# 47<sup>th</sup> Tomato Breeders Roundtable













Come to the Wooster campus of The Ohio State University. This campus is home to Ohio State and affiliated entities: the Ohio Agricultural Research and Development ther (OARDC), Ohio State ATI, Northeast Region Ohio State University Extension, Bio tearch Park, and USDA Agricultural Research Services (ARS).

DC facilitates the research conducted by the university's College of Food, Agricultural Environmental Sciences (CFAES) and is dedicated to enhancing the well-beilgr ans through research on food, agriculture, family and the environment. At any given our researchers are engaged in more than 400 research projects eimed at producing heatity, affordable food and agricultural products, while promoting stewardship of al resources and the environment. This work keeps Ohio positioned favorably in the economy and benefits you and your family.

to State ATI ranks number one in the nation among institutions awarding two-year grees in agriculture and related sciences. Choosing from 31 areas of study, students can rsue an associate degree at Ohio State ATI and also transfer credits to complete a fourir degree on Ohio State's Columbus campus. If you have any questions or want additiona smatton about Ohio State ATI please call 330-287-1330 during normal business hours.

crest Arboretum is a 90-acre outdoor laboratory and landscape garden, home to usands of native and introduced species and cuttivars of trees, shrubs and other plants. a Aboretum is open daily from dawn to dusk. Parking and admission is free. The Miller villon, located within the arboretum, as well as Shisler Conference Center and Fisher ditorium offer meeting, training and large event amenities for business, industry and the heral bublic.

We encourage you to explore our campus and facilities here in Wooster and we hope your visit will provide you with a better understanding of the research, education and outreach activities of our faculty, staff and students. Guided tours are available for groups of ten or more during normal operating hours. A minimum two-week advance notce is required, and dates are subject to availability. Contact the Wooster Campus Director's Office for more information on guided tours by calling 330-263-3700 or send an email to oardc-outreach@ osuuedu. We hope you enjoy your visit to the Wooster Campus.



142 E. Liberty St. Unit 10       28. Smoke the Burger Joint         330-601-1880 (coffee/pastry)       206 Beall Ave.         330-601-0206       330-601-0206	503 E. Liberty St. 14. Templeton's Scottish Bakery & 330-601-0742	13. <b>Gionino's Pizzeria</b> 330-264-2604 (NYC diner style) 120 N. Buckeye St. 330-262-0093 27. <b>Rice Bowl</b>	131 N. Market St. 330-201-6777 (espresso drinks, pastries) 26. Muddy's 335 E. Liberty St.	1.01 W. Livery 91.       25. The Hero House         330-601-1800 (Gourmet Sandwiches)       25. The Hero House         12. Gathering Grounds Coffee Shop       330-262-8166 (sandwiches, salads)	330-601-0885 (Asian Fusion & Japanese) 24. The Henry Station 244 E. Henry St. 11.Jimmy John's 330-264-2226 (Casual, Pizza)	10. Basil Asian Bistro 145 W. Liberty St.	211 S. Market St. 9. Local Roots Market & Cafe 330-592-6273 (Wine, Bourbon, Tapas) 140 S. Walnut St. 23 Eloning Inde Market Stroot Coll	8. Spoon Market & Deli     6. Spoon Market & Deli     744 W. Liberty St.     330-262-0880 (deli-style w/full bar)     22. H2 Huth & Harris Wine Merchants	150 W. Liberty St. 330-601-1101 (burgers, gourmet melts, & 21. Sure House Coffee Roasting Co. more) 330-264-3663 (Coffee roasting, full	6. Matsos Family Restaurant 154 W. Liberty St. 330-264-8600 (casual Greek) 7. Gouda Bar & Grill 20. Meatheads Union 147 S. Market St. 330-301-1147 (Butcher shop serving lunc only. Variety of sandwich offerings)	330-264-6263 (3 restaurants in one , offering casual and fine dining)       18. Omahoma Bob's BBQ         offering casual and fine dining)       320-262-9871(casual dining)         5. The Parlor Restaurant       330-262-9871(casual dining)         203 W. Liberty St.       19. City Square Steakhouse         330-262-4971 (casual dining)       330-262-4971(fine dining)         330-262-4971 (casual dining)       330-262-2489 (fine dining)	3. The Olde Jaol Steakhouse     (small plates, brick oven pizza)       215 N. Walnut St.     17. Tulipan Hungarian Pastry       330-262-3333 (fine dining)     & Coffee Shop       4. TJ's Trio     330-264-8092 (coffee/ pastry)       359 W. Liberty St.     330-264-8092 (coffee/ pastry)	2. Olde Jaol Tavern 215 N. Walnut St. 330-262-3333 (casual dining) 330-263-2949	1. Towne Market Café 334 N. Market St. 330-264-6797 (casual dining) 330-264-6797 (casual dining)	Places to Eat & Meet
www.mainstreetwooster.org www.fb.com/mainstreetwooster	Main Street Wooster, Inc. 377 W. Liberty St. 330-262-6222	TS. TS. TS.		W. SOUTH Ж. S 22 М. S 22 П. S E SOUTH	9 20 18 9 21 19	W. LIBERTY 6 7 8 16 15 26 27	4 5 10 11 14 25	W. NORTH ST.	W. LARWILL 2 3 28	ب ب ب ب ب ب ب ب ب ب ب ب ب ب ب ب ب ب ب		<ul> <li>A. McDonald's E. Wendy's</li> <li>B. KFC F. China Garden</li> <li>C. Arby's</li> <li>D. Donato's Pizza</li> </ul>	Grab a bite on Beall Avenue	With great shopping in between!	Downtown Wooster

# Acknowledgements

My sincere thanks to each and every one of you for attending the 47<sup>th</sup> Tomato Breeders Roundtable. I am happy to host you in Ohio, and pleased to feature The Ohio State University's facilities, faculty and students in our program. We will try to strike a balance between formal presentations, hands-on activities, and discussion. A portion of the meeting, focused on tomato rootstocks, will be live-streamed as webinar and will feature work supported through the USDA's Specialty Crops Research Initiative.

The Ohio State University, the Ohio Agricultural Experiment Station and the Ohio Agricultural Research and Development Center in Wooster, OH has a long history of tomato research encompassing innovation in pathology and breeding. Notable milestones include discovery and deployment of the Tm2<sup>a</sup> locus by Leonard Alexander, Professor of Plant Pathology; discovery of resistance and management of Fusarium Crown Rot by teams from Plant Pathology and Horticulture; and the development of humid-environment adapted processing germplasm by Dr. Stan Berry, Professor of Horticulture. The Tm2<sup>a</sup> locus is the most widely deployed resistance in tomato and has been effective for nearly 50 years. Dr. Berry's variety, OH8245, has been used extensively in Brazil as AG45, and as a parent in commercial hybrids in CA, Italy, the Midwestern US, and Brazil. Dr. Berry's early season OH7983 survived as an OP/inbred variety into the late 1990's and may have been the last inbred processing variety used by the canning industry. These breeding contributions have been supported by a research network that encompassed "field to factory" contributions from Plant Pathology and Food Science and Technology. In the last decade, through the leadership of Dr. Steven Schwartz (Food Science and Technology) and Dr. Steve Clinton (James Cancer Center) the research model has expanded to "field to clinic", encompassing production, breeding, management, human nutrition, health, and medical research. The field to clinic research paradigm lives in collaborations through the Food Innovation Center (FIC), the Center for Advanced Functional Foods Research and Entrepreneurship (CAFFRE), the Wilbur A. Gould Food Industries Center, and our University Discovery Themes.

David Francis, Host.

#### Cover Art.

Ed Marthey, Wooster, OH. pencil sketch print, all rights reserved. **Registration and Web Page.** Christine E Cooley, University of Florida, Assistant to Center Director, Media Coordinator, Event Coordinator. **Fisher Conference Center.** Debbie Shaffer, Conference Coordinator, OARDC, Fisher Auditorium **Catering.** Linda Patin, Linda's Local Thyme **Hiltion Garden Inn.** Aimee Welsh, Director of Sales, Hilton Garden Inn, Wooster **Logistical Support.** The tomato team: Jihuen (Jin) Cho, Troy Aldrich, Eduardo Bernal, Sean Fenstemaker, Michael Dzakovich. **Fiscal Administrative Support.** 

Kimberly Nolletti and Laura Williams, Horticulture and Crop Science, OSU.

