CucCAP:

Leveraging applied genomics to improve disease resistance in cucurbit crops

Fourth Annual CucCAP Team Meeting
April 12-13, 2019
Hosted by Boyce Thompson Institute and Cornell University
Ithaca NY
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AGENDA

and

PARTICIPANTS
AGENDA

Fourth annual CucCAP team meeting – April 12-13, 2019

Friday, April 12
8:00-8:15 Arrivals, welcome
8:15-8:30 Overview of project progress, plans for meeting
  - Progress to date
  - Priorities for the coming year
  - Priorities and strategy for the future

Session I – Genomic Tools
Objective I: Develop genomic approaches and tools for cucurbit species
- Develop genomic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and analysis; and genotype, phenotype and QTL databases
- Perform GBS analysis of PI collections and core populations of the four species to provide a community resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website and genomics and bioinformatics workshops

8:30-8:50 Overview of progress: bioinformatics platforms and website, GBS data and analysis, publications (Fei, Reddy)
8:50- 9:30 Status of core panels (seed stocks for first generation; resequencing) and timeline to finish watermelon (Levi)
  - melon (McCreight)
  - cucumber (Weng)
  - squash (Mazourek)

9:30-10:30 Proposed genomics tools objectives for CucCAP 2 – Discussion and feedback from industry
  9:30 -- Pan-genomic analysis, identify sources of individual variation, multiple reference genomes, nanopore resequencing representatives, cucurbit marker sets across genomes; genomics website and bioinformatics capacities (Fei)
  9:50 -- Core populations seed supplies – can we partner with seed companies? Partner with NGS to distribute core populations (Yiqun, Amnon)

10:10-10:30 Break

Session II – Breeding for disease resistance
Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.
- Utilize genomic approaches to map resistance loci for key cucurbit diseases
- Develop and verify molecular markers for efficient trait selection and gene pyramiding
- Introgress resistances into advanced breeding lines
- [E/O] Provide web-based and face to face information via field trials, extension venues, and scientific meetings regarding breeding materials, markers, and breeding progress

10:30-11:30 Watermelon: Status for each disease
  - where were we at the outset? Where are we now? Priorities to finish CucCAP1?
  - breeding lines, mapping/QTL, markers, introgression
    Present by disease:
    - Fusarium, gummy stem blight, Phytophthora, powdery mildew, GCMMV, PRSV-W (Levi, Kousik, Ling, McGregor, Wechter, Wehner)

11:30-12:00 Feedback/priorities from industry (include teleconference if needed with commodity reps)
12:00-1:00 **Lunch**

1:00-1:40 **Melon**: Status for each disease  
– where were we at the outset? Where are we now? Priorities to finish CucCAP1?  
  breeding lines, mapping/QTL, markers, introgression  
  Present by disease: powdery mildew, CMV, CYSDV, Fusarium  
  (McCreight, Kousik, Wechter, Wintermantel)

1:40-2:00 Feedback/priorities from industry (include teleconference if needed with commodity reps)

2:00-2:25 **Cucumber**: Status for each disease  
– where were we at the outset? Where are we now? Priorities to finish CucCAP1?  
  breeding lines, mapping/QTL, markers, introgression  
  Present by disease: downy mildew, Phytophthora  
  (Weng, Grumet, Wehner)

2:25-2:45 Feedback/priorities from industry (include teleconference if needed with commodity reps)

2:45-3:00 **Break**

3:00-3:30 **Squash**: Status for each disease  
– where were we at the outset? Where are we now? Priorities to finish CucCAP1?  
  breeding lines, mapping/QTL, markers, introgression  
  Present by disease: PRSV-W, ZYMV, Phytophthora, powdery mildew?  
  (Mazourek, Wessel-Beaver, Smart, Hausbeck?)

3:30-3:45 Feedback/priorities from industry (include teleconference if needed with commodity reps)

**Session III – Disease control information and Economic impact**

Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.
- Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets
- Use a risk-based simulation model to analyze economic potential of the disease resistant cucurbit cultivars
- [E/O] Develop a centralized cucurbit disease website, including content in English and Spanish, providing information about diagnostics and links to disease clinics; disease control recommendations; disease alerts and links to forecasting tools

3:45-4:15 Extension team: Progress to date, priorities to finish CucCAP1  
  (Schulthies, Hausbeck, Linares, Quesada, Smart, Lorscheider)

4:15-5:15 Socioeconomics team: Progress to date, priorities to finish CucCAP1  
  (Palma, Rivera)

5:15-5:45 Discussion and feedback from industry (include teleconference if needed with commodity reps)

6:00 **CucCAP Networking Dinner**
Saturday, April 13
8:00-8:15 Arrive
8:15-8:30 USDA-SCRI landscape and implications for the future

Session IV – Planning Sessions

8:30-9:15 Working session I: commodity group goals
Watermelon, Melon, Cucumber, Squash
How do we build on our accomplishments?

Utilize genomic approaches to map resistance loci/develop markers/introgress resistance
Possible future steps:
- Refine list of diseases based on industry priorities, emerging diseases, progress to date?
- Diversify sources of resistance (how? GWAS? Does other genetic analysis of PIs help?)
- Identify syntenic regions across species?
- Continue development of markers/develop tools for pyramiding (what would those be?)
- Test performance of identified QTL when incorporated into advanced breeding lines – coordinate with extension/pathologists/industry? coordinated resistance trials?
-- Phenotype core populations/ GWAS – which traits? multiple locations?
  Develop coordinated approach for phenotyping across locations/traits? (Michael?)
- New approaches to consider/highlight?

9:15-10:00 Working session II
A. Genomics tools goals (Fei, lead)
  Priorities, strategies, partnering with industry

B. Extension and Economics goals (Schultheis, Palma)
  Priorities, strategies, partnering with industry

10:00-10:15 Break

10:15-11:15 Priorities and strategies including matches
Report out from each team (5 min each)
Feedback from industry; including new industry partners?

11:15-12:00 Wrap up discussions, feedback from external reviewers
CucCAP Team

Project Director

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Cecilia McGregor  
Assistant Professor  
Department of Horticulture  
1119 Miller Plant Sciences  
University of Georgia Athens, GA 30602  
cmcgre1@uga.edu  
(watermelon team)
<table>
<thead>
<tr>
<th><strong>Organization</strong></th>
<th><strong>Representative</strong></th>
<th><strong>Position</strong></th>
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</thead>
<tbody>
<tr>
<td>National Watermelon Promotion Board</td>
<td>Mark Arney</td>
<td>Executive Director, National Watermelon Promotion Board</td>
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<tr>
<td>National Watermelon Association</td>
<td>Robert Morrissey</td>
<td>Executive Director, National Watermelon Association</td>
</tr>
<tr>
<td>California Melon Research Board</td>
<td>Milas Russell</td>
<td>Chair Elect, California Melon Research Board President, Sandstone Melon Company</td>
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<tr>
<td>California Melon Research Board</td>
<td>Steve Smith</td>
<td>Chair, California Melon Research Board Co-Owner Turlock Fruit Company</td>
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<tr>
<td>Pickle Packers International</td>
<td>Brian Bursiek</td>
<td>Executive Vice President, Pickle Packers International</td>
</tr>
<tr>
<td>Swanson Pickles and Pickle Packers International</td>
<td>John Swanson</td>
<td>President Swanson Pickle Company; Research Board, Pickle Packers International</td>
</tr>
<tr>
<td>Martin Farms (squash grower, shipper)</td>
<td>Mitch Beyler</td>
<td>Partner, John B. Martin and Sons Farms, Inc.</td>
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<tr>
<td>Stony Brook Wholehearted Foods (squash processor)</td>
<td>Greg Woodworth</td>
<td>Founder, Stony Brook Wholehearted Foods</td>
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<tr>
<td><strong>Seed Industry</strong></td>
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<tr>
<td>Bayer Crop Science</td>
<td>Jovan Djordjevic/ Suren Baliji/ Peter Kraan</td>
<td>Global R&amp;D Lead, Melons and Watermelons, Bayer Crop Science</td>
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<tr>
<td>HM Clause</td>
<td>Kishor Bhattarai</td>
<td>Phytopathology Project Manager, HM Clause, Vegetable Seeds Division, Limagrain</td>
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<tr>
<td>Hollar Seed Company</td>
<td>Bruce Carle</td>
<td>Plant Breeder, Hollar Seed Company</td>
</tr>
<tr>
<td>Johnny’s Selected Seeds</td>
<td>Rob Johnston/ Lindsay Wyatt</td>
<td>Chairman, Johnny’s Selected Seeds</td>
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<tr>
<td>Monsanto</td>
<td>Nischt Shetty</td>
<td>NAFTA Cucurbit Lead for Monsanto Vegetable Seeds</td>
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<tr>
<td>Sakata Seeds</td>
<td>Jeff Zischke/ Nihat Guner</td>
<td>Director of Research, Vegetables, Sakata Seed</td>
</tr>
<tr>
<td>United Genetics Seeds Co.</td>
<td>Xuemei Zhang</td>
<td>Melon Breeder, United Genetic Seeds</td>
</tr>
</tbody>
</table>
**Cucurbit Crop Curators**

Robert Jarret - *Citrullus* spp.
USDA-ARS, Plant Genetic Resources Conservation Unit
1109 Experiment Station, Griffin GA 30223
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**External Evaluators**

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CucCAP PROJECT OBJECTIVES,

TIMELINES and METRICS
CucCAP PROJECT OBJECTIVES

Each objective includes integrated research and extension/outreach [E/O] approaches:

**Obj. 1. Develop genomic approaches and tools for cucurbit species.**
- Develop genomic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and analysis; and genotype, phenotype and QTL databases
- Perform GBS analysis of PI collections and core populations of the four species to provide a community resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website, and by genomics and bioinformatics workshops open to all members of the cucurbit scientific and breeding communities

**Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.**
- Utilize genomic approaches to map resistance loci for key cucurbit diseases
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**Disease priorities identified by the cucurbit industries:**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Identified as commodity funding priority</th>
<th>Also affects:</th>
</tr>
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<tbody>
<tr>
<td>Downy mildew</td>
<td>cucumber</td>
<td>melon, watermelon, squash/pumpkin</td>
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<tr>
<td>Fusarium wilt</td>
<td>watermelon</td>
<td>melon, cucumber</td>
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<td>Gummy stem blight</td>
<td>watermelon</td>
<td>melon, cucumber, squash/pumpkin</td>
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<tr>
<td>Phytophthora rot</td>
<td>cucumber, watermelon, squash/pumpkin</td>
<td>melon</td>
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<tr>
<td>Powdery mildew</td>
<td>melon, watermelon, squash/pumpkin</td>
<td>cucumber</td>
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<tr>
<td>Viruses (CMV, CYSDV, PRSV-W, CGMMV)</td>
<td>melon&lt;sup&gt;b,c&lt;/sup&gt;, watermelon&lt;sup&gt;c,e&lt;/sup&gt;</td>
<td>cucumber&lt;sup&gt;c,e&lt;/sup&gt;, squash/pumpkin&lt;sup&gt;b,d&lt;/sup&gt;</td>
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</table>
## Project Structure – Team Organization

<table>
<thead>
<tr>
<th>CucCAP Teams</th>
<th>Team</th>
<th>PD, Co-PDs and Co-PIs</th>
<th>Institution*</th>
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<tbody>
<tr>
<td>Watermelon</td>
<td>PD: Rebecca Grumet</td>
<td>(RG) MSU</td>
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<td></td>
<td><strong>Team Leader: Amnon Levi</strong></td>
<td>(AL) ARS-SC</td>
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<td></td>
<td>Shaker Kousik</td>
<td>(SK) ARS-SC</td>
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<td></td>
<td>Kai-Shu Ling</td>
<td>(KSL) ARS-SC</td>
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<td></td>
<td>Cecilia McGregor</td>
<td>(CM) UGA</td>
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<td></td>
<td>Lina Quesada</td>
<td>(LQ) NCSU</td>
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<td></td>
<td>Pat Wechter</td>
<td>(PW) ARS-SC</td>
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<td></td>
<td>Todd Wehner</td>
<td>(TW) NCSU</td>
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<td>Melon</td>
<td><strong>Team Leader: Jim McCreight</strong></td>
<td>(JM) ARS-CA</td>
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<td></td>
<td>Shaker Kousik</td>
<td>(SK) ARS-SC</td>
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<td></td>
<td>Pat Wechter</td>
<td>(PW) ARS-SC</td>
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<td></td>
<td>Bill Winternmantel</td>
<td>(BW) ARS-CA</td>
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<tr>
<td>Cucumber</td>
<td><strong>Co-PD, Team Leader: Yiqun Weng</strong></td>
<td>(YW) ARS-WI</td>
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<td></td>
<td>Rebecca Grumet</td>
<td>(RG) MSU</td>
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<td></td>
<td>Mary Hausbeck</td>
<td>(MH) MSU</td>
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<td>Todd Wehner</td>
<td>(TW) NCSU</td>
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<tr>
<td>Squash</td>
<td><strong>Team Leader: Michael Mazourek</strong></td>
<td>(MM) CU</td>
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<td></td>
<td>Christine Smart</td>
<td>(CS) CU</td>
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<td></td>
<td>Linda Wessel-Beaver</td>
<td>(LWB) UPR</td>
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<td>Genomics/bioinformatics</td>
<td><strong>Team Leader: Zhangjun Fei</strong></td>
<td>(ZF) BTI</td>
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<td></td>
<td>Umesh Reddy</td>
<td>(UR) WVSU</td>
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<td></td>
<td>Amnon Levi (watermelon)</td>
<td>(AL) ARS-SC</td>
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<td></td>
<td>Mike Mazourek (squash)</td>
<td>(MM) CU</td>
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<td></td>
<td>Pat Wechter (melon)</td>
<td>(PW) ARS-SC</td>
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<td></td>
<td>Yiqun Weng (cucumber)</td>
<td>(YW) ARS-WI</td>
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<td>Socioeconomics</td>
<td><strong>Team Leader: Marco Palma</strong></td>
<td>(MP) TAMU</td>
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<td></td>
<td>Louis Ribera</td>
<td>(LR) TAMU</td>
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<td>Extension/Outreach</td>
<td><strong>Team Leader: Jonathan Schultheis</strong></td>
<td>(JS) NCSU</td>
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<td></td>
<td>Lina Quesada (watermelon)</td>
<td>(LQ) NCSU</td>
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<td></td>
<td>Mary Hausbeck (cucumber)</td>
<td>(MH) MSU</td>
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<td></td>
<td>Jim McCreight (melon)</td>
<td>(JM) ARS-CA</td>
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<td></td>
<td>Angela Linares Ramirez</td>
<td>(ALR) UPR</td>
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<td></td>
<td>Christine Smart (squash)</td>
<td>(CS) CU</td>
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<td></td>
<td>Zhangjun Fei (bioinformatics)</td>
<td>(ZF) CU</td>
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</tbody>
</table>

*Institution abbreviations: ARS-CA (Salinas), SC (Charleston), WI (Madison); BTI-Boyce Thompson Inst; CU-Cornell Univ; MSU-Michigan St Univ; NCSU-North Carolina St Univ; TAMU- Texas A&M Univ; UGA-Univ Georgia; UNH-Univ New Hampshire; UPR-Univ Puerto Rico; WVSU-West Virginia St Univ
TIMELINE CHART

