 2.82SC 3 fl oz LBG-FS2 14 fl oz + Captan 80WDG 20 oz	Pk-PF 1C 2C-6C	12 cd	0.4 ab	0 a	28 ef	0.8 a-c

13 Manzate 75DF 12 oz + LBG-FS2 14 fl oz	Pk-1C	18e	0.6b	0a	24 d-f	0.7 a-c
LBG-FS2 14 fl oz + Captan 80WDG 20 oz	2C-6C					

Mean separation by Waller-Duncan K-ratio t-test ($p=0.05$). All leaves rated on each of 10 shoots/tree from four single-tree reps, 16 Jul or 25-fruit samples picked 27 Sep and rated 1 Oct.

*"Leaf spots" refers to an unidentified symptom; could be inhibited cedar-apple rust, frog-eye leaf spot or

an injury response.

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi.

Treatment application dates: 18 Apr (Pk, pink); 25 Apr (Bl, bloom); 7 May (PF, petal fall);

First-sixth covers (1C-6C): 16 May, 30 May, 15 Jun, 28 Jun, 19 Jul, 5 Aug.

Table 4. Scab and mildew control and fruit finish on Fuji apple, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	Scab		Mildew,	Fruit finish	
		% leaves infected	% fruit	% leaves	russet ratings (0-5)*	opalescence
0 No fungicide	--	24 e	26 b	4.9 c	1.5 a	0.6 a
1 Inspire Super 3 fl oz + Manzate 12 oz Captan 80WDG 20 oz	Pk-1C 2C-6C	6 b-d	1 a	1.3 bc	1.6 a	1.1 bc
2 Excalia 2.84SC 0.75 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	2 ab	0 a	0.6 ab	2.3 b-d	1.0 ab
3 Excalia 2.84SC 1.0 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	<1 a	2 a	0.4 ab	2.4 b-d	1.2 b-d
4 Luna Sensation 1.25 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	2 ab	1 a	0.3 ab	2.4 b-d	1.5 c-e
5 Aprovia 0.83EC 1.39 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	2 a-c	0 a	0 a	2.6 cd	1.6 de
6 Sercadis 2.47SC 0.88 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	5 b-d	1 a	0.3 ab	2.7 d	1.7 e
7 --- Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	No Pk or PF Bl, 1C 2C-6C	9 cd	2 a	0.4 ab	2.4 b-d	1.2 b-d
8 Manzate Pro-Stick 75DF 12 oz A19649B 0.86 fl oz Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	3 b-d	0 a	0.6 ab	2.2 b-d	1.3 b-e
9 Manzate Pro-Stick 75DF 12 oz A19649H 0.86 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	3 b-d	1 a	0.8 ab	2.4 b-d	1.1 bc
10 Manzate Pro-Stick 75DF 12 oz Aprovia 0.83EC 1.38 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	3 b-d	0 a	0.6 ab	2.6 cd	1.2 bc
11 Manzate Pro-Stick 75DF 12 oz+- Luna Sensation 4.17SC 1.25 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C covers	9 d	3 a	0.6 ab	2.1 a-c	1.0 ab
12 Manzate 75DF 12 oz + LBG-FS2 14 fl oz Inspire Super 2.82SC 3 fl oz LBG-FS2 14 fl oz + Captan 80WDG 20 oz	Pk-PF 1C 2C-6C	3 b-d	1 a	0.3 ab	1.9 ab	1.3 b-e
13 Manzate 75DF 12 oz + LBG-FS2 14 fl oz LBG-FS2 14 fl oz + Captan 80WDG 20 oz	Pk-1C 2C-6C	5 b-d	0 a	0.3 ab	1.9 ab	1.5 c-e

Mean separation by Waller-Duncan K-ratio t-test (p=0.05). All leaves rated on each of 10 shoots/tree from four single-tree reps, 16 Jul or 25-fruit samples picked 27 Sep and rated 1 Oct.

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi.

* Fruit finish rated on a scale of 0-5 (0 = perfect finish, 5 = severe russet or opalescence, presumed not to be mildew).

Treatment application dates: 18 Apr (Pk, pink); 25 Apr (Bl, bloom); 7 May (PF, petal fall);

First-sixth covers (1C-6C): 16 May, 30 May, 15 Jun, 28 Jun, 19 Jul, 5 Aug.