# **Schedule** 47<sup>th</sup> Tomato Breeders Roundtable

# April 4th

**Opening reception 5:30-8:00** Hilton Garden Inn Light Hors d' Oeuvres and Bar

# April 5th

47<sup>th</sup> Tomato Breeders Roundtable Fisher Auditorium South Exhibit Area 8:00 Registration

# 8:30-9:30 Roundtable check in/Area reports

9:30-10:00 Break

# 10:00-12:00 Tomato Rootstocks

Matt Kleinhenz, Moderator

Chieri Kubota, OSU "grafting technologies and their trends" (Keynote Speaker)

Cary Rivard, KSU "assessing scion compatibility in tomato as a function of yield improvement"

Sean Fenstemaker, OSU "vigor, resistance, and rootstocks"

Jonathan Kressin, NCSU "colonization dynamics of the tomato-bacterial wilt pathosystem and its implications for resistance selection"

Rafael Lacaz-Ruiz, Monsanto "rootstock for the computer controlled environment, an industry perspective"

Bryan Zingel, Sakata "industry perspective"

# 12:00-1:30 Lunch

# 2:00-5:00 Breeding Technology

Arron Carter "phenotyping and genomic prediction in wheat breeding" (Keynote Speaker)

Erin Steer and Leah Benedict, LGC "KASP service technology for SNP genotyping"

Scott Weigel, AgriPlex Genomics "PlexSeq: a targeted amplicon based sequencing method for SNP genotyping"

Paul Chomet and David Neuman, NRGene "Pan-Genome: Moving beyond a single reference genome: GenoMagic <sup>™</sup>, a novel solution to describe and manage genomic variation"

## 3:15-3:30 Break

Barbara E. Liedl, WVSU, "understanding segregation distortion and reproductive barriers to improve transfer of traits from S. pennellii to cultivated tomato"

Eduardo Bernal, OSU, "background genome selection for rapid introgression and evaluation of quantitative trait loci (QTL) for resistance in tomato to multiple xanthomonas spp."

Andika Gunadi, "rapid progress of CRISPR for targeted crop modification"

David Francis, OSU "experience with genomic selection in processing tomato"

#### **Evening activities:**

#### Dinner on own

5:00-6:30 Discussion of Pan Genome Project (Hilton Garden Inn, Buckeye Board Room)

7:00-9:00 Tomato CGC (123 Williams Hall, ZOOM link for computer video or telephone connection)

# April 6th

47<sup>th</sup> Tomato Breeders Roundtable Fisher Auditorium South Exhibit Area 8:00-8:50 Pest Resistance

John Snyder, UKY, "progress report: introgression of iingiberene and type IV trichome density from *Solanum. Habrochaites* LA2329 into *S. lycopersicum*" (Keynote Speaker)

Mohammad H Dawood, UKY "a new seqquiterpene alcohol from wild tomato repelled the twospotted spider mite, *Tetranychus uritcae*"

Ammar AL-Bayati, UKY "the role of trichome secretions and densities in spider mite behavior for an interspecific population of tomato"

Martha Mutschler, Cornell, "prebreeding tomato for optimized acylsugar-mediated resistance to insects"

# 8:50-9:00 Break

# 9:00-11:00 Disease Resistance

Sam Hutton, UFL "virus resistance" (Keynote Speaker)

Jasmine Lopez, UFL., "identification of a major graywall resistance locus on chromosome 9 of tomato"

Jessica L. Chitwood, UFL., "discovery and improvement of novel and known resistance to fusarium wilt race three of tomato."

Robyn Roberts, Boyce Thompson Institute, "a screen of genetically diverse tomato accessions reveals a range of unusual responses to *Pseudomonas syringae* pv. Tomato'

Rebecca L. Wente, UFL, "fine mapping and candidate gene characterization of the pepper bacterial spot resistance gene bs6"

Pragya Adhikari, NCSU, "bacterial spot of tomato:pathogen story and our efforts of tomato improvement in NC"

Taylor Anderson, Cornell Univ., "Pyramiding resistances to bacterial spot and bacterial speck in elite fresh market tomato"

# 11:00-12:00 Quality in Whole-Peel and Diced Tomato Products

11:00-11:10 Valente Alvarez, OSU "The Wilbur A. Gould Food Industries Center" 11:10-11:25 Ken Martin, Furmano's "quality evaluation for whole peel and diced (discussion)"

# 12:00-1:00 Lunch

# 1:00-2:45 Tomato Quality

Jessica Cooperstone, OSU "tomatoes, health, and the metabolome" (Keynote Speaker)

Steven Loewen, U of Guelph "breeding for processing tomato quality"

Dilip R. Panthee, NCSU, "tomato improvement for fruit quality and disease resistance at NC State University"

Edgar Sierra Orozco, UFL., "fine mapping of a locus in chromosome 12 controlling flat and globe fruit shape in fresh-market tomato"

Reza Shekasteband, UFL., "fruit weight variation caused by the known FW2.2, FW3.2, FAS, and FW11.3 allele in fresh market tomato"

**2:30-3:30 Discussion** Next Place, Next Time

**3:30-5:00 Facilities Tours** See next page

5:00-7:00 Networking Event, Miller Pavilion, Secrest Arboretum (33 on Campus Map)

Dinner on own

#### 3:30-5:00 Facilities Tours

47<sup>th</sup> Tomato Breeders Roundtable Closed-Toe Shoes are recommended We will divide into groups, each with a tour leader. Please stay with your group.

# Station 1 Molecular and Cellular Imaging Center (Building 4, Campus Map, Selby Hall Basement)

Sequencing and Genotyping Equipment (Dr. Tea Meulia)

MCIC Computational Biology Laboratory (MCBL) (Michael Dzakovich (expression analysis), Saranga Wijeratne (SNP pipeline))

Station 2 Williams Greenhouse (Building 7, Campus Map, South/East Wing) Tomato Bacterial Spot Screen (Eduardo Bernal)

Assessment of beneficial bacteria in petunia disease screen (Kaylee South)

## Station 3 Williams GH (North West Wing, Head House, and Gourley Greenhouse)

Wheat Breeding (Nelly Arguello-Blanco, Williams Greenhouse, N. West wing)

Digital Phenotyping (Nathan Nordstedt, Williams Head House)

Barley Breeding and Genetics (Dr. Eric Stockinger, Gourley Greenhouse)

#### 5:00-7:00 Networking Event, Miller Pavilion, Secrest Arboretum (33 on Campus Map)

Dinner on own

April 7, OSU Plant Science Symposium

# ABSTRACTS

# **Rootstock Breeding**

#### Assessing Scion Compatibility in Tomato as a Function of Yield Improvement

David E. Loewen and Cary L. Rivard Department of Horticulture and Natural Resources Kansas State University

Although research with grafted vegetables is fairly common around the US, most of these trials are designed to compare rootstocks or evaluate the effectiveness of specific growing practices or environments. Little research has been conducted to determine if grafting with a particular rootstock are consistent across different scion cultivars. Therefore, our objectives were to 1) examine the effects of a single rootstock on multiple (10) scion varieties, 2) determine which scion varieties showed a high degree of compatibility with the rootstock, and 3) identify grafted or nongrafted varieties that show commercial potential for high tunnel production. Our study employed 'Maxifort' as the rootstock and evaluated ten, determinate, red slicer tomato cultivars as scion. The study was conducted during 2016 and 2017, and took place in one bay of a threeseason, multi-bay high tunnel. Nongrafted and grafted treatments were included for each variety. The study utilized a RCBD with four replications and five plants were grown in each plot. Year had a very strong effect on yield. All ten varieties provided some numerical benefit from grafting with 'Maxifort' rootstock and average marketable yield improvements ranged from 8.2% to 34.6%. 'Red Deuce', 'Skyway', and 'Tasti Lee' may be good candidates for grafting with 'Maxifort' in addition to 'BHN 589'. These varieties displayed the highest degree of grafting compatibility as it relates to fruit yield and ranged from 28.6% to 34.6%. Interestingly, 'Red Deuce' grafted onto 'Maxifort' rootstock displayed the highest marketable yields of any combination tested, but the benefit of grafting was 28.6% when compared to nongrafted plants. Similarly, 'Primo Red' showed insignificant improvements (8.2%) in yield as a result of grafting with 'Maxifort', but was the highest producing nongrafted variety in both years. Elucidating the interactions that occur between scion and rootstock and their impact on fruit yield may be one quantitative way of assessing rootstock/scion compatibility in tomato.