<table>
<thead>
<tr>
<th>Objective</th>
<th>Personnel/Institution</th>
<th>Year</th>
</tr>
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<tbody>
<tr>
<td>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</td>
<td>(initials as in Table 3)</td>
<td>1</td>
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<tr>
<td><strong>1.1. Develop genomic and bioinformatics platforms</strong></td>
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<tr>
<td>1.1.1. Genotyping by sequencing</td>
<td>ZF (BTI)</td>
<td>X</td>
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<tr>
<td>1.1.2. Sequence data processing/analysis</td>
<td>ZF (BTI)</td>
<td>X</td>
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<tr>
<td>1.1.3. ICuGI database development</td>
<td>ZF (BTI)</td>
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<tr>
<td>1.1.4 Community standardized nomenclature</td>
<td>YW (ARS-WI), AL (ARS-SC)</td>
<td>X</td>
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<tr>
<td>1.1.5. Genomic, bioinformatics workshops</td>
<td>ZF (BTI), UR (WVSU), members of crop teams</td>
<td>X</td>
</tr>
<tr>
<td><strong>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations</td>
<td>ZF (BTI), RG (MSU)</td>
<td>X</td>
</tr>
<tr>
<td>- watermelon</td>
<td>AL (ARS-SC)</td>
<td>X</td>
</tr>
<tr>
<td>- melon</td>
<td>JM (ARS-CA)</td>
<td>X</td>
</tr>
<tr>
<td>- cucumber</td>
<td>YW (ARS-WI), MM (CU)</td>
<td>X</td>
</tr>
<tr>
<td>- squash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2.2. Population genetics and GWAS analyses</td>
<td>UR (WVSU), ZF (BTI)</td>
<td>X</td>
</tr>
<tr>
<td>- watermelon</td>
<td>AL (ARS-SC)</td>
<td>X</td>
</tr>
<tr>
<td>- melon</td>
<td>JM (ARS-CA)</td>
<td>X</td>
</tr>
<tr>
<td>- cucumber</td>
<td>YW (ARS-WI), RG (MSU)</td>
<td>X</td>
</tr>
<tr>
<td>- squash</td>
<td>MM (CU)</td>
<td>X</td>
</tr>
<tr>
<td><strong>(b) Obj. 2. Genomic assisted breeding for disease resistance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2.1 QTL map resistances:</strong></td>
<td>Screen for resistance (Sc), develop populations (P), phenotype (F), sequence (S), QTL map (Q)</td>
<td></td>
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<tr>
<td>2.1.1. Watermelon</td>
<td>KSL (ARS-SC), AL (ARS-SC)</td>
<td>Sc</td>
</tr>
<tr>
<td>- CGMMV</td>
<td>AL (ARS-SC), PW (ARS-SC)</td>
<td>FSQ</td>
</tr>
<tr>
<td>- Fusarium race 1</td>
<td>PW (ARS-SC), AL (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>race 2</td>
<td>CM (UGA), TW (NCSU)</td>
<td>P</td>
</tr>
<tr>
<td>- gummy stem blight</td>
<td>SK (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>- Phytophthora</td>
<td>SK (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>- PRSV-W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1.2. Melon</td>
<td>SK,PW (ARS-SC), JM (ARS-CA)</td>
<td>PF</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>PW (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>- Fusarium</td>
<td>JM (ARS-CA), WW (ARS-CA)</td>
<td>F</td>
</tr>
<tr>
<td>- CYSDV</td>
<td>JM (ARS-CA), MM (CU)</td>
<td>P</td>
</tr>
<tr>
<td>- CMV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1.3. Cucumber</td>
<td>YW (ARS-WI), TW (NCSU)</td>
<td>PFS</td>
</tr>
<tr>
<td>- downy mildew</td>
<td>RG (MSU)</td>
<td>PF</td>
</tr>
<tr>
<td>- Phytophthora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1.4 Squash</td>
<td>MM (CU), CS (CU)</td>
<td>PF</td>
</tr>
<tr>
<td>- PRSV-W</td>
<td>MM</td>
<td>PFQ</td>
</tr>
<tr>
<td>- CMV</td>
<td>MM</td>
<td>PFQ</td>
</tr>
<tr>
<td>2.2 Marker development and verification:</td>
<td>Refine map (R) develop marker (M), verify (V)</td>
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</tr>
<tr>
<td>2.2.1. Watermelon</td>
<td>AL (ARS-SC), PW (ARS-SC)</td>
<td>R</td>
</tr>
<tr>
<td>- Fusarium race 1</td>
<td>PW (ARS-SC), AL (ARS-SC)</td>
<td></td>
</tr>
<tr>
<td>race 2</td>
<td>CM (UGA), TW (NCSU)</td>
<td></td>
</tr>
<tr>
<td>- gummy stem blight</td>
<td>SK (ARS-SC)</td>
<td></td>
</tr>
<tr>
<td>- Phytophthora</td>
<td>SK (ARS-SC)</td>
<td></td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td></td>
</tr>
<tr>
<td>- PRSV-W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2.2. Melon</td>
<td>SK (ARS-SC)</td>
<td>M</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>PW (ARS-SC)</td>
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</tr>
<tr>
<td>- Fusarium</td>
<td>WW (ARS-CA), JM (ARS-CA)</td>
<td></td>
</tr>
<tr>
<td>- CYSDV</td>
<td>JM (ARS-CA), MM (CU)</td>
<td></td>
</tr>
<tr>
<td>- CMV</td>
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</table>

19
<table>
<thead>
<tr>
<th>2.2.3. Cucumber</th>
<th>YW (ARS-WI), TW (NCSU), RG (MSU)</th>
<th>RM</th>
<th>RM</th>
<th>V</th>
<th>RM</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>- downy mildew</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Phytophthora</td>
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<tr>
<td>2.2.4 Squash</td>
<td>MM(CU), LWB(UPR)</td>
<td>RM</td>
<td>V</td>
<td></td>
<td>RM</td>
<td>V</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td></td>
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</tr>
<tr>
<td>- Phytophthora</td>
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<tr>
<td>- PRSV-W</td>
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<tr>
<td>- CMV</td>
<td></td>
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</table>

### 2.3. Introgress resistance into advanced breeding lines:

Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R).

<table>
<thead>
<tr>
<th>2.3.1. Watermelon</th>
<th>AL (ARS-SC), PW (ARS-SC)</th>
<th>B</th>
<th>I</th>
<th>IA</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Fusarium race 1</td>
<td>AL (ARS-SC), PW (ARS-SC)</td>
<td>B</td>
<td>B</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>- gummy stem blight</td>
<td>CM (UGA), TW (NCSU)</td>
<td>B</td>
<td>B</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>- Phytophthora</td>
<td>SK (ARS-SC)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>A</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>SK (ARS-SC)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>A</td>
</tr>
<tr>
<td>- PRSV-W</td>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>2.3.2. Melon</td>
<td>SK (ARS-SC), JM (ARS-CA)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>IA</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>PW (ARS-SC)</td>
<td>B</td>
<td>B</td>
<td>I</td>
<td>IA</td>
</tr>
<tr>
<td>- Fusarium</td>
<td>JM (ARS-CA), WW (ARS-CA)</td>
<td>B</td>
<td>I</td>
<td>IA</td>
<td>IAR</td>
</tr>
<tr>
<td>- CMV</td>
<td>JM (ARS-CA)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>IA</td>
</tr>
<tr>
<td>2.3.3. Cucumber</td>
<td>YW (ARS-WI), TW (NCSU)</td>
<td>B</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>- downy mildew</td>
<td>RG (MSU)</td>
<td>B</td>
<td>B</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>- Phytophthora</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3.4 Squash</td>
<td>Already exists MM (CU), CS (CU)</td>
<td>I</td>
<td>I</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>Already exists</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- Phytophthora</td>
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<td></td>
<td></td>
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<tr>
<td>- PRSV-W</td>
<td></td>
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<td></td>
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<tr>
<td>- CMV</td>
<td></td>
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</tbody>
</table>

### (b) Obj. 3. Economic impact analyses, disease control information

#### 3.1 Perform economic analysis, cost of production/disease control

<table>
<thead>
<tr>
<th>3.1.1. Define, parameterize, simulate, validate production variables</th>
<th>LR (TAMU), MP (TAMU)</th>
<th>X</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.2. Estimate the potential economic impacts to the cucurbit industry</td>
<td>LR (TAMU), MP (TAMU)</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

#### 3.2 Provide readily accessible information to facilitate disease control

<table>
<thead>
<tr>
<th>3.2.1. Develop a centralized cucurbit disease website</th>
<th>LQ (NCSU), JS (NCSU)</th>
<th>X</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster</td>
<td>LQ (NCSU), MH (MSU), CS (CU), ALR (UPR)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3.2.3 Provide disease alerts and forecasting tools</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3.2.4. Field days and demonstration plots</td>
<td>Crop and extension teams</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
### Status of resistance breeding for the priority cucurbit diseases at project outset.

<table>
<thead>
<tr>
<th>Crop and disease</th>
<th>Sources of resistance</th>
<th>Elite germplasm for introgression</th>
<th>Field testing locations</th>
<th>Resistant parental line</th>
<th>Phenotypic data for GWAS</th>
<th>Segregating populations</th>
<th>Analysis of inheritance</th>
<th>QTL analysis segregating populations</th>
<th>Marker development</th>
<th>Introgression into cultivated types</th>
<th>Advanced breeding lines for release</th>
<th>Cultivars for release to farmer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watermelon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fusarium race 2 (Fus)</td>
<td>PI 482246-USVL246&lt;sup&gt;SR2&lt;/sup&gt;; PI 482252-USVL252&lt;sup&gt;SR2&lt;/sup&gt;</td>
<td>SC</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>Fusarium race 1</td>
<td>Calhoun Gray</td>
<td>SC</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>Gummy stem blight (GSB)</td>
<td>PI 482276-UGA1081; PI 526223-UGA157</td>
<td>SC, NC</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phytophthora (Phyt) Powdery mildew (PM)</td>
<td>PI 494531-USVL531MDR; PI 560003-USVL003MDR</td>
<td>Standard: Charleston Gray Icebox: Sugar Baby</td>
<td>SC, NC, SC, SC, SC</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CGMMV</td>
<td>Currently evaluating</td>
<td>GH&lt;sup&gt;®&lt;/sup&gt;</td>
<td>SC</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>PRSV-W</td>
<td>PI 595203</td>
<td></td>
<td></td>
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<tr>
<td><strong>Melon</strong></td>
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</tr>
<tr>
<td>Powdery (PM)</td>
<td>MR-1</td>
<td>Cantaloupe: TopMark, Impac Honeydew: Green Flesh Honeydew or PMR Honeydew</td>
<td>CA1,2, AZ, CA1</td>
<td>x</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fusarium (Fus)</td>
<td>MR-1</td>
<td></td>
<td>CA1</td>
<td>x</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>CYSDV</td>
<td>PI 313970; TGR1551</td>
<td></td>
<td>CA1, AZ</td>
<td>x</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>CMV</td>
<td>PI 161375; Freeman cucumber</td>
<td></td>
<td>CA1,2, AZ</td>
<td>x</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Cucumber</strong></td>
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<tr>
<td>Downy mildew (DM)</td>
<td>PI 197088; PI 330628</td>
<td>Slicer: Poinsett 76</td>
<td>WI, NC</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Phytophthora (Phyt)</td>
<td>PI 109483</td>
<td>Pickling: NC-25, GY14</td>
<td>MI, NY</td>
<td>x</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><strong>Squash</strong></td>
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<tr>
<td>Phytophthora (Phyt) Powdery (PM)</td>
<td>PI 211996; PI 483347; PI 634693</td>
<td>Butternut: Burpee Butterbush Tropical pumpkin: Soler, Taina Dorada</td>
<td>NY, PR</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td>Powdery (PM)</td>
<td>C. martenezii</td>
<td></td>
<td>PR</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
<td></td>
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<tr>
<td>PRSV-W</td>
<td>Menina, Nigerian Local</td>
<td></td>
<td>PR</td>
<td>X</td>
<td>X</td>
<td>x</td>
<td>X</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CMV</td>
<td>Menina, Nigerian Local</td>
<td></td>
<td>PR</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>X</td>
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</tbody>
</table>

<sup>SR2</sup>, <sup>SR3</sup>, <sup>SR4</sup>, <sup>SR5</sup> denote resistance sources and additional information.
## PROJECT METRICS

<table>
<thead>
<tr>
<th><strong>Metrics to be used in CucCAP project evaluation</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short term metrics (1-2 years)</strong></td>
<td></td>
</tr>
<tr>
<td>1. State of the art, genotyping by sequencing (GBS) and data analysis platforms are developed for cucurbit species.</td>
<td></td>
</tr>
<tr>
<td>2. GBS sequence data are obtained for 1000-1600 PIs for each of the four cucurbit crops.</td>
<td></td>
</tr>
<tr>
<td>3. Community-standardized cucurbit gene/trait descriptors and nomenclature are established.</td>
<td></td>
</tr>
<tr>
<td>4. Germplasm lines with resistance to <em>Fusarium r.1,2</em>, <em>Phytophthora</em>, powdery mildew, and PRSV are established for watermelon; for CYSDV in melon, and <em>Phytophthora</em> in cucumber.</td>
<td></td>
</tr>
<tr>
<td>5. Markers developed for KASP-based assay for downy mildew in cucumber and powdery mildew and ZYMV in squash.</td>
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</tr>
<tr>
<td>6. Field trials and field days are held to test and demonstrate disease resistant materials (average 1/yr/crop).</td>
<td></td>
</tr>
<tr>
<td>7. Representative farms are developed for economic analyses for three locations for each of the four commodities.</td>
<td></td>
</tr>
<tr>
<td>8. The CucCAP Cucurbit Disease Extension Website is established.</td>
<td></td>
</tr>
<tr>
<td>9. Participation in outreach to 15-20 stakeholder groups per year via industry events and field days.</td>
<td></td>
</tr>
<tr>
<td>10. A Cucurbit Genomics and Bioinformatics workshop is delivered at PAG 2017 attended by members from at least 20 cucurbit research laboratories.</td>
<td></td>
</tr>
<tr>
<td>11. Field trials and field days are held to test and demonstrate disease resistant materials (average 1/yr/crop).</td>
<td></td>
</tr>
<tr>
<td>12. Representative farms are developed for economic analyses for three locations for each of the four commodities.</td>
<td></td>
</tr>
<tr>
<td>13. The CucCAP Cucurbit Disease Extension Website is established.</td>
<td></td>
</tr>
<tr>
<td>14. Participation in outreach to 15-20 stakeholder groups per year via industry events and field days.</td>
<td></td>
</tr>
<tr>
<td>15. A Cucurbit Genomics and Bioinformatics workshop is delivered at PAG 2017 attended by members from at least 20 cucurbit research laboratories.</td>
<td></td>
</tr>
<tr>
<td><strong>Medium term metrics (3-4 years)</strong></td>
<td></td>
</tr>
<tr>
<td>1. Population structure analysis is performed and molecular-directed core populations are established for the four cucurbit crops.</td>
<td></td>
</tr>
<tr>
<td>2. Breeder-friendly databases to store and distribute genomic, phenotypic, and genotypic information and development of associated data analysis tools are implemented (<a href="http://www.icugi.org">www.icugi.org</a>). Based on current traffic, at least 1000 unique visitors are expected per week.</td>
<td></td>
</tr>
<tr>
<td>3. GWAS analyses are performed for CGMMV, <em>Fusarium</em>, gummy stem blight, <em>Phytophthora</em> and PRSV-W in watermelon; CYSDV in melon and <em>Phytophthora</em> in cucumber.</td>
<td></td>
</tr>
<tr>
<td>4. QTL associated with CGMMV, <em>Fusarium r.2</em>, gummy stem blight, <em>Phytophthora</em>, powdery mildew, and PRSV in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; downy mildew, <em>Phytophthora</em> in cucumber; and CMV, PRSV and powdery mildew in squash have been identified.</td>
<td></td>
</tr>
<tr>
<td>5. Molecular markers have been developed for <em>Fusarium r.1</em> in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; downy mildew in cucumber; and CMV, PRSV and powdery mildew in squash.</td>
<td></td>
</tr>
<tr>
<td>6. Breeding lines with resistance to <em>Fusarium r.1,2</em> and PRSV in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; downy mildew in cucumber; and <em>Phytophthora</em> in butternut squash are available to researchers and seed companies.</td>
<td></td>
</tr>
<tr>
<td>7. Cucumber lines carrying multiple disease resistances (downy mildew/powdery mildew/ZYMV) developed by marker assisted selection.</td>
<td></td>
</tr>
<tr>
<td>8. Field trials and field days are held to test and demonstrate disease resistant materials (average 2/yr/crop).</td>
<td></td>
</tr>
<tr>
<td>9. Cucurbit disease informational materials in English and Spanish are developed and posted on the CucCAP disease website for each of the priority diseases.</td>
<td></td>
</tr>
<tr>
<td>10. Stakeholders use website and social media tools to obtain information about disease outbreaks, diagnosis and control. Based on prior experience with cucurbit disease tracking and informational websites, 1000-2000 hits per week are expected during peak growing season.</td>
<td></td>
</tr>
<tr>
<td>11. Participation in outreach to 15-20 stakeholder groups each year via industry events and field days.</td>
<td></td>
</tr>
<tr>
<td>12. Representative farms are compared to information available through USDA agencies and Extension service budgets for accuracy, and inputs are used to develop stochastic economic feasibility model by region.</td>
<td></td>
</tr>
<tr>
<td>13. Scenarios developed using project findings are run through economic feasibility models.</td>
<td></td>
</tr>
<tr>
<td>14. The Cucurbitaceae 2018 conference is hosted, expected attendance of 200-250 international cucurbit scientists from public and private sector.</td>
<td></td>
</tr>
<tr>
<td>15. Cucurbit genomics workshops are delivered at PAG 2018, 2019 and Cucurbitaceae 2018; expected attendance at Cucurbitaceae 2018, 100-200 people.</td>
<td></td>
</tr>
<tr>
<td>16. 15 graduate students and 3 post-docs are trained in cucurbit genetics, genomics, disease and economic analysis.</td>
<td></td>
</tr>
<tr>
<td>17. 4-5 refereed articles are published by each crop group.</td>
<td></td>
</tr>
</tbody>
</table>
## Long term metrics