Table 5. Sooty blotch and flyspeck and rot control on Fuji apple, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	Sooty blotch		Flyspeck		% fruit inf. 'at harvest'		
		% fruit	% area	% fruit	% area	Any rot	Bitter rot	White rot
0 No fungicide	--	98 b	18 b	78 b	5 c	64 d	17 c	4 b
1 Inspire Super 3 fl oz + Manzate 12 oz Captan 80WDG 20 oz	Pk-1C 2C-6C	1 a	<1 a	0 a	0 a	1 ab	1 a	0 a
2 Excalia 2.84SC 0.75 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	0 a	0 a	0 a	0 a	2 ab	1 a	0 a
3 Excalia 2.84SC 1.0 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	0 a	0 a	0 a	0 a	5 c	0 a	1 a
4 Luna Sensation 1.25 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	0 a	0 a	0 a	0 a	1 ab	1 a	0 a
5 Aprovia 0.83EC 1.39 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	0 a	0 a	0 a	0 a	2 ab	0 a	2 ab
6 Sercadis 2.47SC 0.88 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	1 a	<1 a	0 a	0 a	1 ab	0 a	1 a
7 --- Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	No Pk or PF Bl, 1C 2C-6C	0 a	0 a	0 a	0 a	4 c	0 a	0 a
8 Manzate Pro-Stick 75DF 12 oz A19649B 0.86 fl oz Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	0 a	0 a	0 a	0 a	1 ab	0 a	0 a
9 Manzate Pro-Stick 75DF 12 oz A19649H 0.86 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	0 a	0 a	0 a	0 a	0 a	0 a	0 a
10 Manzate Pro-Stick 75DF 12 oz Aprovia 0.83EC 1.38 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	1 a	<1 a	1 a	<1 b	4 a-c	0 a	1 ab
11 Manzate Pro-Stick 75DF 12 oz Luna Sensation 4.17SC 1.25 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C covers	1 a	<1 a	0 a	0 a	8 c	0 a	2 ab
12 Manzate 75DF 12 oz + LBG-FS2 14 fl oz Inspire Super 2.82SC 3 fl oz LBG-FS2 14 fl oz + Captan 80WDG 20 oz	Pk-PF 1C 2C-6C	0 a	0 a	0 a	0 a	9 c	0 a	2 ab
13 Manzate 75DF 12 oz + LBG-FS2 14 fl oz LBG-FS2 14 fl oz + Captan 80WDG 20 oz	Pk-1C 2C-6C	0 a	0 a	0 a	0 a	3 a-c	3 b	0 a

Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Counts 25-fruit samples picked 27 Sep and rated 1 Oct.

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi.

Treatment application dates: 18 Apr (Pk, pink); 25 Apr (Bl, bloom); 7 May (PF, petal fall);

First-sixth covers (1C-6C): 16 May, 30 May, 15 Jun, 28 Jun, 19 Jul, 5 Aug.

Table 6. Post-harvest rot control on Fuji apple, 2019. Virginia Tech AREC.

Treatment and rate/100 gal dilute	Timing	% infection, post-storage rots*					
		20-day incubation			31-day incubation		
		Any rot	Bitter rot	White rot	Any rot	Bitter rot	White rot
0 No fungicide	--	4 5 c	37 e	10 d	51 f	36 c	17 e
1 Inspire Super 3 fl oz + Manzate 12 oz Captan 80WDG 20 oz	Pk-1C 2C-6C	4 ab	2 a-c	2 a-c	10 b-e	4 ab	6 c-e
2 Excalia 2.84SC 0.75 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	9 b	8 d	1 ab	18 e	10 b	5 b-d
3 Excalia 2.84SC 1.0 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	1 0 b	2 a-c	8 cd	12 c-e	2 a	9 de
4 Luna Sensation 1.25 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	3 a	0 a	2 ab	4 ab	1 a	2 a-c
5 Aprovia 0.83EC 1.39 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	6 ab	2 a-c	4 a-d	9 a-e	3 ab	6 b-d
6 Sercadis 2.47SC 0.88 fl oz + Syl-Coat Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk, PF Bl, 1C 2C-6C	4 ab	3 a-d	2 ab	8 a-e	7 ab	2 a-c
7 --- Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	No Pk or PF Bl, 1C 2C-6C	7 ab	5 d	2 a-c	15 de	9 b	4 b-d
8 Manzate Pro-Stick 75DF 12 oz A19649B 0.86 fl oz Inspire Super 2.82EW 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	2 a	1 ab	1 ab	3 a	2 a	1 ab
9 Manzate Pro-Stick 75DF 12 oz A19649H 0.86 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	3 a	1 ab	0 a	6 a-c	3 ab	0 a
10 Manzate Pro-Stick 75DF 12 oz Aprovia 0.83EC 1.38 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C 2C-6C	4 ab	1 a-c	3 a-d	8 a-d	4 ab	4 a-d
11 Manzate Pro-Stick 75DF 12 oz Luna Sensation 4.17SC 1.25 fl oz Inspire Super 2.82SC 3 fl oz Captan 80WDG 20 oz	Pk Bl-PF 1C covers	7 ab	3 a-d	4 a-d	11 b-e	3 ab	4 a-d