#### Vigor, Resistance, and Rootstocks

Sean Fenstemaker, Nancy Huarachi Morejon, Mohammed Masud, Matt Kleinhenz, Stephanie Wedryk and David Francis, The Ohio State University. Frank Louws, NC State University

Grafting to rootstocks is becoming popular in annual vegetable production to control soil borne diseases, replace fumigation, and impart vigor. We initiated a rootstock (RS) breeding program in 2007 to address the feasibility of using grafted plants in soil-based production, including certified organic systems. A total of 110 rootstocks were developed through pollination of 10 cultivated (Solanum lycopersicum) parental lines as female plants and 11 accessions of wild species as male parents. Under open field conditions using the F1 scion 'Celebrity' we found little evidence for yield increases with the exception of trials where soil-borne disease reduced yields. In contrast RS performance trials conducted with heirloom scions or under season extension exhibited yield increases. We determined that some RS were able to impart vigor, measured as increased growth and canopy density. There was a positive correlation with the genetic distance between parents used to create hybrid RS and vigor. There were negative correlations associated with genetic distance and traits associated with seed quality. Environmental and genetic effects on seed quality were evaluated during two seed production seasons and by harvesting fruit at different maturation stages. Seed quality was assessed based on seed size and germination rates. Seed size was influenced more by parental genetics than environment. Seed germination was influenced by genetics and environmental factors. Using seed weight as selection criterion in breeding might allow early selection for high quality RS. Key selection points in the breeding of new tomato rootstock cultivars were identified.

# 4-DIMENSIONAL COLONIZATION DYNAMICS OF THE GRAFTED TOMATO-BACTERIAL WILT PATHOSYSTEM AND THEIR IMPLICATIONS FOR RESISTANCE SELECTION

\*Jonathan Kressin<sup>z</sup>, North Carolina State University Dept. of Horticultural Science and Dept. of Entomology and Plant Pathology; Dilip R. Panthee, North Carolina State University Dept. of Horticultural Science; Frank J. Louws, North Carolina State University Dept. of Entomology and Plant Pathology; Marc Planas<sup>z</sup>, Nuria Sanchez-Coll, and Marc Valls, Centre for Research in Agricultural Genomics. <sup>z</sup>First co-authors

The breeding history of tomato (Solanum lycopersicum L.) resistance to bacterial wilt (Ralstonia solanacearum species complex) has been riddled with enigmas, conflicting reports, and decades of low genetic gain. The most efficacious resistance has been available for more than 40 years, yet it has only been effectively deployed in rootstock varieties because of the extreme difficulty of introgressing high levels of resistance into large-fruited, pleasant tasting genetic backgrounds. These resistant rootstocks have been a primary driver of the adoption of grafted tomato production in North Carolina and elsewhere, yet the quantitative resistance in tomato does not prevent the bacteria from invading roots and hypocotyl tissue where the graft union resides; rather it predominantly restricts the ability of the pathogen to colonize adjacent xylem cells. We explored this enigmatic aspect of bacterial invasion patterns over time and space in grafted tomato combinations (susceptible to highly resistant) with luciferase- and green fluorescent protein-labeled GMI1000 strains to better understand why vegetable grafting endures as a viable management strategy. Through destructive sampling and novel imaging techniques, our results suggest that tomato resistance induces a series of tug-of-war arenas that are sequentially overcome for the bacteria to effectively colonize the plant-root invasion, root proliferation, radial vascular colonization, vertical xylary translocation (unclear), and pith/cortical tissue invasion. The work also clearly highlights key aspects of this quantitative tug-of-war dynamic that results in a mostly binary disease response-wilt. Wilt development is most related to the tugof-war dynamic in the basal hypocotyl, where effective resistance suppresses bundle invasion, not just invasion within bundles. We have then explored ways to measure key aspects of that interaction in a more high-throughput manner that would be required for breeding programs, specifically the vascular browning symptomology. Although destructive, it could provide a rapid visual metric for assessing colonization resistance at the vascular tissue-system level. The merits and drawbacks of this method will be discussed briefly. Our work provides breeders with new tools and a framework for measuring and selecting for resistance at each important anatomical location.

## Rootstock for the computer controlled environment, an industry perspective

Rafael Lacaz-Ruiz. Ag. Eng. University of São Paulo. Tomato Technical Development Representative for De Ruiter in North America.

The demand for constant supply of top quality fresh tomatoes from high tech greenhouses in North America has been growing constantly in the past decade. Consumers are leading this demand, looking for more and better produce. Consequently, there has been more competition amongst major super marketers that see in this market an opportunity for increased sales. In response, the industry has been aggressively expanding its area in Mexico, Canada and USA, causing a natural price competition. Under this scenario of pressure and opportunity chase, Growers have been innovating aggressively by increasing their efficiency and increasing the size of greenhouses to maintain profitability and remain competitive.

The use of rootstocks is currently widely adopted and of upmost importance in the greenhouse industry because it provides additional vigor for long crops and disease resistances that are commonly present in greenhouses.

Understanding what are the major challenges and needs related to use of rootstocks the greenhouse industry to continue delivering the right tools for growers is key to answering this dynamic growth.

# **Breeding Technology**

# UNDERSTANDING SEGREGATION DISTORTION AND REPRODUCTIVE BARRIERS TO IMPROVE TRANSFER OF TRAITS FROM *SOLANUM PENNELLII* TO CULTIVATED TOMATO

Barbara E. Liedl, Department of Biology, West Virginia State University, Institute, WV, 25112

The germplasm of many crops including tomato is narrow due to constraints imposed during domestication and spread, thus increasing the importance of wild relatives as a source of genetic variation. However, segregation distortion, linkage drag and reproductive barriers impede the transfer of desirable traits from the wild to cultivated species. This project was undertaken to understand segregation distortion and reproductive barriers in the F<sub>2</sub> and backcross (BC) populations from the interspecific cross between tomato, *S. lycopersicum*, and the wild species, *S. pennellii*.

The three populations (F<sub>2</sub>, BCLyc and BCPen) derived from crosses between *S. lycopersicum* M82 and *S. pennellii* LA716 and their interspecific F<sub>1</sub> were evaluated for % pollen stainability, and % seed germination as well as if the plants had produced flower, fruit or seed. Percent stainable pollen varied greatly for the parents: 99.22% M82, 16.31% LA716 and 85.7% of the interspecific hybrid, F<sub>1</sub>. Both backcross populations had similar means, 57.9% BCLyc and 48.9% for BCPen, while the F<sub>2</sub> mean was 31.7% but varied from zero to 99.2%. The F<sub>2</sub>, as expected from previous research had only 27.75% of the population producing fruit, of which, only 9.43% produced seed. As expected, all of the BCLyc flowered and 98% produced fruit with germinable seed. Conversely, the majority of the BCPen produced flowers, but only 8.7% of the plants produced self-fruit and only 3.26% produced seed. Only two populations, F<sub>2</sub> and BCLyc exhibited any abnormal seed germination (18.8% and 1.1% respectively), with all of the germinated seed from the F<sub>2</sub> being abnormal.