1. Sustainable data management, storage, and statistical analysis systems for cucurbit sequence, QTL, marker and phenotype data are available for the cucurbit research and breeding community
2. Sustainable community resources for cucurbit GWAS analysis are available for the four crops
3. Advanced breeding lines with resistance to Fusarium race 1,2, gummy stem blight, *Phytophthora*, powdery mildew, PRSV and GCMMV in watermelon; CMV, CYSVD, *Fusarium* and powdery mildew in melon; and combined downy mildew, powdery mildew, and ZYMV in cucumber.
4. Breeding lines with resistance to critical cucurbit diseases are used in breeding programs to improving/pyramiding resistance into commercial cucurbit cultivars
5. Markers developed from major QTL are used in breeding programs to improve disease resistance in commercial cucurbit cultivars
6. Markers are adopted by at least one fee for service genotyping lab serving the US breeding community
7. A sustainable web-based resource is available for information about cucurbit disease diagnosis and control
8. The cost and time frame for development of cucurbit cultivars with comprehensive disease resistance packages is reduced
9. Cucurbit producers experience reduced losses, improved crop quality and reduced input costs and labor due to increased disease resistance
10. There is reduced pesticide used to control cucurbit diseases.
TEAM PROGRESS REPORTS

and

PLANS FOR THE COMING YEAR
Genomics and Bioinformatics Team

Team members:
Zhangjun Fei (Boyce Thompson Institute)
Umesh Reddy (West Virginia St. Univ.)
Amnon Levi (USDA, ARS)
Yiqun Weng (USDA, ARS)
Michael Mazourek (Cornell University)
Pat Wechter (USDA, ARS)
Rebecca Grumet (Michigan State University)

<table>
<thead>
<tr>
<th>(a) Obj. 1. Develop common genomic approaches and tools for cucurbit</th>
<th>Personnel/Institution</th>
<th>Yr 1</th>
<th>Yr 2</th>
<th>Yr 3</th>
<th>Yr 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Develop genomic and bioinformatics platforms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1.1. Genotyping by sequencing</td>
<td>ZF (BTI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1.1.2. Sequence data processing/analysis</td>
<td>ZF (BTI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1.1.3. ICuGI database development</td>
<td>ZF (BTI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1.1.4. Community standardized nomenclature</td>
<td>YW (ARS-WI), AL (ARS-SC)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>1.1.5. Genomic, bioinformatics workshops</td>
<td>ZF (BTI), UR (WVSU), members of crop teams</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations</td>
<td>ZF (BTI), RG (MSU)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- watermelon</td>
<td>AL (ARS-SC)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- melon</td>
<td>JM (ARS-CA)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- cucumber</td>
<td>YW (ARS-WI)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- squash</td>
<td>MM (CU)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2.2. Population genetics and GWAS analyses</td>
<td>UR (WVSU), ZF (BTI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- watermelon</td>
<td>AL (ARS-SC)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- melon</td>
<td>JM (ARS-CA)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- cucumber</td>
<td>YW (ARS-WI), RG (MSU)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- squash</td>
<td>MM (CU)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Work in progress and plans

1.1. Develop genomic and bioinformatic platforms for cucurbit crops
1.1.1. Genotyping by sequencing
In closely working with Cornell Genomic Diversity Faculty, we have set up the genotyping-by-sequencing (GBS) platform for the cucurbit species.

1.1.2. Sequence data processing/analysis
We have established a GBS data analysis pipeline based on TASSEL-GBS (http://www.maizegenetics.net/tassel).

1.1.3. ICuGI database development
We have re-implemented the ICuGI database (now named Cucurbit Genomics Database (CuGenDB), and the new URL: http://cucurbitgenomics.org/) using the GMOD Tripal system (http://gmod.org/wiki/Tripal) and the Chado database schema (http://gmod.org/wiki/Chado). The newly designed and developed database was released in May 2017. Currently the database contains genome sequences of melon, watermelon (97103 and Charleston Gray), cucumber (Chinese Long and Gy14), wild cucumber (Cucumis sativus var. hardwickii PI 183967), four Cucurbita species (C. pepo, C. maxima, C. moschata and C.
argyrosperma) and bottle gourd. Genome syntenies between any two of the sequenced cucurbits have been identified and a synteny viewer have been implemented in the database. An “expression” module has been developed in the database using RNA-Seq datasets publicly available for cucurbit species, mainly collected from NCBI Sequence Read Archive (SRA). A set of tools to mine and analyze the RNA-Seq datasets, such as heatmap view of expression profiles and differential gene expression analysis, were implemented. The synteny viewer and the expression module have been packed as Tripal extension modules which can be implemented in other genomic databases developed using the Tripal system. Development of tools and interfaces to analyze and integrate genotype and phenotype data is ongoing. A manuscript describing the database has been published (Zheng et al., 2019, Nucleic Acids Research, 47:D1128).

1.1.4 Community standardized nomenclature.
This is in progress.

1.1.5. Genomic, bioinformatics workshops
A workshop on the Cucurbit Genomics Database was held at the Solcuc2017 meeting in Sept. 2017 at Valencia, Spain. A talk on the database was presented at the CUCURBITACEAE 2018 in November 2018 at Davis, California.

1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS)
1.2.1. GBS of cucurbit species, establish molecular-informed core populations
We have finished GBS for all cucumber, melon, watermelon, Cucurbita pepo, C. maxima and C. moschata accessions collected from the USDA National Plant Germplasm System (Table 1). After removing accessions with insufficient reads and merging duplicated accessions, a total of 1,564 cucumber, 2,077 melon, 1,365 watermelon, 852 C. pepo, 463 C. maxima and 314 C. moschata accessions have been genotyped (Table 2). We have finished processing the GBS data and SNP calling for cucumber, melon and watermelon, and analysis of the GBS data for C. pepo, C. maxima and C. moschata is underway.

We obtained a total of 1.71, 1.57 and 0.88 billion GBS reads with expected barcodes for melon, cucumber and watermelon, respectively. From these reads, a total of 54,192,089, 76,860,960 and 34,621,369 unique tags were obtained, and 743,545, 593,678 and 388,298 tags with at least 10 reads were used for SNP calling for melon, cucumber and watermelon, respectively. A total of 89,377, 114,338 and 62,258 SNPs were called in melon, cucumber and watermelon, respectively, and 27,846, 23,828, and 25,930 SNPs were obtained by applying criteria of missing data rate < 0.5 and minor allele frequency (MAF) > 0.01 (Table 3).

<table>
<thead>
<tr>
<th>Batch</th>
<th>DNA plate No.</th>
<th>Multi-plex Level</th>
<th>Crop</th>
<th>DNA Submission Date</th>
<th>Data Release Date</th>
</tr>
</thead>
<tbody>
<tr>
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<td>8</td>
<td>96</td>
<td>cucumber</td>
<td>4/13/2016</td>
<td>7/12/2016</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>96</td>
<td>cucumber</td>
<td>5/2/2016</td>
<td>7/12/2016</td>
</tr>
<tr>
<td>3</td>
<td>11,12,13,14</td>
<td>384</td>
<td>cucumber</td>
<td>8/24/2016</td>
<td>10/18/2016</td>
</tr>
<tr>
<td>4</td>
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<td>9/23/2016</td>
<td>11/21/2016</td>
</tr>
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<td>1,4,7,15</td>
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<td>10/3/2016</td>
<td>11/21/2016</td>
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<tr>
<td>6</td>
<td>31,34,35,36</td>
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<td>10/19/2016</td>
<td>11/21/2016</td>
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<tr>
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<td>1/3/2017</td>
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<tr>
<td>8</td>
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<td>watermelon</td>
<td>11/4/2016</td>
<td>2/15/2017</td>
</tr>
</tbody>
</table>
Note: Those in yellow background are samples from mapping populations.

### Table 2 Summary of cucurbit accessions genotyped using GBS

<table>
<thead>
<tr>
<th></th>
<th>melon</th>
<th>cucumber</th>
<th>watermelon</th>
<th>C. pepo</th>
<th>C. moschata</th>
<th>C. maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>49</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
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<td>10</td>
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<td>cucumber</td>
<td>1/20/2017 &amp; 2/2/2017</td>
<td>5/31/2017</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>50,51,52,53</td>
<td>384</td>
<td>melon</td>
<td>2/14/2017</td>
<td>5/5/2017</td>
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</tr>
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<td>12</td>
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<td>2/22/2017</td>
<td>5/5/2017</td>
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</tr>
<tr>
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<td>melon</td>
<td>3/2/2017</td>
<td>5/5/2017</td>
<td></td>
</tr>
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<td>5/5/2017</td>
<td></td>
</tr>
<tr>
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<td>melon &amp; watermelon</td>
<td>3/23/2017</td>
<td>5/31/2017</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>71,72,73,74</td>
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<td>1melon&amp;3squash</td>
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<td>6/13/2017</td>
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<td>5/31/2017</td>
<td>7/11/2017</td>
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</tr>
<tr>
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<td>squash</td>
<td>8/18/2017</td>
<td>9/25/2017</td>
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<td>3/5/2018</td>
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<td>90</td>
<td>96</td>
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<td>2/8/2018</td>
<td>3/5/2018</td>
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<tr>
<td>22</td>
<td>91</td>
<td>96</td>
<td>watermelon</td>
<td>2/8/2018</td>
<td>3/5/2018</td>
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<tr>
<td>23</td>
<td>92</td>
<td>96</td>
<td>watermelon</td>
<td>2/8/2018</td>
<td>3/5/2018</td>
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<tr>
<td>24</td>
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<td>96</td>
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<td>3/5/2018</td>
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<tr>
<td>25</td>
<td>94</td>
<td>96</td>
<td>watermelon</td>
<td>2/8/2018</td>
<td>3/5/2018</td>
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<tr>
<td>26</td>
<td>81,82</td>
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<td>C. maxima</td>
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<td>27</td>
<td>83,84</td>
<td>192</td>
<td>C. maxima</td>
<td>3/1/2018</td>
<td>3/22/2018</td>
<td></td>
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<tr>
<td>28</td>
<td>27</td>
<td>96</td>
<td>C. maxima</td>
<td>3/20/2018</td>
<td>10/9/2018</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 Summary of GBS sequencing and called SNPs

<table>
<thead>
<tr>
<th></th>
<th>melon</th>
<th>cucumber</th>
<th>watermelon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total good barcoded reads</td>
<td>1,712,021,164</td>
<td>1,565,948,724</td>
<td>884,151,231</td>
</tr>
<tr>
<td>Total reads covering tags (&gt;=10)</td>
<td>1,606,338,621</td>
<td>1,441,002,859</td>
<td>828,388,082</td>
</tr>
<tr>
<td>Mapped reads</td>
<td>1,260,870,721</td>
<td>1,054,491,682</td>
<td>515,465,448</td>
</tr>
<tr>
<td>Unmapped reads</td>
<td>345,467,900</td>
<td>386,611,177</td>
<td>276,922,634</td>
</tr>
<tr>
<td>Total tags</td>
<td>54,192,089</td>
<td>76,860,960</td>
<td>34,621,369</td>
</tr>
<tr>
<td>Tags with &gt;= 10 reads</td>
<td>743,545</td>
<td>593,678</td>
<td>388,298</td>
</tr>
</tbody>
</table>
A core collection selection strategy has been developed. Briefly, a total of ~400 accessions will be selected for each species. Around 300 accessions which represent the majority of the genetic diversity of the germplasm, based on the core collection analysis using GenoCore (Jeong et al., 2017, PLoS ONE 12:e0181420), will be selected. Another ~100 accessions with interesting traits and/or parents of mapping/breed populations will be selected. In the final core collection, if a selected line is known to be derived from a PI accession that is also in the final core collection, then the corresponding PI should be replaced with the most closely related one on the phylogenetic tree. Accessions in the final core collection whose genomes have already been resequenced should also be replaced by the most closely related ones on the phylogenetic tree, unless they harbor very interesting/important traits.

Based on this strategy, core collections of melon and cucumber have been established. The melon core collection contains 384 accessions and captures 98.96% of all allelic diversity in the melon germplasm we have genotyped, and the cucumber core collection contains 395 accessions, of which 354 are from the GBS collection and captures 95.9% of all allele diversity, and 41 are historical varieties with important horticultural and disease resistance traits. Principal component analysis (PCA) of the melon and cucumber core collections showed similar pattern to that of the entire collections (e.g., melon shown in Figure 1). Core collection section is currently underway for watermelon and C. pepo.

1.2.2. Population genomics and GWAS analyses
Using SNPs called from the GBS data, we have performed population genomic analyses for cucumber, watermelon and melon accessions. Phylogenetic, PCA and population structure analyses have been done for accessions of cucumber, watermelon and melon. The results from these analyses for watermelon accessions are shown in Figure 2 as an example. Linkage disequilibrium (LD) decay patterns and population differentiation have also been investigated for these species.
We have collected historical phenotype data from the USDA National Plant Germplasm System for cucumber, watermelon and melon accessions. GWAS have been performed to identify SNPs and regions that are significantly associated with important agronomic traits. GWAS for watermelon resistance to powdery mildew race 2 is shown in Figure 3 as an example.

Manuscripts reporting the results from population genomics and GWAS analyses as well as core collection development for cucumber has been published (Wang et al., 2018, Horticulture Research 5:64), for watermelon has been submitted, and for melon is under preparation. Analysis of the GBS data for Cucurbita species is underway.
Figure 3. Genome-wide association studies (GWAS) of resistance to powdery mildew race 2 in stem (left) and leaf (right) of watermelon.

1.2.3 Genomic resequencing of core collections
We have compared cost-effective services for Illumina genomic library construction to accommodate our budget for genome resequencing of the core collections, and selected the “Nextera skim sequencing WGS library preps (1/3 concentration)” service provided by Cornell Biotechnology Resource Center (http://www.biotech.cornell.edu/brc/genomics/services/price-list#ht), which charges $1,152 per full plate (96 samples) and additional $900 for pooling and Blue pippin size selection ($2,052 in total; $21.4 per sample).

We have sent 21 *C. pepo* samples (one Illumina lane) and a plate of cucumber samples (96 samples; 6 lanes) in the core collection for library construction. The constructed libraries have been sequenced at GENEWIZ (~$1,500 per lane, which generates ~120 Gb paired-end sequence data). We have obtained cleaned sequence data of >10× depth of the coverage for most of the accessions (Figure 4).

![Figure 4](image-url)
Watermelon Team

Team members:
Amnon Levi (USDA, ARS) Cecilia McGregor (Univ. Georgia)
Shaker Kousik (USDA, ARS) Pat Wechter (USDA, ARS)
Kai-shu Ling (USDA, ARS) Todd Wehner (North Carolina St. Univ.)

Overall objectives: Identifying quantitative trait loci (QTL) associated with resistance to major and emerging diseases, developing useful molecular markers and utilizing the genomic tools to incorporate resistance into watermelon cultivars.

Major diseases: Gummy stem blight, Fusarium wilt, Powdery mildew, Phytophthora fruit rot, Papaya ringspot virus (PRSV) and Cucumber green motile mosaic virus (CGMMV).

<table>
<thead>
<tr>
<th>Objective</th>
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<tr>
<td>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</td>
<td>(initials as in Table 3)</td>
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<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</td>
<td>ZF (BTI), RG (MSU)</td>
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<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations</td>
<td>AL (ARS-SC), TW (NCSU)</td>
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<tr>
<td>- watermelon</td>
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<td>1.2.2. Population genetics and GWAS analysis</td>
<td>UR (WSVU), ZF (BTI)</td>
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<td>(b) Obj. 2. Genomic assisted breeding for disease resistance</td>
<td>Screen for resistance (Sc), develop populations (P), phenotype (F), sequence (S), QTL map (Q)</td>
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<td>2.1 QTL map resistances:</td>
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<tr>
<td>- CGMMV</td>
<td>AL (ARS-SC), PW (ARS-SC)</td>
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<td>- Fusarium race 1</td>
<td>PW (ARS-SC), AL (ARS-SC)</td>
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<td>race 2</td>
<td>CM (UGA), TW (NCSU)</td>
<td>F</td>
</tr>
<tr>
<td>- gummy stem blight</td>
<td>SK (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>- Phytophthora</td>
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<td>- powdery mildew</td>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td>FSQ</td>
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<td>- PRSV-W</td>
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<td>2.2 Marker development and verification:</td>
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<td>2.3. Introgress resistance into advanced breeding lines:</td>
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<td>- Fusarium race 1</td>
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<td>race 2</td>
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<tr>
<td>- powdery mildew</td>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
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Work in progress and plans

1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS)

GBS.
The GBS is complete and a manuscript describing the work has been submitted. For further information, see Genomics section.