12 Manzate 75DF 12 oz + LBG-FS2 14 fl oz	Pk-PF							
Inspire Super 2.82SC 3 fl oz	1C	1						
LBG-FS2 14 fl oz + Captan 80WDG 20 oz	2C-6C	1 b	5 a-d	7 b-d	12 b-e	6 ab	7 b-d	
13 Manzate 75DF 12 oz + LBG-FS2 14 fl oz	Pk-1C							
LBG-FS2 14 fl oz + Captan 80WDG 20 oz	2C-6C	7 ab	4 b-d	3 a-c	11 b-e	4 ab	5 b-d	

Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Counts of 25-fruit samples picked from each of four single-tree reps 27 Sep and first rated (“at harvest”) 1 Oct then held at ambient warm temperatures

(65-89°F, 20-day mean 72.5°F; 31-day mean 73.4°F).

* Post-storage rots rated 17 Oct and 28 Oct after 20 and 31 days’ incubation at ambient temperatures.

Dilute rates based on 400 gal/A equivalent. Applied dilute to runoff at 250 psi.

Treatment application dates: 18 Apr (Pk, pink); 25 Apr (Bl, bloom); 7 May (PF, petal fall);

First-sixth covers (1C-6C):16 May, 30 May, 15 Jun, 28 Jun, 19 Jul, 5 Aug.

PATHOGENICITY BEHAVIOR OF *ASPERGILLUS*, *ALTERNARIA*, AND *PESTALOTIOPSIS* ON GRAPE BUNCHES

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Introduction

The fungi *Aspergillus* sp., *Alternaria* sp., *Botrytis cinerea*, and *Pestalotiopsis* sp. were frequently isolated from rotten grape bunches in the mid-Atlantic region in 2018. Botrytis bunch rot is a common disease in the mid-Atlantic caused by the known primary pathogen *Botrytis cinerea*. Although *Aspergillus* spp., *Alternaria* spp., and *Pestalotiopsis* spp. have been previously described as pathogens of grape bunches, little research has been conducted to understand their aggressiveness and pathogenicity. The late-season fruit rots caused by these pathogens are of major concern to vineyard managers. Therefore, this study was conducted to evaluate the pathogenicity of these four fungi on four grape cultivars, at three developmental stages, and with or without wounding.

Materials and Methods

Three isolates of *Aspergillus*, *Alternaria*, *Pestalotiopsis*, and *Botrytis* were isolated onto potato dextrose agar (PDA) from ripe, rotten grapes collected from various Maryland vineyards in 2018. The isolates were identified first by colony morphology and then to the genus level with sequences of the internal transcribed spacer (ITS) region. The primers ITS1 and ITS4 were used to amplify this region, followed with Sanger sequencing conducted by the Biodesign Institute at Arizona State University. The twelve isolates of the four fungal species were then stored on filter paper at -20°C until they were revived on PDA and quarter strength PDA. The isolates were incubated at room temperature in the dark until sporulation. Spore suspensions of each group of three isolates were created by flooding the plates with sterile water and rubbing with an inoculation loop. The suspensions were then diluted to a concentration of 1.0×10^5 conidia per milliliter and were placed in four separate atomizers. One other atomizer with sterile deionized water was also included.

Fifteen rows of grapevines in the Wye Research and Education Center experimental vineyard in Queenstown, MD were used for this study. Each row contained alternating plantings of four cultivars: Chardonnay, Cabernet Franc, Merlot, and Chambourcin, respectively. The vineyard contained two boarder rows of Chardonnay on either side of the fifteen plant rows used in this study. Each row was treated as a block and the following treatments were completely randomized within each block. At bloom, within each block, two inflorescences were misted with each spore suspension or water until runoff. Immediately following inoculation, the clusters were protected from outside infections by covering with a wax paper bag. The above inoculation and bagging protocols were repeated on developing grape clusters at veraison and pre-harvest (two to three weeks before harvest). One of the two clusters inoculated at the veraison and pre-harvest stage was wounded immediately prior to inoculation by inserting a sterile toothpick to a depth of 10 mm in 10 grapes per cluster. One of the two clusters inoculated at the bloom stage was wounded in a similar fashion, but at the pre-harvest stage. The clusters were then left to

mature until harvest. Upon ripening, each bagged cluster was removed from the vineyard to be evaluated for severity (percent of cluster infested) of different diseases.