Of the 1000 SNP markers, no data was obtained for 181 markers with the highest failure rate on chromosome 6 (28.7%). An additional 184 markers were not used based on no variation or incorrect markers found, leaving at most 635 markers to be used for analysis. The F2 population, as expected, had 43% of the markers deviate from the expected 1:2:1 ratio. The BCLyc population had over 63% of their markers deviating from the expected 1:1 ratio with excess tomato alleles and all of the markers on two chromosomes (1 and 8) skewed. The BCPen population, only 21.9% of the markers displayed distorted segregation and one chromosome (1) did not display any skewing from the expected ratio. In addition, most of the skewing BCPen markers favored homozygous LA716 combination, but a few skewed towards the heterozygous combination on chromosomes 5 and 9. Each population was examined for the percent of S. *pennellii* genome present. For the  $F_2$ , the expectation was 50% and we observed 54.4% (ranging from 18.6 to 75.48). The two backcross populations were projected to have 25% (BCLyc) and 75% (BCPen), our analysis found an average of 17.8% for BCLyc (range 0-35.9) and 77.6% for BCPen (range 63.7-90.7). Maps of each population were made, but problems with the large amount of skewed markers in the BCLyc limited the number of markers that could be used to create a map. Analysis of the data is ongoing.

**Time-saving, cost-effective SNP genotyping strategies from LGC Genomics** Leah Benedict, Client Executive, Genomics LGC, 3600 Minnesota St. Alexandria, MN 56308

Single nucleotide polymorphisms (SNPs) have become the molecular marker of choice for many crop improvement programs. Historically, a lack of sufficient SNP markers has been a significant obstacle for tomato breeders. Commendable efforts made in the last 20 years have unlocked this powerful molecular tool, allowing for focus to shift to identification of timesaving, cost-effective SNP genotyping strategies.

In this technology showcase, we will describe an automated SNP genotyping workflow developed to minimize hands-on operation time, reduce reagent costs, and boost productivity. The oKtopure<sup>™</sup>, a DNA extraction robot optimized for use with our sbeadex<sup>™</sup> chemistry with the capacity to extract eight 96-well plates in a single run, will be highlighted. Additionally, we will provide an overview of the IntelliQube<sup>®</sup>, our fully-automated PCR platform that integrates liquid handling, sealing, thermal cycling, signal detection, and data analysis on a single instrument. High-throughput SNP genotyping becomes streamlined when pairing the IntelliQube with our Kompetitive Alelle Specific PCR (KASP<sup>™</sup>) genotyping chemistry. The KASP tomato panel, developed by the Solanaceae Coordinated Agricultural Project, further simplifies the adoption of KASP for tomato breeders. These technologies provide robust and economical solutions to overcome throughput and cost challenges that can hinder tomato breeding programs.

# PlexSeq: a targeted amplicon based sequencing method for SNP genotyping Scott Weigel, Sales Director AgriPlex Genomics

AgriPlex Genomics is a full service genomics laboratory located in Cleveland, OH. Our PlexSeq system can screen 10 to 3000 SNP's across 100 to 100,000 samples simultaneously, making PlexSeq an extremely rapid, low-cost, versatile system for plant breeders.

# Moving beyond a single reference genome: GenoMagic <sup>™</sup>, a novel solution to describe and manage genomic variation

Paul Chomet and David Neuman, NRGene

Next Generation sequencing technologies have opened the door to multiple genome analyses and an increased understanding of the variations present in populations. To date, most of the germplasm analyses have relied on the comparison of sequence reads to one reference genome assembly, limiting our understanding of genomic variation. NRGene has developed novel analytics and approaches to efficiently denovo-assemble genomes and to describe the relevant variation across germplasm using a pan-genome approach. We are promoting a pangenome consortium that will enable full genome comparative analyses for tomato germplasm. The longer-term goal is for the pan-genome to serve as a reference to fully catalog the diversity in Solanum lycopersicum through sequence-based haplotypes. This talk will focus on the need and advantage of a pangenome and sequence-based haplotypes to for breeding and gene discovery applications.

# BACKGROUND GENOME SELECTION FOR RAPID INTROGRESSION AND EVALUATION OF QUANTITATIVE TRAIT LOCI (QTL) FOR RESISTANCE IN TOMATO TO MULTIPLE *XANTHOMONAS SPP*.

\*Eduardo Bernal, Debora Liabeuf, and David Francis, The Ohio State University Department of Horticulture and Crop Sciences.

Bacterial Spot of tomato is a foliar disease caused by four species of Xanthomonas. Identification of genetic resistance in wild tomatoes and breeding has been a focus of our control strategy. Three independent sources of resistance, Hawaii 7998, PI 114490 and LA2533 have been discovered, and genetic studies have identified a major QTL mapping to the same region on chromosome 11. Genome resequencing and analysis suggests that these loci are not identical, though current resolution does not allow us to distinguish alleles from linked genes. To assess whether one QTL provides better resistance to multiple species, we developed near isogenic lines (NILs) using marker-assisted selection and background genome selection. The resistant sources were independently introduced into a susceptible parent, OH88119. Insertion/Deletion markers were used to select for the QTL region and a panel of SNP markers assayed on the KASP platform were used for background genome selection. This approach allowed us to rapidly develop NILs that are 95%-99% genetically identical, except for the QTL on chromosome 11. In 2016 and 2017 we assessed multiple lines developed from each source in independent field trials inoculated with three species causing bacterial spot (X. perforans, X. euvesicatoria, X. gardneri). The NILs were evaluated using the Horsfall-Barrat Scale (1-12), which estimates the percentage of disease on the foliage. Linear Models were used to make comparisons between QTL sources and allelic effects within source. The results show that there are significant differences in both cases, with Hawaii 7998 providing the highest level of resistance to all three species. NILs resistant to multiple species will be released for use by private and public breeding research programs.

## The rapid progress of CRISPR for targeted crop modification

Andika Gunadi and John J. Finer, Plant Transformation and Gene Expression Laboratory, Department of Horticulture and Crop Science, The Ohio State University, Wooster, Ohio

Recently available genome editing tools such as CRISPR have shown great promise for the introduction of valuable traits into targeted genetic locations within plants. The ability to delete, insert, and substitute DNA of various sizes into specific locations within a plant genome have far-reaching application for crop improvements, beyond the capabilities of conventional transgene introduction and breeding methods. With more than 3,000 total peer-reviewed publications on CRISPR in 2017, the rapid pace in the application, development, and ramifications of this technology in various fields of life sciences can be both exciting and alarming. This presentation will provide a brief overview on the process of plant genome editing using CRISPR. Recent advancements in the technology will be highlighted, and several current applications in crop improvement will be discussed.

# Experience with Genomic Selection in Processing Tomato

David Francis, The Ohio State University, OARDC, Wooster, OH

The Ohio State University processing tomato breeding program is predominately focused on trait-based selection for yield, quality and disease resistance under humid growing conditions. At the same time, we are interested in selection strategies that leverage sequence resources and the ability to efficiently query sequence variation in the genome. Robust SNP resources developed to detect polymorphism within cultivated breeding populations have made several approaches accessible. These include routine use of marker-assisted background genome selection to increase the efficiency of trait introgression. Using BC populations of 192 plants, selecting for a trait of interest and applying 2-4 background markers per chromosome arm on 96 selected plants has led to recovery of progeny that are a full generation ahead during each cycle. We have also applied Genomic Selection (GS) strategies to our breeding efforts for bacterial spot and yield. We find that marker coverage in the range of 20-300 markers is sufficient for gains that exceed phenotypic selection. Using prior knowledge of linkage reduces the necessary number of markers. Incorporating knowledge of gene action into GS models increases prediction. Cross validation of GS models within inbred populations show strong prediction (0.4-0.6 for yield traits). Post-hoc analysis of hybrids developed from the same inbreds used to create training models show prediction in the range of 0.2. Recently we have applied GS strategies for parent choice in designing new hybrids using inbred lines that were not in the original training population. Prediction of performance was statistically significant (0.03) though prediction was low (0.1). GS strategies lead to yield increases of 1.2 T/A relative to phenotypic selection. Combining GS and phenotypic selection lead to yield increases of 4.3 T/A relative to phenotypic selection alone. Our experience reinforces the role of markers and GS strategies as a tool to supplement breeder knowledge and improve decision making during the selection process.