Core populations.
We are collecting and increasing Citrullus PI accessions, heirloom cultivars, and gene mutant type-lines. Seed increase of the 2000 PI accessions is being accomplished by seed companies, USDA scientists, and university researchers. Each is increasing 1 to 10 accessions per year using controlled pollination in greenhouse or field.

A core collection was developed, consisting of 420 PI accessions that had traits of interest to researchers. Of those, 250 germinated and were increased by self pollination. Seeds from self pollination and leaf tissue of those core accessions were sent to Michigan State University.

Gene type lines. Collection and seed increase of the watermelon gene type-lines will include all cultivars, breeding lines, and PI accessions in the gene mutant list at Cucurbit Genetics Cooperative. Examples include: PI 189225 (db, Ar-2-1), NC-517 (C), PI 482261 (Citr), Bush Charleston Gray (dw-1), PI 595203 (zym-CH, zym-FL).

Selfing of PIs to form core population is in progress.

2.1, 2.2, 2.3 QTL map resistance, marker development and verification, introgress resistance

Fusarium wilt race 2 (Pat Wechter, Sandra Branham, and Amnon Levi, USDA, ARS, U.S. Vegetable Laboratory (USVL), Charleston, SC)

Genetic mapping of QTL associated with resistance to Fusarium wilt race 2- Two-hundred and twenty F$_{2:3}$ families derived from the cross USVL-252$^{FR}$ x PI 244019-PRSV-R$_{(S3)}$ were constructed in collaboration with Dr. Nihat Guner and team at Sakata Seeds. The 220 families were evaluated for resistance to Fusarium wilt (FW) race 2 resistance (Figure 1) in two separate experiments at the U.S. Vegetable Laboratory. The distribution of FW race 2 resistance in the population indicates polygenic inheritance (Figure 2). Genotyping-by-sequencing (GBS) of the F$_{2:3}$ population identified a major QTL on Chromosome 1 of USVL-252$^{FR2}$ associated with resistance to FW race 2. KASP markers are being developed and will be validated for utility of incorporating the resistance into the genetic background of watermelon cultivars. We conducted a genetic mapping study to identify quantitative trait loci (QTLs) associated with resistance to Fon race 1 in segregating populations (F$_{2:3}$ and recombinant inbred lines) of Citrullus amarus (citron melon) derived from the Fon race 1 resistant and susceptible parents, USVL246-FR2 and USVL114, respectively. A major QTL ($q$Fon1-9) associated with resistance to Fon race 1 was identified on chromosome 9 of USVL246-FR2. This discovery provides an additional host-resistance source of resistance to Fon race 1 in watermelon and as it co-locates with the QTL for Fon race 2 resistance in the same population, may provide non-race specific resistance (Branham et al. 2017, 2019).

Figure 1. Two-hundred and twenty F$_{2:3}$ families derived from the cross USVL-252$^{FR}$ x PI 244019-PRSV-R$_{(S3)}$ being evaluated for Fusarium wilt race 2 in a greenhouse at the U.S. Vegetable Laboratory (Summer, 2017).
Converting QTL to Kompetitive Allele Specific PCR KASP markers tightly linked to Fusarium wilt race 1 resistance - DNA of the resistant and susceptible parents (C. lanatus) and the F2 parental plants of the most resistant versus the most susceptible F2:3 families (Lambel et al. 2014) were used for a QTL-seq analysis. QTL-seq narrowed the Fon race 1 QTL interval on chromosome 1 of watermelon (Lambel et al. 2014) by 500 kb (Branham et al. 2018). SNPs from the interval were converted to KASP primers. The KASP primers were used in genetic mapping of the same population used for the initial mapping of QTL associated with FW race 1 resistance (Lambel et al. 2014). QTL mapping yielded several KASP markers tightly linked to race 1 resistance and narrowed the QTL interval further from 1.56 Mb to 315 kb (Figure 3). In collaboration with the HM.Clause team in Davis, California we conducted QTL-seq and developed KASP markers tightly linked to FW race 1 resistance (Figure 3; Branham et al. 2018). We have developed KASP markers for Fon races 1 and 2 QTL in C. amarus. The FW races 1 and 2 resistant lines USVL246-FR2 and USVL252-FR2 were crossed with Charleston Gray, Calhoun Gray and Sugar Baby to generate F1, F2, BC1 and BC2F2. The KASP markers will be used to incorporate resistance to FW races 1 and 2 into the genome background of watermelon cultivars.

What do you plan to do during the next reporting period to accomplish the goals?
- Complete development and validation of KASP markers and incorporate FW races 1 and 2 resistance from USVL246-FR2FR and USVL-252FR into genomic background of watermelon cultivars (Wechter, Branham and Levi).
Papaya ringspot virus (PRSV) resistance (Amnon Levi, Kai-shu Ling, and Sandra Branham, USDA, ARS, U.S. vegetable Laboratory (USVL), Charleston, SC)

Identifying QTL associated with PRSV resistance
Several F2 and BC1 populations derived from the cross USVL-252Fr x PI 244019-PRSV-R(s3) were constructed in collaboration with Dr. Nihat Guner and team at Sakata Seeds. The genetic populations were evaluated for resistance to PRSV-resistance at the U.S. Vegetable Laboratory. The distribution of PRSV-resistance in the population confirmed inheritance by a single homozygous recessive gene in PI 244019 (Guner, 2004; Guner and Wehner, 2008). Genotyping-by-sequencing (GBS) of an F2 population identified a major QTL on Chromosome 3 of PI 244019 associated with PRSV-resistance (Figure 4). The major QTL interval comprises several ribosomal genes, among them the eukaryotic elongation factor eIF4E known to be associated with resistance to potyviruses in cucurbit crops (Ling et al. 2009). KASP markers are being developed and will be used for incorporating the resistance into the genomic background of watermelon cultivars.

What do you plan to do during the next reporting period to accomplish the goals?
-Complete development of KASP markers tightly linked to PRSV-resistance in PI 244019-PRSV-R(s3) and use them to incorporate resistance into genome background of watermelon cultivars (Levi, Ling, Branham).

Figure 4. A major QTL associated with PRSV resistance identified on chromosome 3 of Citrullus amarus PI 482019 using GBS-SNP data analysis.

Powdery mildew of watermelon (Shaker Kousik, Patrick Wechter, Sandra Barnham, Amnon Levi; USDA, ARS, U.S. vegetable Laboratory (USVL), Charleston, SC)

Inheritance of powdery mildew resistance, identification of QTL and RNAseq
USVL608-PMR (S0), a red fleshed watermelon line with high levels of resistance to PM was used as the female parent (P1) and crossed with USVL677-PMS which is highly susceptible (P2). The parents, F1, backcrosses to both parents (BC1, BC2) and a large F2 population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. All susceptible parent (USVL677-PMS) plants were rated >7 [mean disease severity (DS) = 94%], whereas most resistant parent (USVL608-PMR) plants were rated as 1 (DS=2.5%). Majority of the BC1 plants were rated ≤2 and considered as resistant. Of the 466 F2 plants, 221 were rated ≤2 (DS=3.1%). Of the 76 BC2 plants, 23 were rated ≤2 (DS=2.9%). Chi-square analyses of the observed segregation of phenotypes for the F2 plants indicated that two genes control PM resistance with a good fit for a 7:9 resistance to susceptibility ratio. The proposed model for this ratio is two genes with one recessive for high resistance and one dominant for high resistance. This is supported by a backcrossing segregation ratio of 1:3. We have observed some highly and moderately resistant plants in the F2 indicating the cumulative effect of the two

36
genes. QTL-seq analysis on the most resistant and most susceptible DNA bulks from the F$_2$ populations identified a major QTL in chromosome 2.

We have also completed RNA-seq analysis of the parents during PM infection. Plants of the resistant line USVL608-PMR and the susceptible line USVL677-PMS were with inoculated with 10$^5$ conidia$^{-\text{ml}}$ of \textit{P. xanthii}. Symptom development was observed every day. In addition, leaf samples were collected for microscopy and for RNA extraction. RNA-seq profiling was done on leaf samples collected at 0, 1, 4, and 9 days post inoculation (DAI). Powdery mildew symptoms were visible on USVL677-PMS 4 DAI whereas leaves of USVL608-PMR were clean. We have completed RNA-seq on all these samples. Data analysis is in progress. A quick analysis of the differentially expressed genes (DEG) indicated several resistance genes in chromosome 2.

We also completed inheritance studies on the egusi type watermelon (\textit{C. mucosospermus}) line USVL531-MDR. This line was found to be resistant to 11 PM isolates from across the U.S.A. and was released by USDA ARS in 2018. This line was used as the female parent (P$_1$) and crossed with USVL677-PMS which is highly susceptible (P$_2$). The parents, F$_1$, backcrosses to both parents (BC$_1$, BC$_2$) and a large F$_2$ population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. The susceptible parent (USVL677-PMS) had mean disease severity of 8.14 on the 0-10 scale, whereas it was 1.17 for the resistant parent. Segregation patterns point to single gene inheritance, but also indicated another gene is inherited maternally. Chi-square analyses of observed segregation of phenotypes for the F$_2$ populations fit models for these gene models and were further supported by segregation patterns in the backcross populations. QTL-seq analysis on the extremes from the F$_2$ populations and RNA-seq analysis of the parents during PM infection are being conducted to identify the chromosomal regions involved in resistance. USVL531-MDR will serve as a useful source to incorporate PM resistance into commercial cultivars. We have developed several red fleshed resistant lines (at F$_7$) using USVL531-MDR as the source of resistance.

**Advancing Powdery mildew resistant inbred lines**

Fruit from F$_2$ plants from a cross of USVL531-MDR and USVL677-PMS with powdery mildew resistance, uniform red flesh and decent brix ($>7$) were collected and have been advanced till F$_6$ and further advancement to F$_7$ is in progress. We are currently evaluating 10 red fleshed F$_7$ lines that were homozygous for resistance. We completed assessment of fruit quality from F$_5$ and F$_6$ progenies that were homozygous for resistance to PM and had red flesh and brix $>7$ in 2018.

**Identifying and developing multiple disease resistant lines from accessions**

Majority of the watermelon plant introductions (PI) considered as resistant or tolerant display varying levels of disease resistance. Hence it is important to screen and select for several generations to develop highly resistant lines from these PI. We have developed 36 lines with high levels of resistance to powdery mildew from various PI. Of these 13 are also resistant to Phytophthora fruit rot and can be considered as multiple disease resistant (MDR). These lines were evaluated for resistance to powdery mildew and Phytophthora fruit rot in the field and displayed high levels of resistance compared to susceptible lines including Mickey Lee and USVL677-PMS. These lines will serve as useful sources of resistance for future studies.

In 2018 we released four red fleshed lines with high levels of resistance to powdery mildew. We have completed making crosses with these powdery mildew resistant lines (USVL608-PMR, USVL313-PMR, USVL585-PMR and USVL225-PMR) with USVL677-PMS and ‘Dixie Lee’ to develop populations for conducting inheritance studies in 2019 and developing resistant inbred lines with high fruit quality. A paper documenting the release of these four PM resistant watermelon lines was published in HortScience in 2018.
Phytophthora fruit rot of watermelon (Shaker Kousik; USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC)

Inheritance of resistance, identification of QTL and RNAseq
The U.S. Vegetable Laboratory (USDA, ARS) in Charleston has developed several germplasm lines with high levels of resistance to Phytophthora fruit rot. In these studies we used the germplasm line USVL531-MDR which was resistant to 20 different P. capsici isolates from across the U.S.A. Studies to determine inheritance of resistance to Phytophthora fruit rot using the same population described for powdery mildew (USVL531-MDR X USVL677-PMS) were conducted as USVL531 is resistant to both these diseases. However, based on this study it was difficult to assess the number of genes controlling resistance and hence we are in the process of developing a recombinant inbred line (RIL) population and are currently at the F8 stage.

We completed growing out the F3 families in summer-fall of 2018 (total 40 families, about 600 plants) and screened them for Phytophthora fruit rot. The data is being analyzed. We have extracted DNA from parents and F2 plants for GBS. However, since resistance to powdery mildew is a dominant trait we will pool the DNA from 20 most susceptible lines and 20 most resistant lines and send it out for sequencing. We will perform QTLseq analysis on the resulting data.

We are currently phenotyping the populations from USVL003-MDR x USVL677-PMS for resistance to powdery mildew and Phytophthora fruit rot.

Fruit rind samples were collected from individual fruit after 12h, 24h, 48h, 72h, and 96h after inoculation and immersed in liquid nitrogen to quench all the metabolomics processes. Rind samples were then processed for extraction of RNA and sent to Duke University Genomic center for RNA sequencing. Sequencing has been completed and we are currently analyzing the RNA-seq data. We completed experiments to determine the transcriptomic profile during P. capsici infection of resistant and susceptible genotypes. Our studies with melatonin have also shown that it can suppress the growth of Phytophthora capsici in culture plates. Our research has also indicated that 1000mM melatonin solution is capable of reducing development of Phytophthora fruit rot on cucumbers.

Advancing resistant inbred lines
Advanced germplasm lines of USVL531-MDR, USVL0020-PFR, Charleston, Gray and Sugar Baby were grown in the field and fruit were harvested when mature. Fruit of each of these lines was inoculated with 10^4 zoospores/ml and maintained in a humid chamber (26 ± 1 °C >95%RH). We have identified three red fleshed (plants) with tolerance to Phytophthora fruit rot and high level of resistance to Powdery mildew (at the F5 stage). These will be screened for resistance to both the diseases and advanced further to F6

Project metrics (time line) for research on Phytophthora fruit rot and powdery mildew of watermelon
- Develop germplasm lines with resistance to Phytophthora fruit rot and powdery mildew for watermelon: **Completed.**
- Develop populations for phenotyping resistance to Phytophthora fruit rot and powdery mildew of watermelon: **Completed**
- Sequence and map Phytophthora fruit rot and powdery mildew QTL in watermelon: **In progress.**
- Introgress Phytophthora and powdery mildew resistance into cultivated type watermelon: **In progress**
- Participation in outreach to stakeholder groups per year via industry events and field days: **Completed**
Watermelon gummy stem blight resistance (Luis Rivera and Todd C. Wehner; NC State Univ.; Cecilia McGregor, University of Georgia, Athens, GA)

Inheritance of resistance, identification of QTL

Wehner

Objective: a) Evaluate a RIL population of watermelon (*Citrullus lanatus* × *C. amarus*) for resistance to gummy stem blight and fruit quality traits and b) Map GSB resistant genes through genome-wide association studies (GWAS).

Phenotyping: A watermelon GSB population was developed by intercrossing the most resistant accessions of *Citrullus* four times (I₄), followed by crossing with elite cultivars of watermelon (I₄F₁), followed by intercrossing without selection, while maintaining wild and elite types in the populations (I₄F₁I₄), followed by self-pollinations of plants at random (I₄F₁I₄S₁). The 300 RILs and 20 controls (10 PIs and 10 commercial cultivars) were evaluated for resistance to gummy stem blight in greenhouses at North Carolina State University in Raleigh, North Carolina (Figure 1 and 2), and in the field at the Horticultural Crops Research Station at Clinton, North Carolina (Figure 3). We inoculated plants with *Stagonosporopsis cucurbitacearum* at a concentration of 5×10⁵ spores/ml (Figure 4). To evaluate disease severity, we adopted an ordinal disease assessment scale (Gusmini et al. 2002). Plants were rated four times, in an experiment with, 2 locations, and 10 replications (at greenhouse and field). We also evaluated fruit quality in the gummy stem blight field trial. We also collected data of fruit shape, rind pattern and toughness, seed size and color, flesh color and intensity and hollow heart. We will identify RILs with high yield of excellent fruit quality.

Figure 1. Greenhouse test for resistance to gummy stem blight

Figure 2. Gummy stem blight symptoms during the greenhouse test

Figure 3. Field test for resistance to gummy stem blight
Additionally, genomic DNA of gummy stem blight isolates collected from field outbreaks was extracted, and a PCR-based marker test for distinguishing the three morphologically identical, but genetically distinct species causing gummy stem blight was performed (Figure 4). We used three sets of primers, including *Db05* that produces a 216 to 224-bp fragment in all three species, *Db06* that produces a 283- to 289-bp in *S. citrulli* and a 268-bp and slightly fainter fragment in *S. cucurbitacearum*, and *Db01* that produces a 256- to 364-bp fragment in *S. citrulli* (Brewer et al. 2015). Two of the isolates were *S. cucurbitacearum* (syn. Didymella bryoniae) and one isolate was *S. caricae*.