Results

Upon evaluation of the diseases in each bagged cluster, multiple diseases were observed such as *Aspergillus* fruit rot, *Alternaria* fruit rot, *Pestalotiopsis* fruit rot, *Botrytis* bunch rot, ripe rot, sour rot, and *Fusarium* fruit rot (data not shown). Occasionally, multiple diseases had developed on the same cluster. However, the most severe disease on each cluster was almost always caused by the inoculum. Overall, the highest mean disease severity was caused by *Aspergillus* (9.9%), followed by *Botrytis* (9.4%), *Alternaria* (4.1%), and *Pestalotiopsis* (3.2%). Grape clusters treated with water had the lowest amount of disease in all treatments. Wounded clusters had a much higher overall amount of each disease than nonwounded clusters at each treatment timing (Figure 1).

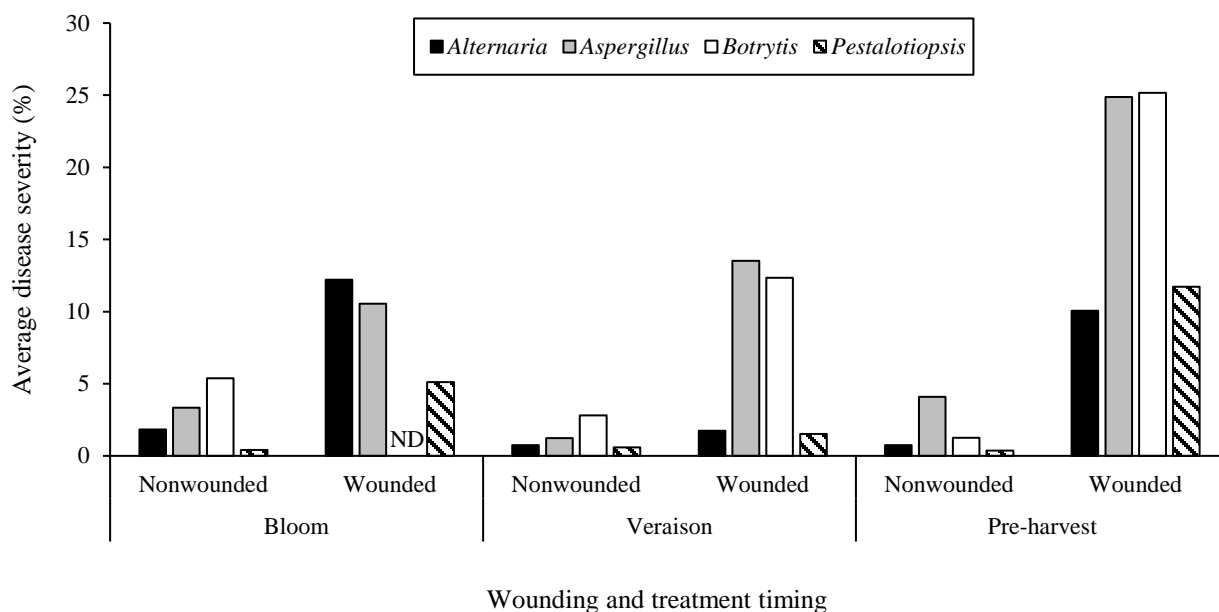


Figure 1. Average disease severity (%) of four diseases caused by four inocula, respectively, with different wounding and timing treatments. ND = no data.

The inoculation timing also greatly influenced the level of disease, with pre-harvest being the most susceptible inoculation timing for *Aspergillus* and *Pestalotiopsis*, and bloom being the most susceptible inoculation timing for *Alternaria* and *Botrytis* (Figure 1). *Botrytis* appeared to be the most pathogenic out of the four fungi because of its ability to cause disease on

nonwounded clusters at bloom and veraison (Figure 1). The veraison timing was the most resistant to *Alternaria* and *Pestalotiopsis*.

The different cultivars also had different levels of susceptibility to each disease. Chardonnay appeared to be the most susceptible, with the highest average disease severity in nonwounded clusters for each inoculum, while Chambourcin appeared to be slightly more resistant than Cabernet Franc and Merlot. Within wounded clusters, the most susceptible cultivar was not consistent amongst the different inocula (Figure 2).