# **Pest Resistance**

# Introgression of Zingiberene and Type IV Trichome Density from *Solanum. Habrochaites* LA2329 into *S. lycopersicum* – Progress Report

# John Snyder, Ammar Al-Bayati and Mohammad Dawood, Dept. of Horticulture, University of Kentucky

In summer 2012, we grew out approximately 1500 BC2F1 individuals. Donor was a BC1F1 having high zingiberene concentration, a sesquiterpene hydrocarbon present in trichome secretions on leaves, high type IV trichome density and high resistance to spider mites. All BC2F1 plants were evaluated for type IV density, fruit set in the field and extent of spider mite infestation in the field. Approximately 10% of the plants had type IV trichome density similar to that of the wild accession, and approximately 10% had fruit set in the field. Cuttings of 19 individuals of interest were rooted and then grown in the greenhouse in fall of 2012 and winter 2013. Trichomes, trichome secretion composition and abundance and self fruit set were assessed on these genotypes. Of the 19 genotypes, only 10 produced sufficient seed (BC2F2) for field production in 2013. These plants were evaluated for type IV trichome density, fruit set and trichome secretion composition and abundance. Cuttings were obtained from a few outstanding individuals. After additional evaluation in the greenhouse, we identified one individual having a concentration of zingiberene and type IV trichome density similar to those of the wild progenitor, but no fruit set. This donor plant was used as female to generate the BC3 generation. At present the population is at BC5. Progress to date, including methods used for assessing zingiberene concentration and type IV trichome density will be presented. The overall goal is to combine reliable fruit and seed set with high zingiberene concentration and high type IV trichome density.

# A NEW SESQUITERPENE ALCOHOL FROM WILD TOMATO REPELLED THE TWO-SPOTTED SPIDER MITE, TETRANYCHUS URTICAE

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Tomato (Solanum lycopersicom L.) is one of the most economically important world-wide grown vegetable. However, tomato is a host for numerous pests that reduce productivity. Tomato breeders have focused more on increasing fruit quantity and quality and less on enhancing crop resistance to herbivores. Many accessions of the wild tomato relative, Solanum habrochaites strongly resist arthropod attacks. The monocyclic sesquiterpene hydrocarbon known as zingiberene is found in *Solanum habrochaites* type IV glandular trichomes. To investigate if other components of type IV trichome exudates are responsible for S. habrochaites resistance to arthropods, seeds of S. habrochaites LA 2329 were germinated and grown under greenhouse conditions. The question pursued was "Do other compounds present in trichome secretions have repulsive activities against the two spotted-spider mite Tetranychus urticae, similar to that of zingiberene? Accordingly, LA 2329 trichome leaf exudates were extracted and a portion of their extracts was injected into a gas chromatograph equipped with a mass selective detector (GC/MSD) to separate and identify trichome exudates of LA 2329. The results revealed the presence of two predominate chromatographic peaks. One of these was 7-epi zingiberene and the other was an unidentified compound, but, based on its mass spectrum is likely a sesquiterpene-alcohol, related to zingiberene. To investigate the relative activities of these two major chemical constituents, crude extracts of trichome secretions were separated by silica gel column chromatography and the resulting fractions were evaluated for repellency to spider mite using bridge bioassays. Results revealed that the activity of sesquiterpene alcohol was significantly higher than that for zingiberene. These results support the idea that the degree of repellency may differ among components of trichome exudates and also highlighted the potential value of integrating the sesquiterpene alcohol into cultivated accessions of tomato to improve arthropod resistance in commercial tomato cultivars.

Keywords: Trichome, Zingiberene, Sesquiterpene-alcohol, Tomato, Spider mites, Repellency

# THE ROLE OF TRICHOME SECRETIONS AND DENSITIES IN SPIDER MITE BEHAVIOR FOR AN INTERSPECIFIC POPULATION OF TOMATO

Ammar AL-Bayati, John Snyder, Dept. of Horticulture, University of Kentucky, Lexington, KY

There is significant necessity for assay platforms to assess and identified levels of resistance and insect performance, as demonstrated by recent publications reporting this subject. The whole leaf bioassay requires very limited physical space and labor to assess and identify levels of plant resistance and insect behavior. An interspecific population between Solanum lycopersicum and a wild relative, Solanum habrochaites LA2329, provided the plant material used in this research and was maintained in the greenhouse and field in summer 2017 at the University of Kentucky, Lexington, Ky. The population has been produced to improve arthropod resistance of tomato by introgression of high zingiberene (ZGB) concentration and high type IV trichome density from the wild to cultivated tomato. The objective of this research was to (1) observe the differences of mite behavior among a range of hybrids compared to the positive and negative controls, (2) identify the interactions between genotypes and leaf surfaces with spider mites with respect to presence and absence of type IV trichomes and chemical profiles and, (3) determine the interaction of zingiberene and type IV trichomes on mite performance. Thirteen tomato genotypes with three replications ( $BC_3F_3 \& BC_3F_4$ ) were selected based on presence or absence of trichome type IV, and ZGB. A whole tomato leaf, consisting of five leaflets was transferred to the laboratory, inserted into a 250-ml flask filled with tap water and supplied with fluorescent light., . Then a bean leaf infested by about thirty two-spotted spider mites, Tetranychus urticae Koch, was placed at the base of the tomato leaf. Leaflet position (1-5) and surface infested by mites, mite webbing score (0-3), feeding damage score (0-3), leaflet position of webbing and feeding damage (1-5), egg density per cm<sup>2</sup> as well were recorded and analyzed by SAS using GLM & CORR procedures. Three leaflets from the leaf adjacent to the bioassayed leaf were analyzed for ZGB and monoterpene (MTP) by GC-FID plus another leaflet was taken for counting number of type IV trichomes on abaxial and adaxial surfaces using a microscopic grid. There were significant negative correlations between total type IV trichome densities and number of leaflets and surfaces infested by mites, mite webbing, number of leaflets with mite webbing, feeding damage, number of leaflets with feeding damage, egg counts and density per cm<sup>2</sup>. ZGB was negatively correlated with egg counts and densities. Based on reduced mite success on some of the genotypes, we concluded that resistance has been successfully transferred from the wild accessions to the interspecific population. This bioassay demonstrated behavioral differences of mites associated with the presence or absence of leaf compounds and trichome densities, as well as supported the idea that introgression of type IV trichome and ZGB will lead to greater spider mite resistance. New plant versions that can produce toxic or repellent chemicals on their own can defend themselves against certain types of arthropods and insects which in turn reduce or eliminate extensive synthetic pesticide utilization and cost.

# Prebreeding Tomato for Optimized Acylsugar-Mediated Resistance to Insects

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Acylsugars produced by the wild tomato *Solanum pennellii* control major pests of tomato. Prior breeding created the first tomato lines producing acylsugar levels impacting major pests. Tomato lines producing different levels of the same acylsugars showed the importance of acylsugar level. Using the benchmark line CU071026, tomato lines were bred that possess additional *S. pennellii* introgressions containing QTL that impact fatty acid components of acylsugars; these lines vary for the fatty acid profiles or sugars of their acylsugar. Testing these tomato lines performed by cooperators has lines that produce different acylsugar chemotypes also vary in efficacy of insect control. Furthermore, the optimal lines for control of vector insect species also reduce the likelihood of virus presence after plant/plant tissue is exposed to viliferous insects.

Use of acylsugar mediated resistance in commercial hybrids requires that any negative horticultural traits maintained in acylsugar lines through linkage drag are removed. The most severe of these negative traits was lack of normal fruit set; this trait has been resolved by alterations on chromosome 3 and chromosome 8, resulting in creation of the new benchmark line CU17NBL-1. Deconstruction of CU070126 created a series of mono and di introgression lines, and recovered plants with recombinations in each of the 4 targeted introgressions, allowing mapping of QTL responsible for the other negative horticultural traits: small fruit, short branchy plant type, and off flavor to fruit, facilitating their removal of each of these traits. This work is progressing very rapidly. Small fruit QTL were located on chromosomes 2, 3 and 10; a line in which the chromosome 10 small fruit QTL has been removed has already been created. The off flavor trait is governed by a QTL on chromosome 3; several lines lacking this QTL have been identified. As each case of linkage drag is rectified, the altered introgression is backcrossed into a common line, which eventually will possess all of the altered introgressions needed for acylsugar production in a line with good horticultural type.