*Genotyping:* The 300 RILs were planted in spring 2018, at greenhouses of NC State, to sample leaf tissue for DNA extraction. The DNA was sent for SNP discovery through genotyping by sequencing (GBS) method at Cornell University. We expect to get several thousand of SNPs for association analysis (GWAS). Resistance to GSB and fruit quality are being evaluated in 3 years (2017, 2018, 2019), 2 locations (field, greenhouse), and 10 replications on 300 lines (I4F1I4) at the S4, along with 20 controls (10 PIs and 10 commercial cultivars).

*Association analysis:* The phenotypic and genotypic data is being analyzed using R packages: GWASTools, GWASdata, SNPassoc,.snpMatrix, GenABEL and pbatR. The result of the analysis will allow us to locate and identify SNP markers associated with GSB resistance.

*Figure 4.* Gummy stem blight spore mass production and identification through PCR and electrophoresis.
Inheritance of resistance, identification of QTL

McGregor

Population Development:
Three populations are currently being used for this research: WPoP GSB1 (PI 482276 x Crimson Sweet), WPoP GSB2 (PI 189225 x Sugar Baby) and the ZxD population (ZXRM x PI 244019). The later population was previously developed.

- WPoP GSB1: PI 482276 x Crimson Sweet population of 225 F2:3 lines. Complete
  - Backcross population: WPoP GSB 1BC: BCF2 for PI 482276 x Crimson Sweet (recurrent) for trait introgression and marker validation. Complete
- WPoP GSB2: PI 189225 x Sugar Baby population of 140 F2:3 lines. Complete
  - Backcross population: WPoP GSB 2BC: BC for PI 189225 x Sugar Baby (recurrent) for trait introgression and marker validation. In progress

Phenotyping:
- WPoP GSB1: PI 482276 x Crimson Sweet population of 178 F2:3 lines (15 plants x 178 lines = 2670 plants) was phenotyped in a growth chamber using C. citrilli isolate 12178A (GA). Disease symptoms for each seedling were scored on a 0 – 5 scale and BLUPs calculated. Parents and F1 and 4 other control genotypes were also included. Complete
- WPoP GSB2: PI 189225 x Sugar Baby. This population is currently being phenotyped. In progress
- ZxD population (ZXRM x PI PI 244019). This population is currently being phenotyped. In progress

Genotyping and QTL mapping:
- WPoP GSB1: PI 482276 x Crimson Sweet population of 178 F2 plants were genotyped by GBS. The reads were aligned (Fei lab) to the C. amarus PI 296341 reference genome. A genetic map consisting of 1,237 high quality markers were created. Three QTL for GSB resistance was identified: qClGSB1.1 (R2 = 17%), qClGSB1.2 (R2 = 13%), qCLGSB8.1 (R2 = 10%).

Goals for 2018-2019
- Develop KASP markers to span QTL regions identified in WPoP GSB1 (PI 482276 x Crimson Sweet population.). Validate makers in BCF2 and start introgression.
- Complete phenotyping for WPoP GSB2, and use the data to create bulks for QTL-seq. Samples will be sent for sequencing (Georgia Genomics Facility), and ΔSNP index will be calculated to identify significant regions.
- Phenotype ZxD population and map QTL using existing SNP map.

Cucumber green mottle mosaic virus (Kai-shu Ling and Amnon Levi USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC)
- We have completed the initial screening of USDA watermelon germplasm (~1,400 accessions). In the repeated test, several selected lines showed promising level of tolerance to CGMMV (without visible symptom). However none of them was immune to CGMMV, the virus titer were detectable in the tolerant plants using ELISA tests.
- We made single plant selection of the promising lines and are developing segregating populations through crossing. S2 seeds have been generated from one of the most promising Citrullus colocynthis line.
• Seeds from seven PI lines with potential for resistance (tolerance) to CGMMV have been sent to the collaborator to generate plant tissue for support the re-sequencing efforts under the CucCAP project.
• We submitted a release notice ‘Virus-resistant desert watermelon (Citrullus colocynthis) germplasm line ‘USVL18-157VR’ useful for enhancing CGMMV-resistance in watermelon cultivars. The release notice is currently in the process of review and approval by USDA, ARS, National Program Leaders (NPL).

Table 1. Selected lines with potential tolerance to CGMMV were selected for re-sequencing

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<td>145</td>
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<td>151</td>
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<tr>
<td>714</td>
<td>Citrullus lanatus</td>
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**What do you plan to do during the next reporting period to accomplish the goals?**
We are advancing through single plant selection of the most promising Citrullus colocynthis line to S3. Those seeds will be provided with the sponsoring seed companies to make crosses to your elite materials. In addition, once the F2, BC1 seeds are generated, materials from segregating populations will be used for Genotyping-by-sequencing or similar study to identify SNPs in association with the tolerance to CGMMV.
Melon Team

Team members:
Jim McCreight (USDA, ARS)
Shaker Kousik (USDA, ARS)
Michael Mazourek (Cornell Univ.)
Pat Wechter (USDA, ARS)
Bill Wintermantel (USDA, ARS)

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<td>1.2.2 Population genetics and GWAS analyses - melon</td>
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1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations

Melon

- Genotyped the available NPGS melon accessions and heirloom (n= 2,084) by GBS methods. Population structure, pattern of LD, and redundant accessions were analyzed using the genotype data.
- Selected 384-member molecular-informed core population, i.e., functional panel or diversity panel, from the 2,084 based on molecular data analysis and historic importance.
  - Validated the utility of the diversity panel for identification of loci that determine quantitative and qualitative traits based on GWAS of 100-seed weight, fruit characteristics, and flower sex expression.
  - The panel was phenotyped in a 2018 field test in Imperial Valley, with particular respect to those traits that define melon horticultural groups.
  - A manuscript is in preparation to report results of population structure analyses using a suite of tools, LD decay, Core collection selections, and GWAS using historical and project-generated data.
- The melon core population was planted in a greenhouse at Salinas in December 2108 for selfing and subsequent resequencing; one plant per member. Seeds of each member will be increased for deposit in USDA, NPGS. Fruits from 57 members have been harvested to date (3/28/19).
- GBS and endorna virus analysis of 42 C. melo ssp. agrestis var. texanus, and bona fide C. melo ssp. melo var. chito and var. dudaim accessions confirmed the unique identity of var. texanus among the 19 melon horticultural groups in the recently revised melon classification scheme.
2.1.2. Melon

Powdery Mildew (*Podosphaera xanthii*) resistance in MR1xAY RIL

- Awaiting for growth chamber space availability for Race 2 test.
- Growth chamber test for resistance to race S is underway.
- Growth chamber test for resistance to race 1 scheduled to follow the race S test.

Powdery Mildew (*Podosphaera xanthii*) California Field Tests

Two replicated field tests of cucurbit powdery mildew race differentials subjected to natural infection were planted in

- Imperial Valley. University of California, Desert Research and Extension Center, Holtville; watered 8 March 2018; insufficient infection to evaluate.
- Central Valley. University of California, Westside (Westside Research and Extension Center, Five Points; Planted 25 June 2018; insufficient infection to evaluate.

*Downy Mildew*

- Initiated phenotyping resistance in the MR1 x AY RIL.

**CYSDV**

- PI 313970 x Top Mark–Produced 200 F2:F3 progenies. GBS analysis completed. Evaluated for CYSDV reaction replicated, naturally-infected field tests in Imperial Valley at the University of California, Desert Research and Extension Center, Holtville; watered 16 August 2018.
- PI 313970 x TGR 1551 (PI 482420)–Produced 184 F2:F3 progenies. GBS analysis completed. Evaluated for CYSDV reaction replicated, naturally-infected field tests in Imperial Valley at the University of California, Desert Research and Extension Center, Holtville; watered 16 August 2018.

**CMV**

Evaluated 25 advanced Cornell University CMV-resistant melon lines developed by M. Kyle-Jahn and H.M. Munger. Limited quantities of seed were produced in 2017 and transferred via MTA to USDA-ARS, Salinas.

- Greenhouse evaluation–Controlled-inoculation tests at Salinas, Spring 2018: nine lines exhibited resistance: six lines were asymptomatic, with limited or no virus accumulation (Figure 1, Left), three lines exhibited only local lesions against a subgroup 1 CMV isolate from melon; the other 16 lines were susceptible with 15 lines exhibiting mosaic reactions.
• Field evaluation–Central Valley, University of California, Westside (Westside Research and Extension Center, Five Points; Planted 25 June 2018 for disease reactions to natural CMV-infection, adaptation, and fruit quality. Field was infested with melon aphid that was controlled with insecticide application. CMV was not present in the field; sampled plants were negative for the virus. The lines appeared to be poorly adapted to the Central Valley, CA, as indicated by plant size and condition. None of the lines exhibiting resistant reactions in the greenhouse were U.S. western shipper (USWS) type melons (Figure 2). Additional backcrossing is needed to combine CMV resistance with USWS type melon.

Figure 1. Susceptible and resistant reactions to CMV inoculation: Left, virus-free, asymptomatic plants of resistant line 17-4065-1; Right, Susceptible line 17-4028-1 showing mosaic symptoms.

Figure 2. Fruits of two CMV-resistant Cornell breeding lines did not exhibit western U.S. shipping type, orange flesh melon characteristics.
2.2 Marker development and verification

<table>
<thead>
<tr>
<th>2.2.2. Melon development and verification:</th>
<th>Refine map (R) develop marker (M), verify (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- powdery mildew</td>
<td>SK (ARS-SC)</td>
</tr>
<tr>
<td>- Fusarium</td>
<td>PW (ARS-SC)</td>
</tr>
<tr>
<td>- CYSDV</td>
<td>WW (ARS-CA), JM (ARS-CA)</td>
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<tr>
<td>- CMV</td>
<td>JM (ARS-CA), MM (CU)</td>
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<td></td>
<td>M</td>
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<td>RM</td>
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<td></td>
<td>V</td>
</tr>
<tr>
<td>Powdery Mildew</td>
<td>• Identified QTL on Chromosome 5 and 12 for resistance to Powdery mildew race 1.</td>
</tr>
<tr>
<td></td>
<td>• KASP markers for both Powdery mildew QTL have been developed and are in the process of being validated.</td>
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<tr>
<td></td>
<td>• KASP markers for sulfur resistance have been developed and are in the process of being validated.</td>
</tr>
<tr>
<td>Fusarium wilt</td>
<td>• KASP markers have been developed for resistance to Fusarium wilt race 1 and Fusarium wilt race 2, and are being validated</td>
</tr>
<tr>
<td>CYSDV</td>
<td>• No progress to date.*</td>
</tr>
<tr>
<td>CMV</td>
<td>• No progress to date.</td>
</tr>
</tbody>
</table>

*Cucurbit chlorotic yellows virus.* During the summer of 2018, melon plants from a germplasm diversity study in the Imperial Valley, CA were found infected with *Cucurbit chlorotic yellows virus* (CCYV; genus *Crinivirus*, family Closteroviridae). Two melon plants were found exhibiting interveinal yellowing and chlorotic spot symptoms similar to those caused by a crinivirus, but varying from symptoms normally observed during infection by *Cucurbit yellow stunting disorder virus* (CYSDV; genus *Crinivirus*). Total nucleic acid was extracted from leaves of both plants and tested negative for CYSDV, but positive for CCYV by RT-PCR using primers specific to portions of RNA2 of each virus encoding the virus coat protein genes. The CCYV amplicon was sequenced and shared 99% sequence identity with most of the CCYV isolates from around the world sequenced to date. A second set of CCYV-specific primers were designed to a region within RNA1 encoding the RNA-dependent RNA polymerase (RdRp) gene and amplification of a 370 nt amplicon was confirmed. This 370 nt RdRp amplicon sequenced was a 100% match to 20 CCYV isolates from around the world. Due to the similarity in symptoms between CCYV and CYSDV, several archived and frozen total nucleic acid and RNA extracts from Imperial Valley melon, collected over the course of 9 years (2010-2018), were re-analyzed for CCYV to determine whether the virus was newly emerged or if it had evaded detection due to similarity in symptoms to CYSDV. Nineteen of 23 samples collected between 2014 and 2018 were positive for CCYV, and many samples contained mixed infections of CCYV with CYSDV and/or the ipomovirus, Squash vein yellowing virus (SqVYV). All eighteen archived samples collected from 2010 to 2013 tested negative for CCYV, but extracts were confirmed as viable because parallel amplification of CYSDV from these samples was successful. Therefore, CCYV most likely emerged in the Imperial Valley during in 2014 but remained undetected due to similarity with CYSDV in symptoms on cucurbit host plants and vector transmission. CCYV is prevalent in East Asia, the Middle East, and North Africa, and is transmitted efficiently by the whitefly, *Bemisia tabaci*. Both CCYV and CYSDV have long retention times in their whitefly vector, facilitating transmission throughout the region. Further studies
will be necessary to evaluate epidemiology of CCYV in the southwestern US desert production region, and to determine its impact on melon production and development of crinivirus-resistant cultivars. (See Wintermantel et al. 2019)

2.3 Introgress resistance into advanced breeding lines

<table>
<thead>
<tr>
<th>2.3. Introgress resistance into advanced breeding lines:</th>
<th>Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.2. Melon</td>
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<tr>
<td>- powdery mildew</td>
<td>SK (ARS-SC), JM (ARS-CA) B I I IA</td>
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<tr>
<td>- Fusarium</td>
<td>PW (ARS-SC) B B I IA</td>
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<tr>
<td>- CYSDV</td>
<td>JM (ARS-CA), WW (ARS-CA) I I IA IAR</td>
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<tr>
<td>- CMV</td>
<td>JM (ARS-CA) I I I IA</td>
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Melon USVL206 (derived from an MR-1 x AY cross) with resistance to Fusarium wilt race 1 and 2, sulfur resistance, powdery mildew race 1 resistance, and has orange sweet flesh has been crossed into Top Mark, Charentais and backcrossed into AY.
Cucumber Team

Team members:
Yiqun Weng (USDA, ARS)
Rebecca Grumet (Michigan St. Univ.)
Todd Wehner (North Carolina St. Univ.)

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Personnel/Institution</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
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<tbody>
<tr>
<td>1. Develop genomic approaches and tools</td>
<td>ZF (BTI), RG (MSU)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>1.2. GBS PI lines; establish GWAS core</td>
<td>YW (ARS-WI)</td>
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<tr>
<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations - cucumber</td>
<td>ZF (BTI), RG (MSU)</td>
<td>X</td>
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<tr>
<td>- cucumber</td>
<td>YW (ARS-WI)</td>
<td>X</td>
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<tr>
<td>1.2.2. Population genetics and GWAS analysis - cucumber</td>
<td>UR (WSVU), ZF (BTI)</td>
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<tr>
<td>- cucumber</td>
<td>YW (ARS-WI), RG (MSU)</td>
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</tbody>
</table>

2. Genomic assisted breeding

2.1 QTL map resistances

2.1.3. Cucumber - downy mildew - Phytophthora

2.2 Marker development and verification

2.2.3. Cucumber - downy mildew - Phytophthora

2.3. Advanced breeding line development

1.2 GBS of PI collection, establish GWAS core
Personnel: Weng (Wang Y, Tan J, Dymerski R), Grumet (Grumet R, Hammar S.) and Wehner (Wehner T., Silverman EJ) Labs

GBS of PI lines and GWAS panel selection
GBS has been completed for 1234 cucumber accessions including plant introduction (PI) lines and historical cultivars or landraces of cultivated (Cucumis sativus var. sativus) and wild (C. sativus var. hardwickii) cucumber lines. Data analysis was been performed by the bioinformatics team to identify SNPs, determine minor allele frequency, perform phylogenetic, population genomic, and linkage disequilibrium (LD) analysis. A core collection consisting of 392 lines was constructed which captures >95% of allelic diversity as well combined with representation of key disease resistance, fruit quality and agronomic features. This part of work was recently published in the journal, Horticultural Research (Wang et al., 2018).

Seed increase and selfing was started for the GWAS panel. Among the 390 lines, we requested fresh seeds from USDA collection for 119 lines. The rest have gone through at least one-generation of selfing. We also re-sequenced one plate of samples (96 lines) at >10× coverage.

Phenotyping of morphological traits and DM resistance in cucumber natural populations
Three hundred cucumber lines were grown in the University of Wisconsin Hancock Agricultural Research Station (HARS) for collection of morphological data. Meanwhile, 300 cucumber lines (2 reps, 6 plants per rep) were planted in North Carolina State University experimental field in summer 2018. Data for responses to DM natural infestation were collected. Unfortunately, only data form one rep were collected due to unforeseen natural disaster.
2019 work plan
1. Continue selfing and seed increase of the GWAS panel lines.
2. Seed increase of cucumber lines by self-pollination.
3. Prepare GWAS panel lines for re-sequencing (partial).
4. Approximately 250 lines in the GWAS panel will be planted in 2019 summer season in North Carolina State University fields for collecting data for responses to natural DM infestation.