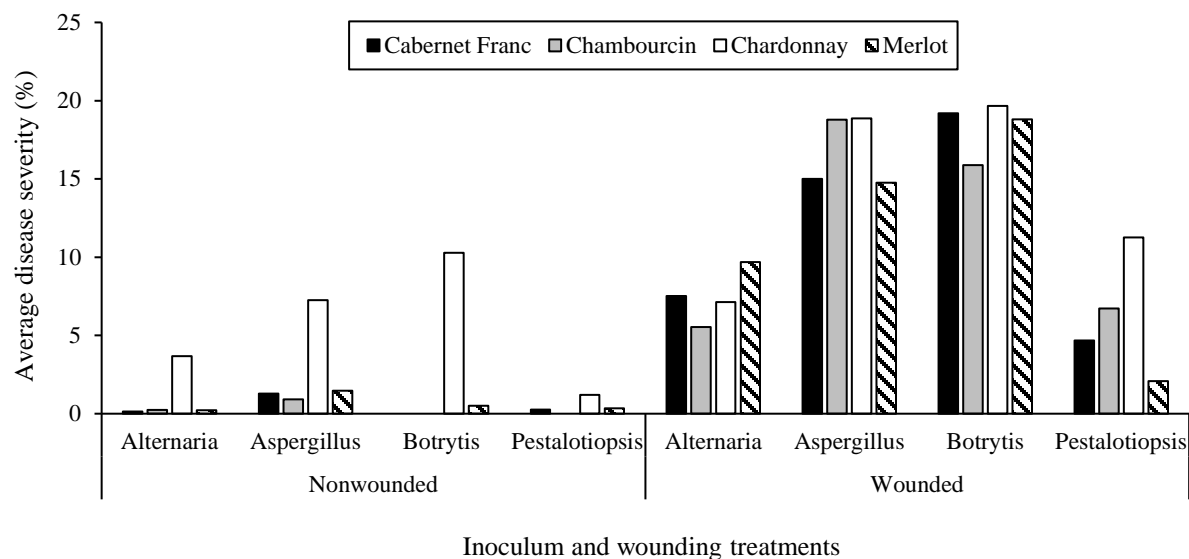


Figure 2. Average disease severity (%) of four diseases caused by four inocula, respectively, on wounded and nonwounded grape clusters of four grape cultivars.

Discussion

In this study, we observed different levels of disease depending on the inoculum. *B. cinerea* is known to be a devastating primary pathogen, while the three others are not as notorious for causing disease. Our results in nonwounded grapes appear to confirm the known pathogenicity of *B. cinerea*, which caused the most disease followed by *Aspergillus*, *Alternaria*, and *Pestalotiopsis*. *Aspergillus* caused close to as much disease as *Botrytis* on nonwounded grapes (Figure 1), and this pathogen could be more pathogenic than *Alternaria* and *Pestalotiopsis*. The clusters were wounded to mimic insect, bird, or physical damage to the fruit, and inoculated to mimic a secondary infection by necrotrophic or opportunistic pathogens. In all treatments, wounded grapes had much higher disease severity than non-wounded grapes. Similar to nonwounded grapes, *Botrytis* and *Aspergillus* caused higher disease than *Alternaria* and *Pestalotiopsis*. Even though not all of the fungi caused the same severity of disease on wounded grapes, they all caused enough disease to be considered secondarily pathogenic. Our results

demonstrate that every fungus included in this study would be problematic when grapes have been damaged.

There was also a difference in severity of disease amongst the four cultivars and three infection timings. On nonwounded grapes, Chardonnay was by far the most susceptible cultivar, while the average disease severity was very low in the other cultivars. This is consistent with the fact that Chardonnay is known to be more susceptible to other fruit rot diseases like black rot and Botrytis bunch rot than the other three cultivars. With wounding, the differences in susceptibility between cultivars were less pronounced, except for a stark difference between Chardonnay and Merlot that was inoculated with *Pestalotiopsis*. Due to the high susceptibility of Chardonnay to the pathogens tested in this study, growers will likely face fruit rot issues with Chardonnay in high disease pressure years. The timing of the infection also affected the amount of disease that was observed, with bloom being the most susceptible time for *Alternaria* and *Botrytis* while pre-harvest was the most susceptible timing for *Aspergillus* and *Pestalotiopsis*. *Botrytis* is known to be a pathogen that affects the bloom stage, and a protective fungicide application at this timing is common practice. This new knowledge of the preferred infection timings of *Alternaria*, *Aspergillus*, and *Pestalotiopsis* can be used to help aim protective fungicide applications for these pathogens.