# **Disease Resistance**

# Discovery and improvement of novel and known resistance to Fusarium wilt race three of tomato.

Jessica L. Chitwood and Sam Hutton, Gulf Coast Research and Education Center, University of Florida, Balm, FL, USA.

Tomato (Solanum lycopersicum) is one of the most important vegetables in the U.S., but both fresh-market and processing tomato production is increasingly threatened by Fusarium wilt race 3 (Fol3) caused by the soil-borne fungus, Fusarium oxysporum f. sp. lycopersici. Fumigation has traditionally been used to help manage many soil-borne diseases including fusarium wilt, but the phase-out of methyl bromide left the industry without an effective replacement and cultural control methods are inadequate. Host resistance to Fol3 through the I-3 gene is the most effective management strategy. However, producers often choose to grow susceptible cultivars due to the association of I-3 with detrimental traits, including bacterial spot sensitivity and small fruit size. We are using a dual strategy to produce more durable Fol3 resistance that is free from these problems. In order to address potential linkage-drag effects, we reduced the I-3 introgression from 5 Mb to approximately 120 Kb through successive recombinant screening and crossing efforts. The reduced introgression was crossed into elite Florida breeding lines and evaluated for its effects on bacterial spot sensitivity and fruit size. Current data shows the reduced introgression results in significantly less bacterial spot and larger fruit size than the original 4 Mb introgression, and it has no effect compared with Fol3 susceptibility. Taken together, these indicate that linkage with these negative traits has been broken. To promote greater durability of resistance, we also sought to identify novel Fol3 resistance. The wild tomato relative S. pennellii has been shown to be highly resistant to all races of Fusarium wilt, and we subjected 42 accessions to disease screens to identify resistance. Molecular markers were used to screen away from the I-3 and I-7 resistance loci as Fol3 resistance was introgressed from these accessions into elite, susceptible tomato backgrounds via seedling disease assays and backcrossing. After four generations of backcrossing, resistant plants were evaluated in the field for horticultural traits.  $F_2$  progeny of single-plant selections representing 36 of the accessions were screened for resistance to Fol3, and ratios of healthy to infected plants were used to identify accessions most likely to have contributed single, dominant resistance genes. Phylogenetic relationships were also considered, and four backcross populations with resistance derived from accessions in three phylogenetic clusters were chosen for mapping novel resistance alleles. The novel alleles will be characterized for efficacy against races 1 and 2 as well as a collection of diverse Fol3 isolates; and will also be pyramided with currently available alleles. These efforts will result in the development of improved *Fol3* resistant cultivars and more durable resistance against this pathogen.

# A SCREEN OF GENETICALLY DIVERSE TOMATO ACCESSIONS REVEALS A RANGE OF UNUSUAL RESPONSES TO *Pseudomonas syringae* pv. Tomato

\*Robyn Roberts, Samantha Mainiero, Adrian F. Powell, Sarah R. Hind, Susan R. Strickler, Boyce Thompson Institute; and Gregory B. Martin, Boyce Thompson Institute and the School of Integrative Plant Science - Plant Pathology and Plant-Microbe Biology, Cornell University

Pseudomonas syringae pv. tomato (Pst) causes bacterial speck disease of tomato. The only genetic resistance to Pst is conferred by the R gene Pto, which recognizes the Pst effectors AvrPto and AvrPtoB. However, Pst strains have recently emerged which lack these effectors, rendering Pto-mediated resistance ineffective. Our study aims to identify new sources of genetic resistance against Pst that can be incorporated into tomato variety improvement programs. Using available whole-genome resequencing data, we leveraged natural variation among tomato heirlooms, breeding lines, and a wild species to screen 216 accessions for new sources of resistance. We inoculated the accessions by spraying Pst wildtype and mutant strains which lack specific effectors and/or flagellin to help elucidate whether observed host responses involve effectortriggered (ETI) or pattern-triggered (PTI) immune pathways. Interestingly, our screen uncovered new Pst disease phenotypes beyond the typical speck symptoms, and we have found that some of these phenotypes are simply inherited. Using an assay that measures the production of PTIrelated reactive oxygen species, we discovered new accessions that have increased, or conversely, no response to the flagellin peptides flg22 or flgII-28, which are recognized by the pattern recognition receptors FLAGELLIN SENSING 2 (FLS2) or FLAGELLIN SENSING 3 (FLS3), respectively. These results provide a gateway for the study of the molecular mechanisms acting downstream of these two pattern recognition receptors. We are currently using available wholegenome resequencing data and genetic approaches to identify gene candidates that underlie novel resistance or susceptibility to Pst.

## FINE MAPPING AND CANDIDATE GENE CHARACTERIZATION OF THE PEPPER BACTERIAL SPOT RESISTANCE GENE *bs6*

# \*Rebecca L. Wente, Jian Li, Samuel F. Hutton, Gulf Coast Research and Education Center, University of Florida; Jeffrey B. Jones, Gerald V. Minsavage, Robert E. Stall, Department of Plant Pathology, University of Florida

Bacterial spot, caused by multiple Xanthomonas spp., is a major disease of tomato (Solanum lycopersicum L.) and pepper (Capsicum annuum L.) in warm, humid environmental conditions. The disease is characterized by necrotic lesions on the leaves and fruit, and can result in substantial crop losses. Host resistance has been an objective in many tomato and pepper breeding programs; and dominant resistance genes that elicit a hypersensitive response have been identified in both crops. However, these genes have lacked durability in the field, resulting in continued efforts to pyramid known resistance genes and to identify novel sources of resistance. Conversely, the recessive pepper resistance gene, bs5, has demonstrated more durable resistance to X. euvesicatoria races 0-10. A second recessive gene, bs6, is known to provide a higher level of resistance when combined with bs5, and may further promote the durability of resistance to this pathogen. Whereas the bs5 locus was recently discovered, the location of bs6 remains unknown. To map and fine map bs6, an  $F_2$  population and  $F_{2:3}$ recombinant inbred lines (RILs) were created from the cross between the susceptible cultivar 'Early Calwonder' (ECW) and an ECW near-isogenic line that contains bs6 (60R). Genotyping by sequencing of ECW, 60R, and 93 F<sub>2</sub> plants mapped bs6 to a 27 Mb region on chromosome 6. RILs were phenotyped by successive disease screens and genotyped with markers saturating this interval, delimiting bs6 to a 666 Kb interval. Candidate genes were sequenced and subjected to gene expression experiments for characterization. These results will improve breeding and selection efforts in pepper, and further work to clone the bs6 gene could accelerate efforts to engineer bs6-based resistance in tomato.

#### Tomato Improvement for Fruit Quality and Disease Resistance at NC State University

#### Dilip R. Panthee

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As a continuous effort to improve tomatoes for disease resistance and fruit quality, we are making some progress in large-fruited, plum and grape hybrid development at NC State University. As a result, we have developed NC13506 which is resistant to *Verticillium* wilt (VW; *Ve* gene), *Fusarium* wilt (FW; races 1, 2 and 3) (*I* and *I-2* and *I-3* genes), *Tomato spotted wilt virus* (TSWV; *Sw-5* gene), *Tomato mosaic virus* (ToMV; *Tm2* gene), and root-knot nematode (RKN; *Mi* gene). Both parents of NC13506 carry the recessive *og<sup>c</sup>* gene for crimson fruit color resulting in expression of improved red color and increased lycopene content in the F<sub>1</sub> hybrid. The combination of a high level of resistance to the TSWV along with resistances to VW and races 1, 2 and 3 of FW, the high marketable yield of large fruit desired for vine-ripe production and improved red color make NC13506 a unique hybrid. More importantly, this hybrid matures about a week earlier than standard control, which may be beneficial for tomato growers.

A new plum potential hybrid tomato NC11265 has been developed, which is resistant to VW, FW (*I* and *I*-2 genes), late blight (LB; *Ph*-2 and *Ph*-3 genes), and TSWV. Both parents of NC11265 carry the recessive *og*<sup>c</sup> gene for crimson fruit color resulting in expression of improved red color in the F<sub>1</sub> hybrid. The combination of a high level of resistance to TSWV, VW and races 1 and 2 of FW, early maturity, and improved red color makes NC11265 unique hybrid. Results from multiple replicated trials indicated that the yield performance of NC11265 was highly stable in North Carolina.