2.1 and 2.2: QTL mapping, marker development for DM and PFR resistances

**Downy mildew (DM)** (Weng and Wehner Labs)

2018 progress

We aim to conduct QTL mapping of DM resistance from two resistant sources: PI 330628 (WI7120) and PI 197088. We previously identified two major-effect QTL dm4.1 and dm5.2 for DM resistance from WI7120 (Wang et al. 2016). Using the PI 197088×Coolgreen RIL population, we also identified 4 major-or moderate-effect QTL, dm4.1, dm5.1, dm5.2, and dm5.3 for DM resistance in PI 190788; dm5.3 is co-localized with pm5.1 (syn. CsMLO1 or CsMLO8, pm-h), which is a major-effect QTL for PM resistance in cucumber (Wang et al. 2017). We focused on three major-effect DMR QTL, dm4.1, dm5.2 from WI7210 and dm5.3 from PI 197088 for fine mapping.

F2 and RIL plants carrying respective QTL regions were selected to backcross with the susceptible cucumber line 9930. Near isogenic lines (NILs) for each QTL were developed in the susceptible 9930 genetic background. We have completed marker-assisted development of NILs for dm4.1 and dm5.2. Secondary F2 populations from crosses between resistant and susceptible NILs were developed, which were genotyped for DM inoculation responses in both field and controlled environments. The development of NILs for dm5.3 has been advanced to BC2.

In 2018, through QTL analysis in the secondary F2 populations, the dm4.1 and dm5.3 loci have been delimited to ~60-80 kb intervals on chromosomes 4 and 5, respectively.

2019 work plan
1. Narrow down the QTL region (1.5 LOD interval) of target QTL regions through continued fine genetic mapping and GWAS
2. Identify candidate genes for dm4.1 and dm5.2.
3. Growth chamber and field evaluation of DM resistance of the NILs.

**Phytophthora capsici fruit rot resistance in cucumber** (R Grumet lab - B Mansfeld, Y-C Lin)

Young fruit resistance to *P. capsici*

2018/19 progress:

QTL-seq analysis. SNP-based linkage analyses are being performed to identify disease resistance QTL from crosses between the susceptible, sequenced pickling cucumber breeding line, Gy14, and two PI109483-derived breeding lines using three populations:

i. An F2 population (n=397) of Gy14 X PI109483-53B from field grown plants, in summer 2017.

ii. An F2 population (n=222) of Gy14 X doubled haploid (DH) line A4-3 grown in the greenhouse in spring 2018. The DH lines were generously produced by Rijk Zwaan from three resistant breeding lines derived from PI109483. Based on tests in summer 2017, DH A4-3, was chosen for further population development.

iii. An F2 population (n=362) of Gy14 X DHA4-3 tested from field grown plant, summer 2018.

Three sets of harvests were performed for each experiment to provide replication in sampling dates, and at least 10 fruit per F2 individual. The populations exhibited a normal distributions for disease scores, consistent with a quantitative trait. Individuals from each end of the distribution, representing the
most resistant and most susceptible plants were selected for bulk segregant QTL-seq analysis. Cleaned reads were aligned to the cucumber Gy14 version 2 reference genome (Weng et al., http://cucurbitgenomics.org/) and the Genome Analysis Toolkit pipeline (GATK; v3.6) (McKenna et al., 2010; https://software.broadinstitute.org/gatk/) used for identification of SNPs and indel variations. SNP-indices were calculated as described in Takagi et al. (2013) using QTL-seqr (Mansfeld and Grumet, 2018) and mapped across the cucumber genome. There was good correspondence between peaks observed on chromosomes 5 and 6 in the two field seasons. The greenhouse trial gave different peaks, suggesting possible environmental effects on response.

![Figure 1. QTL-seq analysis of response of young cucumber fruit to *P. capsici* (data from field trial, summer 2018). Red and green lines - significance P<0.05, 0.01, respectively.](image)

2019/20 work plan
Design KASP markers to validate and narrow the QTL regions found in association with resistance to *P. capsici* for field grown plants. Screen large F2 population, identify recombinants in regions of interest, and test recombinant individuals in the field.

Age-related resistance (ARR) to *P. capsici*
2018/19 progress:
Defense response in ARR+ fruit. The transcriptomic analysis performed in 2017 using samples collected from susceptible- and resistant- age fruit (8 dpp and 16 dpp, respectively) at 0, 4, 24 and 48 hours post-inoculation (hpi) suggested that in ARR-expressing fruit, a successful defense is mounted within the first 24 hours. To understand the dynamics of infection during the first 24 hours, inoculated and control samples were collected from 8 and 16 dpp fruit peel for 3’RNAseq transcriptomic analysis and scanning electron microscopy at 0, 2, 4, 8, 12, 18, 24 hpi. SEM of resistant peels showed evidence for infection failure as early as 4 hpi, including aberrant long germ tubes, and un-germinated, deflated and/or disintegrated spores and hyphae, that were not observed on susceptible fruit. PCA of the transcriptome data showed strong transcriptional changes from 4 hpi and beyond for the inoculated 8 dpp. In contrast, marked changes occurred in the resistant samples by 2 hpi, suggesting an earlier response to infection in the resistant-aged fruit, with only minor changes after 4 hpi. Weighted co-expression networks identified several modules with differential, earlier response to infection at the resistant ages.

QTL mapping of ARR. Doubled haploid (DH) lines derived from F1 progeny of ‘Gy 14’ (ARR-) X ‘Poinsett 76’ (ARR+) were kindly produced by Rijk Zwan and used for QTL-seq analysis. Seed from 79 lines were planted in the greenhouse in 5 replicated blocks along with the two parental lines and F1 progeny. Flowers were hand pollinated, and a single fruit per plant was harvested at 17 days post pollination. Fruits were inoculated with 12 equally spaced 30 µL droplets (10^5 zoospores/ml) and scored at 7 days post inoculation (dpi) using a 0-5 point disease score (0 – no symptoms, 5 – extensive sporulation).
A total of 424 fruit were phenotyped with a mean disease rating of 2.0±0.1. The parental lines were consistently either ranked resistant or susceptible with mean disease ratings of 0.3 and 3.0 for ‘Poinsett 76’ and ‘Gy 14’, respectively. Consistent with our prior studies suggesting a dominant major factor, the RIL population was bimodally distributed and F1 fruit were largely resistant (1.1). High within-line variability of disease rating was observed in lines showing intermediate susceptibility, highlighting the need for reproducibility made available by using a fixed DH population. Fifteen of most resistant and susceptible lines were selected for a second trial in the greenhouse. The disease rating distributions of the two groups separated (Welch’s T test, \( P = 0.003 \)), with means of 2.1 (Resistant) and 4.0 (Susceptible). Eight resistant and susceptible lines which were consistently ranked in both experiments were selected for QTL-seq analysis.

A major ~9Mb QTL passing the 99% confidence interval and a \( \Delta \)SNP-index maximum of 0.88, was identified on chromosome 3. This region also was identified in a prior screen of F2 progeny.

**Figure 2.** QTL-seq analysis of ARR of cucumber fruit to *P. capsici*. Red and green lines - significance, \( P < 0.05, 0.01 \), respectively.

### 2019/20 work plan:
Prepare publication describing infection response to *P. capsici* and early expression of defense on ARR-expressing fruit.

Begin to examine QTL region on chromosome 3.

#### 2.3 Advanced line development for downy mildew resistance

**Marker-assisted QTL pyramiding (Weng and Wehner Labs)**

Our objective is to develop a new version of the elite pickle cucumber inbred line Gy14 with improved DM resistance to the post-2014 DM strain. We focused on marker-assisted pyramiding of the two major-effect QTL (\( dm4.1 \) and \( dm5.2 \)) of DM resistance from WI7120 into Gy14 genetic background. Crosses were made between Gy14 and plants carrying \( dm4.1 \) and \( dm5.2 \) QTL from WI7120/PI 197088. In 2017-2018 period, homozygous lines carrying both \( dm4.1 \) and \( dm5.2 \) were developed. In 2017 summer trial, these plants were grown in the University of Wisconsin Hancock Agricultural Research Station for preliminary observations. The plants were also tested for DM inoculation responses in controlled environments.

In 2019, we will:
1) Continue marker-assisted backcrossing in Gy14 genetic background for pyramiding of \( dm4.1 \) and \( dm5.2 \) QTL from WI7120/PI 197088. Combine \( dm3 \) into Gy14+\( dm4.1+dm5.2 \) genetic background through marker assisted selection.
2) Conduct field and greenhouse screening tests to evaluate DM resistance and performance of horticulture traits.
3) Prepare public release of the introgression lines carrying \( dm4.1, dm5.2 \), and \( dm4.1+dm5.2 \) (in Gy14 background).
**Breeding line development for DM resistance**

*Wehner lab: T Wehner, EJ Silverman*

**RIL development and evaluation of DM resistance.**

The RILs population was developed in 2007 by a cross PI 197088 (HR) × Coolgreen (S). A total of 200 F₂ lines were generated and self pollinated in the greenhouse in 2009. The RILs have been tested in 7 years of field evaluations under high disease intensity. The 2017 population contains 146 lines; 71 at S12 generation, 35 at S11 generation, 32 at S10 generation, and 8 at the S9 generation. Several lines are being recovered and advanced for use in genetic studies.

In 2016, we evaluated for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white).

In 2017, we evaluated for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs ranged from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs ranged from 2.7 to 8.0 for fruit quality rating (9-1). Five RILs had DM resistance of 2.0 to 4.3 and fruit quality of 5.0 to 8.0, making them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2018, we evaluated the 127 sublines in S₈ to S₁₁ for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Sublines were rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs ranged from 2.0 to 7.7 for best rating (0-9 scale) for DM resistance. The RILs were tested for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2019, we will evaluate sublines for high resistance to the new downy mildew in the field in North Carolina. The design will be a randomized complete block with 3 replications and 4 disease ratings. Sublines will be rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs usually range from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs will be tested for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars. We will also advance nine sublines that had high resistance and good fruit quality for use by industry.

**Inbreds with resistance and quality**

The population PI 197088 (HR) × Poinsett 76 (MR) contains 72 lines. The plants have been self-pollinated in the greenhouse 8 generations and tested in the field for evaluation of yield, quality and resistance. We recovered 9 lines of the 72 that did not advance to S₈ in the past greenhouse cycle. We were not able to recover 3 lines last greenhouse cycle and these lines are in the S₇ generation. Lines in S₆ and S₇ are being tested in the field for yield, earliness and quality for release to the industry.

We selected and self-pollinated sub-lines from 41 lines that are at the S₈ to S₉ generation in the greenhouse in 2016. The lines were evaluated for high resistance to the new downy mildew, as well as fruit quality, in the field in North Carolina. The most resistant lines were crossed in the greenhouse using parents that had intermediate fruit quality, with the objective of improving fruit quality among the highly resistant lines.

In 2017, we evaluated sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. A total of 38 sublines were evaluated in a randomized complete block with 3 replications and 4 disease ratings. The RILs ranged from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs ranged from 2.7 to 8.0 for fruit quality rating (9-1). Five RILs had DM resistance of 2.0 to 4.3 and fruit quality of 5.0 to 8.0, making them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

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In 2019, we will evaluate sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. Lines will be evaluated in a randomized complete block with 3 replications and 4 disease ratings. The RILs will be selected for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

Develop inbred cucumber populations. Three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) are being developed for inbred development of pickling and slicing type. Eight to 10 lines each have been selected with yield, earliness, quality and resistance. They will be released to industry for use cultivar development. In 2016, we advanced the most resistant families that also had acceptable fruit quality by self pollination in the greenhouse. There were 3 populations of 8, 9 or 10 families each (S1 to S4 generation) to make 1 or 2 sublines each. The resulting 50 families were tested for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also evaluated for fruit quality. Lines were evaluated for fruit quality on a 1 to 9 scale (1=poor, 9=excellent). A total of 3 lines were selected based on field data collected in 2016. The selected lines were self pollinated and also cross pollinated in pairs in fall 2016 to develop more highly resistant cucumber populations with better fruit quality.

In 2017, 54 lines (including checks) from the three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). Of those, 4 lines from Gy14, 3 lines from NC-25, and 2 lines from Poinsett 76 were advanced since they had resistance of 3 to 5 and quality of 5 to 7.

In 2018, lines (including checks) from the three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The most resistant lines with high fruit quality were advanced.

In 2019, lines (including checks) from the three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The most resistant lines with high fruit quality and high yield were advanced. Those were 2 lines of Gy14, 4 lines of NC-25, and 2 lines of Poinsett 76.

Identify new sources of resistance.

A new population derived from PI 605996 (HR) × 'Poinsett 76' is being developed to provide new sources of high resistance to downy mildew. The F2 progeny will be self-pollinated and the S1 lines tested in the field for high resistance to natural disease incidence of downy mildew at the Clinton, NC research station. In addition to resistance, lines will be selected for yield, earliness and quality.

In 2017, we produced sublines (S2) and backcross lines (BC1S1) from PI 605996 x Poinsett 76 that will be tested for high resistance to DM as well as fruit quality.

In 2018, we produced sublines (S4) and backcross lines (BC1S3) from PI 605996 x Poinsett 76 for testing for high resistance to DM, as well as fruit quality.

In 2019, we produced eight sublines (S4) and backcross lines (BC1S3) from PI 605996 x Poinsett 76 for testing for high resistance to DM, as well as fruit quality
Establish core GWAS populations

1.2.1.1.2.2 GBS of cucurbit species, establish molecular-informed core populations and 1.2.2.
Population genetics and GWAS analysis

The core set of accessions representing *Cucurbita pepo* diversity in the NPGS has been self-pollinated and is being combined with heirloom cultivars to anchor market classes and enrich for cultivar genetics. Sources of resistance and other representatives of diversity in other species to extend the utility of the panel and being selfed pollinated. The goal of representatives from other species is both because squash improvement often involves crosses between species and to extend the benefits of CucCAP investments to those that work with other species such as *C. moschata* (Puerto Rico). The second round of self pollination will likely take place with a subcontractor. The process is now including strain purification within the stocks for accessions that do not match their descriptors for hull-less seeded accessions that aren’t, we are creating new selfed stocks from hull-less segregants.

Three projects are already taking advantage of the GWAS population. For CucCAP, given the lack of phenotypic data, we have phenotyped the collection for qualitative traits of bush growth habit and hull-less seeds. Markers were created with this material validated in breeding populations to include as part of
the MS. A separate study is mapping cotyledon eucurbitacin content to support results from biparental populations.

**Powdery mildew resistance in squash**

2.2.4.1 Marker development and verification (Mazourek lab –K. LaPlant)
Completed in 2017

**Virus resistance in squash**

2.1.4.3, 2.1.4.4. Mapping resistance (M. Mazourek lab-K. LaPlant)

'Whitaker' is a *C. pepo* summer squash from Cornell that is resistant to PRSV and CMV, as well as powdery mildew and ZYMV. The pedigree of 'Whitaker' contains *C. ecuadorensis* and *C. okeechobeensis* subsp. *martinezii*, and therefore it contains several introgressions from each species within its genome. By using 'Whitaker' as a guide to common introgressions from *C. ecuadorensis*, we have tentatively identified a genomic region on chromosome 16 with a length of approximately 1 Mb that may be associated with resistance to PRSV. 'Whitaker' has been used extensively in many breeding programs. We have developed 'Whitaker'-based biparental mapping populations to further refine and validate any identified genomic regions associated with resistance.

2.3.4.3, 2.3.4.4. Introgress resistance into advanced breeding lines (L. Beaver, A. Linares labs – M. Miranda, W. Seda)

*Inheritance of resistance to PRSV:*

Two sources of resistance are well known in *C. moschata*: ‘Nigerian Local’ and ‘Menina’. The inheritance of resistance from ‘Nigerian Local’ has been previously studied, but inheritance studies have not been reported for ‘Menina’, nor is it known if resistance to PRSV in ‘Nigerian Local’ is allelic to that in ‘Menina’. In the inheritance study susceptible genotypes were ‘Verde Luz’, ‘Taina Dorada’ and ‘TP411’. The third to fifth leaf of inoculated seedlings were rated on a 0 to 4 scale for disease severity and scores were combined to convert to a 0 to 12 scale. Resistant x susceptible F2 populations using ‘Nigerian Local’ as the source of resistance (distributions on the left-hand side of Figure 1) had nearly normal distributions with an average disease severity of 5.23 in Nigerian Local x Taina Dorada and 6.25 in Verde Luz x Nigerian Local. In contrast, F2 populations with ‘Menina’ (distributions on the right-hand side of Figure 1) were strongly skewed towards resistance with an average severity of 3.38 in Menina x Taina Dorada, 2.27 in Verde Luz x Menina and 2.80 in TP411 x Menina. The resistant x resistant Nigerian Local x Menina F2 population was very highly skewed, with an average combined severity of 0.840 (Figure 2).