In the improved grape tomato hybrid NC10259, we have combined disease resistance including FW race 3 (*I*-3 gene), TSWV and LB (*Ph-2* and *Ph-3* genes) resistance. Yield performance of this hybrid was consistently higher than control, and total soluble solids (TSS) content was comparable to other hybrids. These hybrids may be a new addition to our efforts to improve tomato for fruit quality and disease resistance in our program.

# BACTERIAL SPOT OF TOMATO: PATHOGEN STORY AND OUR EFFORTS OF TOMATO IMPROVEMENT IN NC

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Bacterial spot of tomato is a serious disease in NC and many other States, caused by multiple bacterial species and physiological races within the genus Xanthomonas. The disease potentially causes up to 66% yield loss. However, practical management of this disease is challenged by lack of effective chemical compounds and commercial resistant cultivar. Field surveys were conducted in major tomato production areas of western North Carolina during 2015/2016 field seasons to assess the pathogen diversity causing bacterial spot disease (Bsp) of tomato. A total of 284 Bsp strains were collected and assessed for sensitivity to copper and streptomycin (100 and 200 ppm) and genetic diversity using BOX-PCR assay. Forty-six representative Bsp strains were subjected to real-time PCR and assessed for a hypersensitive response (HR) to differentiate species and races. Overall, nearly 95.0% of the Xanthomonas strains were resistant to copper and 25%-44% resistant to streptomycin. All Xanthomonas isolates analyzed in both years had similar genetic profiles, suggesting that these bacterial isolates from NC are genetically similar, despite from different seed and transplant sources. Real-time PCR results demonstrated that the bacterial spot pathogens in NC belong to a single species X. perforans. HR assay identified 93.5% of Xanthomonas strains as race T4 and 6.5% as race T3. Results suggest that emergence of copper and streptomycin insensitive bacterial phenotypes in NC and a need for the investigation of alternative strategies for crop management. Genetic resistance to race T4 has been identified in the S. pimpinellifolium LA3707 derived line NC22L-1(2008) through greenhouse and field screening in previous studies. We carried out QTL analysis using 110 recombinant inbred lines (RIL) from a cross between NC- NC 30P x 22L-1 (2008. A linkage map with 886 SNP loci was constructed covering 739.5 cM of 12 chromosomes of tomato, with an average of 0.83 cM between markers. Two major QTLs were identified on chromosome 6 at positions 11.83 cM-14.33 cM and 27.49 cM with LOD values of 4.0 and 8.0, respectively explaining 16%-26% of the total phenotypic variance. These QTLs were consistent in at least two environments. Two QTLs were detected on chromosome 1 at positions 49.58 cM and 95.21 cM with LOD value of 3.0, explaining 23% and 16% of phenotypic variance, respectively. Another QTL is detected on chromosome 12 explaining 9%-14% of phenotypic variance. The markers linked to these QTLs may be valuable in a marker-assisted tomato breeding program against bacterial spot disease.

# PYRAMIDING RESISTANCES TO BACTERIAL SPOT AND BACTERIAL SPECK IN ELITE FRESH MARKET TOMATO.

\*Taylor Anderson, Plant Breeding and Genetics Section, SIPS, Cornell University; Martha A. Mutschler, Plant Breeding and Genetics Section, SIPS, Cornell University; David Francis, The Ohio State University

Significant losses are incurred in tomato due to the bacterial diseases bacterial speck and bacterial spot. The need for resistant breeding stocks is increasingly important given widespread resistance to copper-based control. The Cornell University, in cooperation with the OSU breeding program, is currently pyramiding several disease resistance loci to the underlying pathogens in fresh market tomato. Characterized resistance loci to bacterial speck and spot, on chromosomes 5 and 11, are being pyramided in an elite, disease-resistant fresh market breeding line that already contains resistances to the oomycete disease late blight, and the fungal diseases early blight, and Septoria leaf spot. The marker-assisted backcross breeding approach pairs forward and reverse selection on all chromosomes to accelerate the recovery of the elite, recurrent lines. Several of these genes are in relatively close proximity to each other, reducing the number of linkage groups which must be tracked to move the resistances to new breeding materials. The first of the lines, possessing a bacterial spot resistance gene and resistance-associated QTL on chromosome 11, were completed in the winter of 2017, and will be trialed in the summer of 2018 to evaluate the impact of these loci on bacterial spot disease control and on fresh market tomato market qualities. The lines possessing the chromosome 5 bacterial spot and bacterial speck resistances are nearing completion and would be tested starting 2019.

#### Quality

# PURÉE TO PEAKS IN 15 MINUTES: A RAPID CAROTENOID EXTRACTION AND UHPLC-PDA ANALYSIS WORKFLOW FOR TOMATO BREEDING (POSTER)

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Tomatoes (Solanum lycopersicum) are an economically and nutritionally important crop that owe their color to carotenoid pigments such as lycopene and  $\beta$ -carotene. Public and private breeding programs have focused on manipulating fruit carotenoid content to improve consumer acceptability and enhance potential health benefits associated with consuming tomatoes. However, accurate phenotyping of carotenoids requires lengthy extraction protocols and chromatographic separation methods creating analytical bottlenecks that can reduce genetic gain per year. We developed both a rapid tomato carotenoid extraction protocol and an ultrahigh performance liquid chromatography photodiode array detector (UHPLC-PDA) analysis method. To validate our extraction and quantitation methods, backcross populations of processing and cherry tomatoes were created with varying fruit concentrations of lycopene and  $\beta$ -carotene by exploiting natural variation in the fruit-specific lycopene beta cyclase (*Cyc-B*). These populations were phenotyped using established extraction and high performance liquid chromatography photo diode array detector (HPLC-PDA) methods in addition to our rapid tomato carotenoid workflow. We estimated variance components for genetic and environmental variables for both methods and determined that our new extraction and UHPLC-PDA analysis method performed comparably to established protocols while taking substantially less time. Notably, our methods were approximately 2x (~5 minutes/sample) and 5x (4.2 minutes/sample) faster than current extraction and HPLC-PDA methods, respectively. Our UHPLC-PDA method was able to resolve most cis-carotenoid isomers from all-trans carotenoids. Our rapid carotenoid extraction and analysis workflow could greatly enhance tomato breeding programs by drastically increasing throughput without sacrificing analytical performance or heritability.

#### **BREEDING FOR PROCESSING TOMATO QUALITY**

#### Steve Loewen, University of Guelph Ridgetown Campus

The pre-breeding effort at the University of Guelph's Ridgetown Campus has the overarching goal of increasing genetic diversity for processing tomatoes. The work builds on the accomplishments of previous introgression of wild tomato species into cultivated tomato by Vaino Poysa. Through a strategy of backcrossing and phenotypic selection, the semi-wild lines are developed into breeding lines that retain genetic diversity from the wild species while resembling regionally adapted, processing tomatoes. Historically the work has been developing breeding lines targeted toward whole-peel end-use. The fruit quality attributes required for this end-use will be discussed. Early results of our work using hp-1 to improve colour and nutritional value of fruit will be presented. We have made some progress improving fruit maturity. The progress in our work with high-anthocyanin fruit in a processing background, developed independently of the fresh-market work by others, will be reviewed. Studies have been conducted that begin to link anthocyanins in fresh tomatoes, and possibly processed tomato products, with potential benefits for human health. The discussion will conclude with some reflections on breeding for flavour in tomatoes. Despite tomato breeders' good intentions, public discourse about tomato flavour is fraught with challenges. We propose that for many years tomato flavour has been a symbol for everything that people dislike about modern agriculture.