Resistant to susceptible segregations in F2 populations were variable, depending on how severity scores were grouped into the resistant versus susceptible classes. The most consistent results over similar types of crosses occurred when we grouped plants with an overall severity rating of ≤4 as resistant and grouped plants with an overall severity rating of ≥5 as susceptible. This grouping system also made biological sense since plants with ratings of ≥5 had high individual leaf severity scores, especially in leaves 4 and 5. Both F2 crosses made with ‘Nigerian Local’ fit a 7:9 (R:S) genetic model while all three crosses using ‘Menina’ fit a 13:3 model (Table 1). The resistant x resistant cross (Nigerian Local x Menina) fit a 15:1 model. These segregations suggest that at least two genes are involved in the inheritance of resistance to PRSV for both ‘Nigerian Local’ and ‘Menina’. The data clearly indicate that at least some of the genes for resistance in ‘Nigerian Local’ and ‘Menina’ are different. The resistance conferred by ‘Menina’ seems to be superior to that of ‘Nigerian Local’.

An important consideration when evaluating disease resistance in the greenhouse is the association between greenhouse readings and readings taken in the field. In breeding for PRSV resistance both symptom severity and ELISA readings can be used as a way to evaluate resistance. In a previous CucCAP report we reported correlations between greenhouse and field ELISA readings to be poor. However, we have since looked at this issue from a different point of view. A high correlation per se is not important as long as plants judged resistant (or susceptible) in the greenhouse are also judged as
resistant (or susceptible) in the field. Figure 2 presents greenhouse and field data from 2017. Data from 2016 showed a similar trend. All plants of genotypes known to be susceptible (Mos166, Waltham Butternut and Taína Dorada) fell into the upper right-hand quadrant, meaning they were classified as susceptible in both the greenhouse and field according to their ELISA reading. The results for genotypes known to be resistant (Nigerian Local and Menina) were not as clear. For these genotypes, ELISA readings in the greenhouse were often expectantly high (positioned in the lower right-hand quadrant), while readings in the field were low. However, it should be noted that in this trial greenhouse readings were taken at 18 days post-inoculation (dpi) on the 3rd leaf. Since carrying out this study we have found that greenhouse ELISA readings for PRSV are best taken on the 4th leaf at about 21 dpi (PRSV ELISA readings tend to be high for all genotypes in the first few leaves).

Figure 1. Distributions of severity ratings in F2 populations of tropical pumpkin (*Cucurbita moschata*) inoculated with *Papaya ringspot* virus (PRSV). Populations developed with resistant parent ‘Nigerian Local’ are shown on the left; populations developed with resistant parent ‘Menina’ are shown on the right. For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.

Nigerian Local x Taina Dorada (F2)
Mean severity = 5.23

Menina x Taina Dorada (F2)
Mean severity = 3.38

Verde Luz x Nigerian Local (F2)
Mean severity = 6.25

Verde Luz x Menina (F2)
Mean severity = 2.27

TP411 x Menina (F2)
Mean severity = 2.80

Figure 2. Distribution of combined severity ratings of plants (n=238) from the Nigerian Local x Menina F2 population inoculated with *Papaya ringspot* virus (PRSV). For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.
Table 1. Number of plants evaluated and observed segregations in parental, F1 and F2 populations. 'Nigerian Local' was the resistant parent in the F1 and F2 crosses. Goodness-of-fit in F2 populations was tested with chi-square.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Population</th>
<th>Observed segregation</th>
<th>Tested ratio (R:S)</th>
<th>$\chi^2$</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigerian Local</td>
<td>Res. parent</td>
<td>34</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menina</td>
<td>Res. parent</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taina Dorada</td>
<td>Sus. parent</td>
<td>2</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verde Luz</td>
<td>Sus. parent</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP411</td>
<td>Sus. parent</td>
<td>0</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Resistant x susceptible crosses with Nigerian Local as resistant parent:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Population</th>
<th>Observed segregation</th>
<th>Tested ratio (R:S)</th>
<th>$\chi^2$</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigerian Local x Taina Dorada</td>
<td>F1</td>
<td>8</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verde Luz x Nigerian Local</td>
<td>F1</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigerian Local x Taina Dorada</td>
<td>F2</td>
<td>47</td>
<td>64</td>
<td>7:9</td>
<td>0.0894</td>
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<tr>
<td>Verde Luz x Nigerian Local</td>
<td>F2</td>
<td>42</td>
<td>68</td>
<td>7:9</td>
<td>1.3859</td>
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</tbody>
</table>

Resistant x susceptible crosses with Menina as resistant parent:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Population</th>
<th>Observed segregation</th>
<th>Tested ratio (R:S)</th>
<th>$\chi^2$</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menina x Taina Dorada</td>
<td>F1</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verde Luz x Menina</td>
<td>F1</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP411 x Menina</td>
<td>F1</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menina x Taina Dorada</td>
<td>F2</td>
<td>91</td>
<td>29</td>
<td>13:3</td>
<td>2.3110</td>
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<tr>
<td>Verde Luz x Menina</td>
<td>F2</td>
<td>101</td>
<td>17</td>
<td>13:3</td>
<td>1.4611</td>
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<tr>
<td>TP411 x Menina</td>
<td>F2</td>
<td>91</td>
<td>20</td>
<td>13:3</td>
<td>0.0390</td>
</tr>
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</table>

Cross between two resistant parents:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Population</th>
<th>Observed segregation</th>
<th>Tested ratio (R:S)</th>
<th>$\chi^2$</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigerian Local x Menina</td>
<td>F1</td>
<td>20</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Nigerian Local x Menina</td>
<td>F2</td>
<td>224</td>
<td>14</td>
<td>15:1</td>
<td>0.0549</td>
</tr>
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</table>

For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12. Plants were then categorized R for resistant (overall severity rating of <4) or S for susceptible (overall severity rating of ≥5).

Figure 3. Scattergram of enzyme-linked immunosorbent assay (ELISA) readings for Papaya ringspot virus (PRSV) in six genotypes of tropical pumpkin inoculated with PRSV. Each data point represents readings for a single plant at 18 days post-inoculation (dpi) and 54 dpi. MEN='Menina', NL='Nigerian Local', MOS='Mos166', TD='Taina Dorada’, SOL='Soler’, WAL='Waltham’. Readings above the horizontal line are considered to be positive readings for the virus. Readings to the right of the vertical line (18 dpi) or above the horizontal line (54 dpi) are considered positive for the presence of PRSV.
Phytophthora blight resistance in butternut squash

2.1.4.2 Mapping resistance to Phytophthora blight – Smart, Vogel, Kousic

Raw genotypes calls from TASSEL for the NPGS C. moschata collection were filtered using vcftools to retain just biallelic SNPs. This SNP set was then filtered for minor allele frequency (MAF) > 0.05, sample call rate > 0.20, and mean read depth per site < 44 (corresponds to 95th percentile). The filtered data set included 311 accessions and 40,428 SNPs.

Missing genotypes were imputed with the LD-k nearest neighbors algorithm implemented in TASSEL. 1000 genotype calls with a read depth > 8 were masked to estimate the imputation accuracy rate, which was estimated to be 78%. Any genotypes not imputed by the algorithm were then imputed with the mean. The imputed SNPs were then filtered again for MAF > 0.05, resulting in a final set of 36,568 SNPs.

Ratings at 12 and 41 days post inoculation (dpi) were analyzed separately, only using plots with 3 or more non-missing data points. The mean rating per plot was used as the response variable in a mixed linear model, with accession treated as a random effect because of unbalanced data. Rep was included as a random effect with Dpi12 but not with Dpi41 because the variance explained by Rep was effectively 0. Best linear unbiased predictions (BLUPs) for accession effects were then used in GWAS. The line mean heritability was 0.66 for Dpi12 average plot rating and 0.29 for Dpi41 average plot rating.

GWAS was performed using the rrBLUP R package. Genotype data was available for 265 accessions with BLUPs for Dpi12 and 264 accessions with BLUPs for Dpi41. Population structure and relatedness among accessions were controlled by including the first principal component of the genotype matrix as a fixed effect and treating a random effect for accession and including a relationship matrix to model their effect and treating a random effect for accession and including a relationship matrix to model their covariance.

No significant SNPs were identified using the ratings from 12 dpi. The qqplot shows a good fit of the observed p-values to the expected under the null hypothesis. (Figure 4).

With the ratings at 41 dpi, there are three significant SNPs at a false discovery rate of 5% on chromosomes 10 and 18. However, the qqplot shows that the Type I error is inflated. This is likely related to the highly non-normal data used for GWAS which violates model assumptions. These three significant SNPs have low minor allele frequencies (<0.10).. (Figure 6).
Figure 5. Manhattan plot and qqplot for disease ratings for *C. moschata* GWAS panel inoculated with *P. capsici* 41 days post inoculation.

Figure 6. Boxplots of effect of the most significant SNP from the two chromosomes on the average plot rating at both 12 and 41 dpi.

Figure 7. Prediction accuracy reported is the correlation between observed phenotypes and predicted phenotypes.

Genomic prediction using a GBLUP model was performed with the rrBLUP package. Eighty percent of the accessions were used as a training set to predict the phenotypes of the remaining twenty percent, whose phenotypic values were masked. This five-fold cross validation scheme was repeated 100 times.

The mean prediction accuracy for dpi12 BLUPs (0.51) was considerably higher than the mean prediction accuracy for dpi41 BLUPs (0.25). (Figure 7)
Economic Team

Team members:
Marco Palma (Texas A&M Univ.)
Luis Ribera (Texas A&M Univ.)

<table>
<thead>
<tr>
<th>(b) Obj. 3. Economic impact analyses, disease control information</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>3.1 Perform economic analysis, cost of production/disease control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1.1. Define, parameterize, simulate, validate production variables</td>
<td>LR (TAMU), MP (TAMU)</td>
<td>X</td>
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<tr>
<td>3.1.2. Estimate the potential economic impacts to the cucurbit industry</td>
<td>LR (TAMU), MP (TAMU)</td>
<td>X</td>
</tr>
</tbody>
</table>

3.1 Perform economic analysis, cost of production/disease control
3.1.1. Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets

Completed:

- Macro and micro economic variables were collected to develop the economic model, such as interest rates, input costs, production windows and existing crop budgets.
- Graduate students were selected to work on the project and were trained on how to collect data to develop representative farms.
- Faculty and graduate students have IRB clearance to collect information from producers.
- Developed 11 representative farms in California (3 watermelon and 3 cantaloupe), Florida (3 watermelon) and Texas (1 watermelon and 1 cantaloupe)
- Estimated the economic impact of diseases to cantaloupes, fresh cucumbers, pickles, squash and watermelons.

In progress:

- Identifying Extension budgets in the Northeast region
- Work with CucCap pathologists to estimate yield and quality changes due to CucCap work
- Validate economic impact of diseases
- Validate all representative farms

Publications

Extension/Outreach Team

Team members:
Jonathan Schultheis (N. Carolina St. Univ.)
Mary Hausbeck (Michigan St. Univ.)
Angela Linares (Univ. Puerto Rico)
Jim McCreight (USDA, ARS)
Lina Quesada (N. Carolina St. Univ.)
Chris Smart (Cornell Univ.)
Linda Wessel Beaver (Univ. Puerto Rico)

(b) Obj. 3. Economic impact analyses, disease control information

<table>
<thead>
<tr>
<th>3.2 Provide readily accessible information to facilitate disease control</th>
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</thead>
<tbody>
<tr>
<td>3.2.1. Develop a centralized cucurbit disease website</td>
<td>JS (NCSU)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster</td>
<td>LQ (NCSU), MH (MSU), CS (CU), ALR (UPR)</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>3.2.3 Provide disease alerts and forecasting tools</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>3.2.4 Provide diagnostic and disease management assistance</td>
<td>LQ (NCSU), MH (MSU), CS (CU)</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>3.2.5 Field days and demonstration plots</td>
<td>Crop and extension teams</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

3.2. Provide readily accessible information to facilitate disease control

The CucCAP Extension team communicates the grant’s goals, progress, results and its applications from those directly involved in the grant to stakeholders including breeders, seed company personnel, allied industry partners, growers, and other interested persons. Leadership for extension in each commodity is provided mainly by Mary Hausbeck (cucumber), Lina Quesada (watermelon), Chris Smart (squash), and Jim McCreight (melon). The focus is on aspects related to disease. Jonathan Schultheis complements the plant pathology PIs with pertinent cultural management information. He is also providing leadership with respect to the Cucurbit CAP webpage in conjunction with Mary Lorscheider, the web manager for this project.

Extension activities include both stakeholders and extension personnel via field days, workshops, and commodity meetings at the local, state, national, and international levels. The following information provides updates for April 2018 through March 2019 regarding the objectives and their associated results or outputs. Pertinent extension or research activity inadvertently missed in previous reports has been included with this report.

3.2.1 Develop a centralized cucurbit disease website.

The CucCAP website was first presented at the CucCAP Annual meeting in March 2017. News about cucurbit disease management including disease outbreaks, current CucCAP research activities, announcements of new publications, upcoming presentations by CucCAP researchers at scientific meetings and cucurbit commodity events is posted on the website throughout the year. An email newsletter called the CucCAP Chronicle was sent monthly since June 2017. The newsletter reports recent news and events posted on the CucCAP website. The newsletter is also shared on the CucCAP Facebook and Twitter sites. A monthly post featuring important CucCAP team accomplishments was added to the website and newsletter in October 2018. The number of subscribers to the CucCAP Chronicle has grown from the initial 21 members of the CucCAP team to 93 subscribers in March 2019. A link to previous installments of the CucCAP Chronicle is available at: https://us15.campaign-archive.com/home/?u=925e5a7bece071d0c087e746f&id=e0b5619a11
Google Analytics was set up for the website on September 1, 2017 and 1 ½ years of website visitor data has been collected.

![Site user and session data for the CucCAP website from Sept. 1, 2017 to March 28, 2019.](image)

Figure 1. Site user and session data for the CucCAP website from Sept. 1, 2017 to March 28, 2019. (Users 11,651; new users 11,623; sessions 16,013; sessions per user 1.37; page views 34,875; pages / session 2.18; average session duration 2:35; bounce rate 72.6%).

![CucCAP website page views from Sept. 1, 2017 to March 28, 2019.](image)

Figure 2. CucCAP website page views from Sept. 1, 2017 to March 28, 2019. (page views 34,845; unique page views 26,474; average time on page 2:11; bounce rate 72.6%; exit rate 45.92%).

### 3.2.2. Develop and post diagnostic resources and disease control information

Cucurbit disease factsheets, crop production manuals, and integrated pest management resources for the Northeast, Southeast and the Midwest are maintained and updated on the website. Notices of regional commodity meetings and Extension education sessions are posted on the CucCAP website events calendar. News from CucCAP researchers is reported on the website and in the CucCAP Chronicle, the monthly newsletter. The CucCAP website shares weekly reports from The Cucurbit Downy Mildew Forecast and Melcast throughout the growing season.
3.2.3. Provide disease alerts and forecasting tools

Weekly conference calls, NCSU Vegetable Team (Quesada): These calls occurred from June 7 to September 27 in 2016, from April 4 to October 31 in 2017, and from April 13 to October 12 in 2018. Weekly conference calls, Cucurbit ipmPIPE (Hausbeck, Quesada, Smart): These calls begin in May and continue through August every year and include plant pathologists from the eastern US. Smart has active Facebook and Twitter accounts, and is also active in the Cornell Vegetable alerts blog (which sends messages to vegetable extension educators). As soon as diseases of cucurbits are first reported in NY, she alerts growers through these avenues. Additionally, any new advances made through CucCAP are also shared through these methods.

3.2.4 Provide diagnostic and disease management assistance.

In 2018, Quesada provided diagnostics and disease management recommendations for 19 cucumber, 24 watermelon, 10 melon, 21 squash, and 5 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. In 2017, Quesada provided diagnostics and disease management recommendations for 22 cucumber, 31 watermelon, 9 melon, 10 squash, and 6 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. In 2016, Quesada provided diagnostics and disease management recommendations for 12 cucumber, 33 watermelon, 8 melon, 12 squash, and 9 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. In 2015, Quesada provided diagnostics and disease management recommendations for 40 cucumber, 28 watermelon, 10 melon, 13 squash, and 11 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. Quesada has also been involved in providing disease management recommendations through oral presentations, social media (Twitter: 1,922 followers, Facebook: 705 followers, LinkedIn: 1,641 followers), and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US Vegetable Crop Handbook. Smart diagnosed over 60 samples during the 2017 growing season, in addition to over 100 disease issues diagnosed via photo through email or text message. Of the cucurbits, 30 were pumpkin, 15 summer squash, 10 winter squash, and 5 cucumber. She also provides management recommendations through oral presentations and updates to regional extension educators (both conventional and organic). Smart diagnosed 118 samples during the 2018 growing season, in addition to over 80 disease issues diagnosed via photo through email or text message. Of the cucurbits, 24 were pumpkin, 20 summer squash, 17 winter squash, and 10 cucumber. She also provides management recommendations through oral presentations and updates to regional extension educators (both conventional and organic).