## IDENTIFICATION OF A MAJOR GRAYWALL RESISTANCE LOCUS ON CHROMOSOME 9 OF TOMATO

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Graywall (GW) is a disease of unknown etiology that affects ripening of fresh market tomato (Solanum lycopersicum L), causing uneven ripening and vascular browning on the fruits. There are no biotic or abiotic factors known to cause GW, but incidence is associated with environmental conditions such as low light, cool weather during fruit ripening, and nutrient management practices such as high nitrogen, or low potassium. Symptoms usually appear after mature green fruit have been harvested, stored and delivered to the retail level. GW can cause substantial losses to marketable yield, and the only effective control strategy is the use of resistant cultivars. Resistance breeding efforts rely on phenotypic selection in seasons when disease symptoms are expressed. However, seasonal variation in disease incidence and severity contribute to reduced selection accuracy, resulting in a lengthier and more expensive selection program. An understanding of the genetics of GW resistance, together with the availability of molecular markers linked to resistance genes would greatly improve resistance breeding efforts. Field observations made in fall 2015 suggested that GW resistance/susceptibility may be associated with the Frl locus on chromosome 9. To test this hypothesis, 20 resistant and 15 susceptible recombinant inbred lines (RILs) from the cross between Fla. 8000 (GW susceptible) and Fla. 8111B (GW resistant) were genotyped with 9 markers spanning chromosome 9 and corresponding to SNPs developed through the USDA SolCAP project. Single marker analysis confirmed linkage of a major GW resistance locus with three markers on the short arm of the chromosome ( $P \le 0.0005$ ), near the Frl locus. Trials conducted in winter 2016-2017 likewise demonstrated coupling phase or repulsion phase linkage of GW resistance with Frl in the (Fla. 8828 x Fla. 8590) and (Fla. 7946 x Fla. 8442) F<sub>2</sub> populations, respectively. Using a map-based approach for fine mapping, 35 recombinants corresponding to a 6 Mbp interval were identified from an (Fla. 8000 x Fla. 8111B) F<sub>3</sub> population. Progeny of recombinants were genotyped to select plants that were homozygous for the recombination events for the development of RILs. Results from evaluation of RILs in winter 2017-2018 delimited the resistance locus in Fla. 8111B to a 1.5 Mbp interval. Resistance in Fla. 8111B is dominant, based on testing of this parent along with Fla. 8000 and the F<sub>1</sub> in winter 2016-2017 and 2017-2018 seasons. The chromosome 9 locus was also tested in a separate population developed from the cross between Fla. 8570 (GW resistant) and Fla. 8059 (GW susceptible). 25 resistant and 22 susceptible RILs from this population were genotyped with the initial 9 markers corresponding to chromosome 9. Single marker analysis indicated no significant association of chromosome 9 markers with GW resistance, suggesting that resistance in Fla. 8570 may be controlled by a separate locus (loci). Mapping of GW resistance in this population is currently underway.

## FINE MAPPING OF A LOCUS IN CHROMOSOME 12 CONTROLLING FLAT AND GLOBE FRUIT SHAPE IN FRESH-MARKET TOMATO

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Relative to wild tomato, fruit size of cultivated tomato (Solanum lycopersicum L.) has been tremendously enlarged, and its shape has greatly diversified during the domestication process. About 30 QTLs have been found to account for most of this variation. Within specific tomato markets, however, fruit morphology is less explored, and it is believed that rather than known major QTLs, other loci are responsible for variation in shape and size. Within large-fruited germplasm, breeders have long recognized differences in flat (oblate) and globe (round) fruit shape, especially as this is thought to influence size and marketability characteristics. In general, globe fruits tend to be larger, whereas flat fruits retain better marketability. Breeders often make crosses between flat and globe parents, and in the resulting hybrids, flat shape is observed to be dominant, while size is intermediate. Large fruit size is an important characteristic for many markets, and in some of those, growers receive higher prices for larger fruit. Knowledge of the location of the gene(s) controlling this trait may improve cultivar development efforts for freshmarket tomato. The objectives of this study were (1) to identify and fine map the locus (loci) controlling flat and globe fruit shape in large-fruited germplasm, and (2) to characterize the effect of this trait on fruit shape, size, and marketability, as well as on other morphological traits. Eleven flat and 12 globe-fruited F<sub>6</sub> recombinant inbred lines (RILs) from the Fla. 8000 (flat) x Fla. 8111B (globe) population were genotyped using the 7,720 SNP Illumina Infinium SolCAP SNP array. Composite interval mapping (CIM) detected a single locus on the upper arm of chromosome 12 controlling fruit shape, and further CIM using 240 F<sub>2</sub> plants from the same cross confirmed this region. Using a map-based approach, recombinant plants for this interval were identified, evaluated for fruit shape, and genotyped using markers saturating the region. Results delimited the locus to an approximately 400 KB interval containing a single candidate gene. In a survey of 29 flat and 21 globe UF/IFAS breeding lines, a marker corresponding to this gene always segregated with shape. For three different backgrounds, homozygous flat, heterozygous, and homozygous globe near-isogenic lines were used to characterize the effects of this locus. Fruit shape attributes were measured using the Tomato Analyzer 2.2 software. Significant differences were observed among genotypes for fruit weight and for multiple fruit shape traits such as fruit height, fruit shape index, and shoulder characteristics. Heterozygous genotypes were generally intermediate to the homozygous genotypes for fruit weight and other traits, but the significance of those differences depended on the trait and on the background. For two of the three nearisogenic backgrounds, plant biomass was lower for globe genotypes compared to flat genotypes. In all backgrounds, pedicels were longer and thinner for globe genotypes than for flat genotypes and pedicels of heterozygous genotypes were intermediate but very similar to those of flat genotypes. Based on one season of trialing, this locus had no consistent effect on fruit defects such as cracking and checking.

### FRUIT WEIGHT VARIATION CAUSED BY THE KNOWN FW2.2, FW3.2, FAS, AND FW11.3 ALLELES IN FRESH MARKET TOMATO

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Previous studies have shown that the mutant alleles of FW2.2, FW3.2, and FW11.3 account for the majority of fruit weight increase in cultivated tomato; FW2.2 is practically fixed, but the last two genes are not, even though they are common in cultivated tomato germplasm. The mutant allele of FAS also regulates locule number and fruit weight, resulting in significantly larger fruit size. However, this allele is relatively rare within fresh market germplasm due to excessive fasciation and rough blossom end scarring, resulting in fruit that are too distorted and unmarketable. We sought to study potential interactions among these fruit weight genes in order to determine if introduction of wild type (WT) alleles at FW2.2, FW3.2 and/or FW11.3 might allow the utilization of FAS for increased fruit size without compromising fruit marketability. We developed two BC3 populations in the large-fruited Fla. 8059 and Fla. 8111B backgrounds for studying these effects: 'LYC1894' was used as the donor of the mutant FAS allele (linked with WT FW11.3) and the WT FW2.2 allele for the Fla. 8059 background; and 'Rosato Tondo' was used as the donor of the mutant FAS allele and the WT FW3.2 allele for the Fla. 8111B background. For each background, BC3F2 plants were genotyped at seedling stage to select the nine possible genotypic combinations of mutant and WT alleles. Recurrent parents and twentyfour plants per genotype were transplanted to the field at GCREC in a randomized complete-block design with three blocks. The trial was harvested twice at maturity, and fruit were graded for size and marketability. In the Fla. 8111B background, homozygosity for the WT FW3.2 allele reduced fruit size by 59 g relative to homozygosity for the mutant allele. Although homozygosity for the mutant FAS allele gave an increase in average fruit weight, misshapen fruit and rough blossom scars resulted in approximately 50 percent culls. Heterozygosity for the mutant FAS allele had no effect on fruit size when plants were homozygous for the WT FW3.2 allele, but resulted in an average increase of 39 g when plants were either heterozygous or homozygous for the mutant allele at FW3.2. In the Fla. 8059 background, FW2.2 had no significant effect on fruit size. Homozygosity for the mutant FAS allele again resulted in increased fruit weight, but for the Fla. 8059 background, 100 percent of the fruit were unmarketable. Heterozygosity for the mutant FAS allele had no effect on fruit size for plants that were homozygous for the WT FW2.2 allele, but an average 18 g increase in fruit weight was observed for plants that were either heterozygous or homozygous for the mutant allele at FW2.2. Heterozygosity for the FAS mutant allele had no significant effect on total marketable yield in either background. Results suggest that utilization of the mutant FAS allele in heterozygous state may help to increase fruit size without compromising marketability, but the substitution of WT alleles at FW2.2 and FW3.2 provides little or no advantage in this.