3.2.5. Field days and demonstration plots

Hausbeck hosted a series of Phytophthora and Downy Mildew workshops for growers in Michigan. Smart and Quesada were guest speakers at two of the Michigan workshops. McCleirght Hosted a Cucurbit Field Day at the University of California Desert Research and Extension Center, Holtville, CA. The event focused on cucurbit powdery mildew differentials and the proposed melon core collection.

Powdery Mildew. The replicated test included 47 entries, including the standard powdery mildew race differentials, candidate accessions, and the 21-line triple septet differential set proposed by International Cucurbit Powdery Mildew Initiative.

Core Collection. This includes ca. 384 melon cultigens tentatively selected to represent the genetic variation in the larger set of 2000+ USDA, GRIN accessions and ‘heirloom’ lines.

Schultheyis conducted Variety trials on watermelon, melon, squash, and pumpkins in North Carolina in 2018.

Quesada is evaluating commercial watermelon varieties for anthracnose resistance and supported demonstration plots to evaluate fungicides for disease control and combinations of tolerant varieties and fungicide applications.

Smart has yearly demonstration plots at the Phytophthora blight farm with variety trials for squash (winter squash and summer squash) and other vegetables.
2018 – 2019 Production guides

2018 – 2019 Web content
2. Hausbeck, M.K. Project GREEEN 20th Anniversary MSU AgBioResearch Video about Downy Mildew on Pickling Cucumbers Published on Sep 20, 2018
   https://www.canr.msu.edu/news/time-for-downy-mildew-protectant-sprays-for-cucumbers
   https://plantpathology.ces.ncsu.edu/2018/06/cucumber-anthracnose-reported-in-north-carolina/
   https://plantpathology.ces.ncsu.edu/2018/05/watermelon-powdery-mildew-found-in-south-carolina/

2018 – 2019 Publications from demonstration plots


Cumulative CucCAP

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS
PUBLICATIONS

REFEREED PUBLICATIONS, BOOK CHAPTERS and CONFERENCE PROCEEDINGS

Refereed Publications  (total-92 ; added this year-32)


Dhillon, N.P.S., S. Phethin, S. Sanguansil, and J.D. McCreight. 2017. Early staminate flowering monoecious lines have potential as pollenizers for gynoecious hybrid bitter gourd cultivars Pak. J. Agri. Sci. 54:27–33. 10.21162/PAKJAS/17.4354


Fall LA, Clevenger J, McGregor C. 2018. Assay development and marker validation for marker assisted selection of *Fusarium oxysporum* f. sp. **niveum** race 1 in watermelon. Molec Breed 38:130

Fall LA, Clevenger J, McGregor C. 2018. Assay development and marker validation for marker assisted selection of *Fusarium oxysporum* f. sp. **niveum** race 1 in watermelon. Molec Breed 38:130


Mansfeld BN, Colle M, Kang Y, Jones AD, Grumet R. 2017. Transcriptomic and metabolomic analyses of cucumber fruit peels reveal a developmental increase in terpenoid glycosides associated with age-related resistance to *Phytophthora capsici*. Horticulture Research. 4:17022


Pan, Y.P., Qu, S.P., Bo, K.L., Gao, M.L., Haider, K.R., Weng, Y. 2017. QTL mapping of domestication and diversifying selection related traits in round-fruited semi-wild Xishuangbanna cucumber


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Conference Proceedings


**Book/Book Chapters**


Many members of CucCAP contributed chapters to:


  Comparative genomics of the Cucurbitaceae. Chapter 12.

Other

Plant variety protection (PVP)
watermelon rootstock with resistance to Fusarium wilt and root-knot nematodes.
EXTENSION and OUTREACH RESOURCES

**Disease Control and Extension Resources**


February 2016


Web Content

Anthracnose of cucurbits: http://content.ces.ncsu.edu/anthracnose-of-cucurbits
Cucurbit downy mildew: http://content.ces.ncsu.edu/cucurbit-downy-mildew
Cucurbit powdery mildew: http://content.ces.ncsu.edu/cucurbit-powdery-mildew
Fusarium wilt of watermelon: http://content.ces.ncsu.edu/fusarium-wilt-of-watermelon
Gummy stem blight of cucurbits: https://content.ces.ncsu.edu/gummy-stem-blight-and-phoma-blight-on-cucurbits
https://cucurbits.ces.ncsu.edu/2018/05/watermelon-powdery-mildew-found-in-south-carolina/

**Spanish Factsheets – Accessible on Website**

https://cuccap.org/espanol/manejo-de-phytophthora-en-calabaza-de-invierno-y-en-calabaza/

https://cuccap.org/espanol/monitoreo-y-manejo-del-anublo-lanoso-de-las-cucurbitaceas/

https://cuccap.org/espanol/manejo-de-phytophthora-en-cantalupe-melon-y-sandia/

https://cuccap.org/espanol/manejo-de-phytophthora-en-pepino/

https://cuccap.org/espanol/manejo-de-phytophthora-en-calabaza-de-verano-y-calabacín/

https://cuccap.org/espanol/monitoreo-y-manejo-del-anublo-lanoso-de-las-cucurbitaceas/

https://cuccap.org/espanol/manejo-del-anublo-lanoso-de-la-cucurbitaceas-para-los-agricultores/

https://content.ces.ncsu.edu/pudricion-gomosa-del-tallo-en-cucurbitaceas/

https://content.ces.ncsu.edu/anublo-polvoriento-en-cucurbitaceas

https://content.ces.ncsu.edu/la-antracnosis-de-las-cucurbitaceas

**Webinars**
Smart, C.D. Vegetable Diseases (for beginning growers), March 15, 2017. This was a 1.5 hour webinar.
Smart C.D. Managing cucurbit downy mildew in organic systems in the northeast. December 6, 2016. 1.5 hour webinar with 135 participants.
Smart, C. D. Vegetable Diseases (for beginning growers), March 16, 2016. 1 hour webinar.
**PRESENTATIONS**

**Scientific Conferences and University Presentations (149)**


Ando, K. and McCreight, J.D. 2018. Potential for Production of Turkmen Melons in California, Nation Association of Plant Breeders annual meeting, Davis, CA, 7-10 August 7-10 2018.


D’Arcangelo K., Miles T., and Quesada-Ocampo L. M. (2018) Pseudoperonospora cubensis populations infecting wild and commercial cucurbit hosts display host-specific sensitivity to fungicides. Phytopathology


Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Dept. of Plant Biology, Cornell University. March

Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. College of Horticulture, Shandong Agric. Univ. April

Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. College of Food Science and Engineering, Hefei University of Technology. May

Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Texas A&M University. September, 2016

Fei, Z. 2016. Genome sequencing of sweetpotato wild progenitors. Institute of Vegetables and Flowers, CAAS. April, 2016

Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Nanjing Agricultural University. July, 2016

Fei, Z. 2017. Genome variation elucidates evolution and domestication of fruit ripening and quality traits in watermelon. PAG. January, 2017


Northeast Agricultural University. August

Agricultural Genomics Institute of Shenzhen. May


P. Reeves, X. Wang, T. Wehner, Y. Weng, S. Wu and R. Grumet. 2018. Characterization of the USDA 

Genomics Joint Conference. September.

with Gummy Stem Blight Resistance in Watermelon. IPBGG Retreat 2018, Pine Mountain, GA.

Gummy Stem Blight Resistance in Watermelon. Southern Region American Society for Horticultural 
Science, Birmingham, AL.

Grumet, R. 2016. Introduction to CucCAP - developing genomic resources for the cucurbit community. 
Plant and Animal Genome Conference. San Diego, CA. 
https://pag.confex.com/pag/xxiv/webprogram/Paper18951.html

Grumet R. 2017. The USDA-SCRI CucCAP project: Leveraging applied genomics to increase disease 
resistance in cucurbit crops. SCRI Advisory Board Meeting, Aug. 17, Traverse City MI

Grumet R. 2018. The CucCAP project: leveraging applied genomics to increase disease resistance in 
cucurbits. Fifth International Research Congress, Beijing China

Grumet R. 2018. Cucumber fruit development and resistance to Phytophthora capsici. Nanjing 
Agricultural University, Nanjing China

Grumet R. 2018. Cucumber fruit development and resistance to Phytophthora capsici. Beijing Vegetable 
Research Institute, Beijing China

Science, Washington DC

Grumet R. 2018. Genomic analysis of cucurbit PI collections. NC-7 Meeting, Regional Plant Introduction 
Station, Ames IA

Grumet R. 2018. Cucumbers – the CucCAP project, genetic diversity, and resistance to Phytophthora 
capsici. University of Illinois, Champaign IL

Mazourek, S. Kousik, K-S Ling, C. McGregor, P. Wechter, L. Wessel-Beaver, W. Wintermantel, M. 
Hausbeck, A. Linares-Ramirez, L. Quesada-Ocampo, and C. Smart. 2018. The CucCAP project: 
Genomic Tools and Resources to Facilitate Breeding for Disease Resistance in Cucurbits. 

mefenoxam of Phytophthora capsici in Michigan. American Phytopathological Society Annual 
Meeting, Boston, MA, 29 Jul-3 Aug. Poster presentation.


Kousik, C.S. 2017. Presented an invited seminar on “Progress and challenges in managing watermelon diseases”. Department of Plant Pathology, University of Georgia Athens, GA, Aug. 2017. >50 attendees


Kousik, C.S. and Ikerd, J.L. 2015. Reaction of Phytophthora fruit rot resistant germplasm lines to a broad range of Phytophthora capsici isolates from across United States of America. International soilborne Oomycete conference, Duck Key, FL. December


Lin YC, Grumet R. 2018. QTL-seq of young fruit resistance to Phytophthora capsici in cucumber. Cucurbitaceae 2018, Davis CA

Linares-Ramirez, A.M. 2016. Cucurbits: From to the field to the lab. Agricultural Experimental Station, University of Puerto Rico.

Ling, K.-S. 2017. Presented invited seminars on “Developing genome-guided strategies to manage viral diseases of cucurbit crops” in four institutions throughout China, including: Zhengzhou Fruit Research Institute, Beijing Vegetable Research Center (China, Israel, and the U.S. Workshop on Cucurbit Research), Zhejiang Academy of Agricultural Sciences Fujian Agricultural and Forestry University.


Mansfeld B, Grumet R. 2018, QTLseqr: An R package for bulk segregant analysis with next generation sequencing. PAG XXVI, San Diego CA.


Mansfeld B, Grumet R. 2018. Inhibitory effects of cucumber fruit age-related resistance to Phytophthora capsici manifest within 24 hours of infection. Cucurbitaceae 2018, Davis CA


Noel N. and Quesada-Ocampo L. M. (2017) Characterizing Colletotrichum orbiculare, the causal agent of cucurbit anthracnose, for fungicide efficacy and host susceptibility in North Carolina. Phytopathology 107: S5.77.


Quesada-Ocampo LM. 2017. From the field to the lab and back: translational strategies to improve disease control in vegetable crops. VIB-PSB-NC State Plant Sciences Workshop, Ghent, Belgium


Smart, C.D. SUNY Potsdam November 2017, Potsdam NY. Genomic approaches to understand and manage plant disease epidemics.


Wechter, W.P. 2019. Cucurbit and Brassica pathology research at the U.S. Vegetable Laboratory: Old-school and New-school approaches to plant disease resistance. Invited seminar at the Department of Plant Pathology, University of California-Davis.


Weng Y. 2017. Improve QTL detection power: cucumber downy mildew resistance. An invited talk at China Agricultural University (Beijing, China, July 111, 2017)


Weng Y (2018) ‘Cucumber Molecular Breeding- current status and perspectives’. Invited talk in the Institute of Vegetable Research, Sichuan Academy of Agricultural Sciences (May 7, 2018, Chengdu, China)


Wu S. 2018. Cucurbita genome sequences provide insights into polyploid genome evolution and heterosis in interspecific hybrid. PAG. January.


Extension and Outreach Presentations (145)


Grumet, R. 2015. Update on resistance to Phytophthora capsici in cucumber. PPI Annual Meeting October 30, 2015, Fort Worth, TX


Grumet R. 2017. CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops PPI Spring Meeting, April 19, Milwaukee WI

Grumet R, Lin YC, Mansfeld B. 2017. Resistance of cucumber fruit to Phytophthora capsici. PCIC/PPI, Nov. 1, Chicago IL


Hausbeck, M. 2017. A smorgasbord of vegetable diseases is on today’s menu. MSU Extension and AgBioResearch State Council Meeting, Lansing, MI, Mar. 30 attendees.


Kousik, C.S. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. Indiana Horticultural Congress, Indianapolis, IN. January. 45 attendees (at the talk)

Kousik C.S. 2016. Managing Phytophthora fruit rot of watermelon. Georgia Watermelon Association, St. Simmons, GA. January. Over 100 attendees

Kousik, C.S. 2016. Breadth of resistance of USVL developed Phytophthora fruit rot resistant germplasm lines to Phytophthora capsici isolates from across USA. Watermelon Research and Development Group meeting. San Antonio, TX. Feb >50 attendees


Kousik, C.S. 2017. Presented information on Phytophthora fruit rot and powdery mildew of watermelon to the U.S. Secretary of Agriculture, Dr. Sonny Purdue and his team when they visited the U.S. Vegetable Laboratory, USDA, ARS in Charleston, SC. August 21, 2017.

Kousik, C.S. 2017. Provided information to Sarah Mock, Washington D.C. Bureau Chief for RFD-TV on research being conducted on watermelon at the U.S. Vegetable Laboratory and details of the visit of Dr. Sonny Purdue to USVL. August, 21, 2017. The interview was aired by RFD-TV and is located at website: https://youtu.be/R4tHGZSJqRI


McCreight, J.D. 2015. Melon host plant resistance to powdery mildew and CYSDV. Fall Desert Crops workshop, sponsored by the University of California ANR Cooperative Extension, Imperial County, and University of Arizona Cooperative Extension, Yuma County. El Centro, CA, Oct.


McCreight, J.D. 2017. AgKnowledge class annual visit to U.S. Agricultural Research Station, Grower-Shippers Association of Central California, Salinas, CA, June 2017.

McCreight, J.D. 2017. Assisted Seed Central (http://www.seedcentral.org) hosting 100 persons from ag related companies with research updates and provided laboratory and greenhouse tours, Salinas, CA, April.

McCreight, J.D. 2017. U.S. Plant Breeding: Lettuce, Spinach, Melon, and Sugar beet. Seed Central (an initiative of the Seed Biotechnology Center at the University of California Davis, and Seed Quest), Salinas CA, April.


McGregor, C.E. 2016. Advances in Watermelon Breeding. Southeast Regional Fruit & Vegetable Conference, 8-10 January 2016, Savannah, GA.


Miller N, Druffel A, Adams M, Quesada-Ocampo LM. 2017. Control options for Fusarium wilt of watermelon. 32nd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December


Ribera, Luis A. Trade impact talks:
C-FARE, Washington, DC, April 6, 2017.
Viva Fresh 2017, Austin, Texas, April 21, 2017.
Texas A&M AgriLife Program Planning Meeting, Rosenberg, Texas, May 9, 2017.
Moosejaw, Canada, June 27, 2017.
Imperial Valley EDC, Calexico, California, August 15, 2017

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Ag. Economics Extension Tailgate Workshop, College Station, Texas, September 30, 2017.
Extension Outlook Conference, Stillwater, Oklahoma, October 20, 2017
Imperial Valley EDC Annual Banquet, Calexico, California, November 16, 2017


Smart, C. 2015. Disease problems common during the 2015 growing season. Twilight meeting, Eden Valley, NY.


Smart, C.D. 2016. Understanding and controlling diseases of cucurbits. Crop Consultant Meeting, December 1, Syracuse NY.

Smart, C.D. 2016. Why is the Phytophthora blight from important? New York State Ag Experiment Station Task Force, October 10, 2016.


Smart, C.D. 2016. How the NY Farm Bureau helped established the Phytophthora blight farm. Midwest Farm Bureau visit to NYSAES, June 24, 2016.


Smart, C.D. 2017. On-farm discussion of methods to control Phytophthora blight in summer squash. Seneca Falls NY, July 2017


VandenLangenberg K, Wehner T. 2015. High resistance over the production season to the new downy mildew in cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)


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Weng, Y. 2015. QTL Mapping for downy mildew resistance in WI7120 cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX).


